frontiers | Research Topics

# Zoonoses- a rising threat to healthcare system

**Edited by**
Wenn-Chyau Lee, Yang Cheng, Varakorn Kosaisavee and Laurent Rénia

**Published in**
Frontiers in Microbiology

ISSN 1664-8714
ISBN 978-2-8325-2838-9
DOI 10.3389/978-2-8325-2838-9

## About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

## Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

## Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

## What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

# Zoonoses- a rising threat to healthcare system

**Topic editors**

Wenn-Chyau Lee — University of Malaya, Malaysia
Yang Cheng — Institute of Biomedical Sciences, Academia Sinica, Taiwan
Varakorn Kosaisavee — Mahidol University, Thailand
Laurent Rénia — Nanyang Technological University, Singapore

**Citation**

Lee, W.-C., Cheng, Y., Kosaisavee, V., Rénia, L., eds. (2023). *Zoonoses- a rising threat to healthcare system*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-2838-9

# Table of contents

frontiers | Frontiers in Microbiology

TYPE Editorial
PUBLISHED 13 June 2023
DOI 10.3389/fmicb.2023.1232183



OPEN ACCESS

EDITED AND REVIEWED BY
Axel Cloeckaert,
Institut National de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), France

*CORRESPONDENCE
Wenn-Chyau Lee
leewc_88@hotmail.com

RECEIVED 31 May 2023
ACCEPTED 02 June 2023
PUBLISHED 13 June 2023

CITATION
Lee W-C, Cheng Y, Kosaisavee V and Renia L (2023) Editorial: Zoonoses-a rising threat to healthcare system.
*Front. Microbiol.* 14:1232183.
doi: 10.3389/fmicb.2023.1232183



# Editorial: Zoonoses-a rising threat to healthcare system

Wenn-Chyau Lee[1,2]*, Yang Cheng[3], Varakorn Kosaisavee[4] and Laurent Renia[2,5,6]

[1]Department of Parasitology, Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia, [2]A*STAR Infectious Diseases Labs (A*STAR ID Labs), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore, [3]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, [4]Department of Parasitology and Entomology, Faculty of Public Health, Mahidol University, Bangkok, Thailand, [5]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore, [6]School of Biological Sciences, Nanyang Technological University, Singapore, Singapore

KEYWORDS

**zoonoses, infectious diseases, parasite, virus, bacteria**

Editorial on the Research Topic
Zoonoses-a rising threat to healthcare system

Zoonoses are infections caused by pathogens that are transmitted from animals to humans. They contribute to significant healthcare burden in many parts of the world. The incidence of spillover infections from animals to humans may increase and spread to wider geographical areas in future, due to the changes of climate, ecology, population structure, and socioeconomic activities (Ellwanger and Chies, 2021; Lee et al., 2022). Additionally, immigration and traveling further complicate the transmission biology of zoonoses (Mavroidi, 2008), imposing challenges to the management and control of such outbreaks. Notably, many zoonotic pathogens cause asymptomatic infections to their natural hosts but produce severe pathology in humans (Owen et al., 2004; Evangelista and Coburn, 2010; Hu et al., 2022). As healthcare workers may not be familiar with the diagnosis and pathogenesis of different zoonoses in humans, delayed clinical interventions are relatively common, compromising prognosis. Importantly, research attention dedicated to many zoonotic outbreaks has been shown to wane over time. Thus, a Research Topic of articles covering different aspects of several zoonoses and infections with animal reservoirs were brought together, to offer a convenient reference platform for scientists and healthcare workers.

Monkeypox was undeniably one of the most concerning zoonoses in 2022. Panda and Mukherjee provided their opinions regarding the transmission dynamics of monkeypox in humans, as well as the treatment and management of this infection. Bragazzi et al. compiled a mini review on factors that lead to the underestimation of sexually transmitted diseases, with a special focus on monkeypox. In addition, Ullah et al. put together a comprehensive review article on the epidemiology of monkeypox and its potential threat to public health sector. In contrast to monkeypox that received relatively high public attention, leptospirosis is a low key, yet highly fatal bacterial zoonosis. To better understand the pathobiology of *Leptospira* infection, Pětrošová et al. investigated the structural diversity of *Leptospira* lipid A, the hydrophobic component of endotoxin that is responsible for much of the endotoxin toxicity. Adding to this, van der Westhuizen et al. studied the prevalence of occupational exposure of farmworkers to zoonotic pathogens such as *Brucella* sp., hantaviruses, and *Leptospira* sp. in South Africa.

This Research Topic also received a number of articles related to several zoonotic parasites, some of which are neglected tropical diseases. *Fasciola gigantica* is a large liver fluke of ruminants that readily infects humans, causing fascioliasis. Zheng et al. deciphered the proteins that constituted *F. gigantica* excretory and secretory products (FgESP) derived from the sera of infected buffalos at different time points of infection. Mano et al. reported the correlation between amphotericin B resistance and the increased fitness of *Leishmania martiniquensis*, an autochthonous vector-borne zoonosis in Thailand. Phang et al. (a) investigated *Plasmodium knowlesi*, a potentially fatal vector-borne zoonosis that is prevalent in Southeast Asia. The team predicted the transmission risk of *P. knowlesi* by using machine learning-based ecological niche modeling approaches. A corrigendum for this work was also published by Phang et al. (b) in this Research Topic. Akoolo et al. reviewed the influence of protozoan coinfections on the efficacy of vaccines against the bacterial and viral pathogens. Several coinfection models with relevance to human epidemiological situation were highlighted, such as the coinfection of *Plasmodium* and non-typhoidal *Salmonella* (an important group of zoonotic bacteria), Rotavirus and *Cryptosporidium* coinfection, as well as *Babesia* spp. and *Borrelia burgdorferi* coinfection (both are vector-borne zoonoses). In addition, Wong et al. presented a review on vector management in the control and elimination of vector-borne zoonoses and vector-borne infections with animal reservoirs.

Zoonosis transmission is a broad topic with various knowledge gaps remained to be filled. Obviously, the articles assembled in this Research Topic do not fully reflect the complete picture of this Research Topic. Nevertheless, this article Research Topic contributed new insights and knowledge to this field, which may inspire new studies to improve the understanding on the transmission biology of zoonoses.

## Author contributions

W-CL drafted the editorial. YC, VK, and LR contributed to the editing. All authors provided the final approval of the version to be published.

## Funding

We would like to thank the Ministry of Higher Education, Malaysia, for the Fundamental Research Grant Scheme (FRGS/1/2022/SKK12/UM/02/9) awarded to W-CL. YC was supported by the Institute of Biomedical Sciences Core Funding.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## References

Ellwanger, J. H., and Chies, J. A. B. (2021). Zoonotic spillover: Understanding basic aspects for better prevention. *Genet. Mol. Biol.* 44, e20200355. doi: 10.1590/1678-4685-gmb-2020-0355

Evangelista, K. V., and Coburn, J. (2010). *Leptospira* as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. *Fut. Microbiol.* 5, 1413–1425. doi: 10.2217/fmb.10.102

Hu, G., Du, H., Liu, Y., Wu, G., and Han, J. (2022). Herpes B virus: History, zoonotic potential, and public health implications. *Biosafety Health* 4, 213–219. doi: 10.1016/j.bsheal.2022.05.005

Lee, W. C., Cheong, F. W., Amir, A., Lai, M. Y., Tan, J. H., Phang, W. K., et al. (2022). *Plasmodium knowlesi*: the game changer for malaria eradication. *Malar J.* 21, 140. doi: 10.1186/s12936-022-04131-8

Mavroidi, N. (2008). Transmission of zoonoses through immigration and tourism. *Vet. Ital.* 44, 651–656.

Owen, S. F., Edwards, J. W., Ford, W. M., Crum, J. M., and Wood, P. B. (2004). Raccoon roundworm in raccoons in central West Virginia. *Northeastern Natur.* 11, 137–142. doi: 10.1656/1092-6194(2004)011(0137:RRIRIC)2.0.CO;2

frontiers | Frontiers in Microbiology

TYPE Opinion
PUBLISHED 31 August 2022
DOI 10.3389/fmicb.2022.983076



OPEN ACCESS

EDITED BY
Laurent Rénia,
Nanyang Technological University, Singapore

REVIEWED BY
Huibin Yu,
Yale University, United States
Francesco Bellinato,
University of Verona, Italy

*CORRESPONDENCE
Anupam Mukherjee
amukherjee@nariindia.org;
mukherjee.a@icmr.gov.in

SPECIALTY SECTION
This article was submitted to Infectious Agents and Disease, a section of the journal Frontiers in Microbiology

RECEIVED 15 July 2022
ACCEPTED 18 August 2022
PUBLISHED 31 August 2022

CITATION
Panda K and Mukherjee A (2022) Is monkeypox a threat to another pandemic?
*Front. Microbiol.* 13:983076.
doi: 10.3389/fmicb.2022.983076



# Is monkeypox a threat to another pandemic?

Kingshuk Panda[1] and Anupam Mukherjee [2]*

[1]Indian Institute of Technology, New Delhi, India, [2]Division of Virology, ICMR-National AIDS Research Institute, Pune, MH, India

KEYWORDS

**monkeypox, outbreak, pandemic, epidemic, infectious disease**

The world has witnessed some of the deadliest viral epidemics and pandemics during this 21st century; HIV, Swine Flu or pH1N1, MERS-CoV, Ebola, Zika, Chikungunya, Dengue, and most recently SARS-CoV-2 or COVID-19 are recorded as few of the many with far-reaching consequences. By Aug 12, 2022, the SARS-CoV-2 virus has been responsible for more than 585 million COVID-19 cases globally, resulting in 6.4 million deaths (WHO, 2022c). In reality, the wounds that the COVID-19 pandemic has inflicted on us are very deep and seem to be long-lasting. Amid this ongoing pandemic situation, the increasing cases of monkeypox incidence are becoming a global threat. The virus called monkeypox causes a rare disease in monkeys and humans, specifically in the regions of central and western African countries. In 1958, the virus was first isolated in a laboratory when scientists found pox-like outbreaks in monkeys that were kept for research purposes (Von Magnus et al., 1959). The major animal reservoir or monkeypox is still not discovered, although few studies suggested Gambian pouched rats and rope squirrels are the most suspected reservoir (CDC, 2003; Brown and Leggat, 2016). Previously, in 2003, an outbreak was recorded in the US regions, where most of the cases were reported in both humans and pet prairie dogs (Ligon, 2004). Some sporadic outbreak of monkeypox have been reported in 2018, 2019, and 2021 (Kraemer et al., 2022). Although the number was minimal, most cases were reported in the same family. As per the latest report on Aug 5, 2022, a total of 28,220 confirmed cases have been reported to the World Health Organization (WHO) from 88 countries, out of that 81 countries were not under the monkeypox virus endemic zone earlier. As of now, there are 2859 active cases in the United Kingdom, 7509 cases in Unites states of America, 4942 cases in Spain, and 2887 cases in Germany.

There are two possible routes for monkeypox transmission. These are animal-human transmission and human-human transmission. The animal-to-human transmission is known as zoonotic transmission, which occurs *via* direct contact or through food, water or the environment (Bunge et al., 2022). A few nosocomial transmissions have also been reported in several regions of Africa. Though the monkeypox virus can enter through large respiratory droplets, close or direct contact with skin lesions, and possibly through contaminated fomites, most of the cases of 2022 outbreaks have been identified in the primary care and sexual health settings, mostly among the men who have sex with men groups (MSM) (Kupferschmidt, 2022; WHO, 2022b). The virus generally replicates at the inoculation site and then spreads to the local lymph nodes. The incubation period generally took around 6 to 13 days with a maximum upper limit of 21 days. There is usually a 0-5 day invasion period, accompanied by fever and lymphadenopathy before lessons begin (WHO, 2022a). Persons may experience flu-like symptoms during the prodrome phase,

such as fever, chills, malaise, headaches, backaches, sore throats, shortness of breath, and swollen lymph nodes. The enlarged lymph nodes are the unique clinical clue of the monkeypox virus, which distinguish it from human smallpox infection caused by the variola virus (Cann et al., 2013). The rash is generally well circumscribed, vesicular, or pustular that are deep-seated, firm or hard. Over time, the lesions may umbilicate or become confluent, forming scabs. Although, recent cases have suddenly started without symptoms like fever, genital lesions, or other prodromal symptoms (Minhaj et al., 2022). As the clinical signs and symptoms of monkeypox are similar to smallpox, chickenpox, measles, coxsackievirus and bacterial infection in the early period, hence it is very important to use a differential diagnosis of monkeypox in the early period. Serological methods are not recommended for monkeypox diagnosis due to the cross-reactivity of other orthopoxviruses, therefor Polymerase Chain Reaction (PCR) is the most recommended method for genome-level identification with higher accuracy and sensitivity (Saxena et al., 2022).

Currently, no licensed treatment is available for monkeypox, although two oral drugs, brincidofovir, and tecovirimat, have been approved to treat smallpox and show antiviral efficacy against monkeypox (Adler et al., 2022). A study published in Lancet reported that one patient treated with tecovirimat, with a dosage of 200 mg twice daily for 2 2 weeks orally, showed a short duration of viral shedding and illness upon comparison with other patients (Adler et al., 2022). In addition, the vaccine named MVA-BN, also known as JYNNEOS™ in the US, has been licensed in the United States to prevent the cases of monkeypox or make it less severe. In Canada, the vaccine is called IMVAMUNE®, while in the European Union it is marketed under the trade name IMVANEX® to reduce monkeypox severity and prevent future infections. Recently, the Center for Disease Control and Prevention (CDC) published a datasheet for monkeypox treatment, where they reported that the smallpox vaccine, cidofovir, ST-246, and vaccinia immune globulin can be used to control the monkeypox outbreak but no supporting data is attached to their claim (CDC, 2022). Therefore, the development of proper vaccines or antivirals against the monkeypox infection is the need of the hour that leads toward detailed research on the infection biology of the virus. Although a few papers have already reported monkeypox's infection biology, there is an urgent need to understand the virus life cycle starting from the role of different cellular organelles in viral entry into the cell, each step of its replication machinery, detailed interactions with the host cells, trafficking, and finally the egress of the mature viruses (Satheshkumar and Moss, 2012; Sivan et al., 2016; Realegeno et al., 2020). Equally, more research on the epidemiological forecasts of the virus, transmission dynamics and therapeutics prospects are required to understand the proper model of disease management for monkeypox infection. Recently, In 2021, a research group designed a mathematical model to understand the transmission dynamics of the monkeypox virus (Peter et al., 2021). Still, there are many more stones to be turned to understand the proper way for the management of monkeypox.

The world has already faced a significant outbreak since the last quarter of 2019. Therefore, there is a panic about whether monkeypox could cause such a COVID-19-like pandemic or not. The SARS-CoV-2 virus spreads through tiny airborne droplets called aerosols, whereas monkeypox is mainly spread from close contact with a body fluid such as saliva and coughing. Although emerging literature points toward the presence of monkeypox DNA in the short-range aerosols, the efficiency of this transmission route is still under subjected to further research and validation. Besides everything, any viral disease's primary concern is its new behavior. If we recall, in the initial pandemic situation of COVID-19, there were a few mutations identified. Nevertheless, later on, scientists cataloged more than 12,000 mutations in the SARS-CoV-2 genome. The monkeypox virus genome consists of linear double-stranded DNA, size of approximate ∼197 kb. The genome of this virus is six times as large and complex to analyze as compared to the genome of the SARS-CoV-2 coronavirus (Kozlov, 2022). As the genome of monkeypox is DNA; therefore, the DNA polymerase contains proofreading skills, which makes DNA viruses prone to being less mutagenic; hence, we hypothesize that the monkeypox infection is less likely to create another pandemic situation like COVID-19. However, there are evidences of certain mutations found in the monkeypox DNA sample (Weaver and Isaacs, 2008; Zhang et al., 2022). Previously, a study demonstrated that COP-C3L is a major gene responsible for the difference in virulence among different monkeypox strains, and predicted that mutation in this gene could raise a significant pathogenic strain of monkeypox (Weaver and Isaacs, 2008). A latest analysis indicated the occurrence of single nucleotide mutations and frame-shift mutations in the samples collected from this outbreak (Zhang et al., 2022). Therefore, whether monkeypox could lead to another pandemic at this time point is still a debatable subject.

Few hypotheses have already risen regarding this sudden increase in monkeypox cases. Few studies say that the current sequences retrieved are mostly similar to those from a smattering of monkeypox cases that arose outside Africa in 2018-2019 and are linked with the traveling history. Another hypothesis says that there must be a possibility that the virus was circulating outside Africa in humans and animals but remains undetected (Kozlov, 2022). Whereas, a third hypothesis says that the virus may be coincidently exposed to the community by sexual networks as the recent unexpected cases in MSMs have increased (Mohapatra et al., 2022).

After decades of quiescence, the human monkeypox disease has become a clinically serious infection (Costello et al., 2022). Since the disease was first reported, no extensive studies have been conducted on the exploitation of the host cells by monkeypox infection. Although, few reports have been

published to identify the mechanism of host susceptibility to monkeypox infection upon exposure, specifically in mouse models. The common inbred strains of mice, including BALB/c and C57BL/c, are remarkably resistant to monkeypox virus infection, however, CAST/EiJ shows greater sensitivity and excellent morbidity and mortality due to their inadequate immune response upon monkeypox infection (Americo et al., 2010; Earl et al., 2012, 2015). The classical inbred mice make a more active interferon γ- response, which makes them less susceptible. It has also been proven that the cytokine IL-15 and the number of NK cells play a critical role in combating monkeypox infection (Earl et al., 2020). At present, there are some crucial gaps in understanding the host-cell biology, pathophysiology and epidemiology of the Monkeypox virus.

Besides all of these, a few standard measures should be taken by the public to prevent infection with monkeypox. Moreover, recommendations from WHO is necessary at this point to increase awareness among the common people. Although, a few common principles are the thumb rule to prevent any such viral diseases; Firstly, separate an infected person from a healthy person; Secondly, utilize appropriate protective equipment and good hand hygiene to protect household members when dealing with the infected individuals or serving as caregiver at home; Thirdly, for disinfection of surface, use an EPA-registered disinfectant; Fourthly, patients should avoid contact with pets and animals while infected, as animals are a potent reservoir of monkeypox; Finally, monkeypox symptoms, including unexplained lesions, should be evaluated by a dermatologist and venereologists, and close contact with others, including sexual or intimate partner, should be avoided until the condition is evaluated (Khanna et al., 2022; Minhaj et al., 2022).

Furthermore, we must avoid stigmatizing any infected individual for the source of their infection. An important reason for this outbreak is the inveterate neglect of diseases primarily affecting the poorest populations and the widespread disregard for communities affected by these diseases (Nakoune and Olliaro, 2022). The increasing evidence requires further research on the virus-cell interactions and investigation to understand the disease dynamics. It is crucial to provide proper interventions that prove effective in monkeypox endemic low-income countries, and not simply stockpile them for potential use in high-income countries. The world has already faced a global pandemic; therefore, it should be essential to be alert and ready to respond rapidly.

## Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

## Funding

This review work has been supported by the Indian Council of Medical Research and ICMR-National AIDS Research Institute, Pune.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## References

Adler, H., Gould, S., Hine, P., Snell, L. B., Wong, W., Houlihan, C. F., et al. (2022). Clinical features and management of human monkeypox: a retrospective observational study in the UK. *Lancet Infect. Dis.* 22, 1153–1162. doi: 10.1016/S1473-3099(22)00228-6

Americo, J. L., Moss, B., and Earl, P. L. (2010). Identification of wild-derived inbred mouse strains highly susceptible to monkeypox virus infection for use as small animal models. *J. Virol.* 84, 8172–8180. doi: 10.1128/JVI.00621-10

Brown, K., and Leggat, P. (2016). Human monkeypox: current state of knowledge and implications for the future. *Trop. Med* 1, 8. doi: 10.3390/tropicalmed1010008

Bunge, E. M., Hoet, B., Chen, L., Lienert, F., Weidenthaler, H., Baer, L. R., et al. (2022). The changing epidemiology of human monkeypox-A potential threat? A systematic review. *PLoS Negl. Trop. Dis.* 16, e0010141. doi: 10.1371/journal.pntd.0010141

Cann, J. A., Jahrling, P. B., Hensley, L. E., and Wahl-Jensen, V. (2013). Comparative pathology of smallpox and monkeypox in man and macaques. *J. Comp. Pathol.* 148, 6–21. doi: 10.1016/j.jcpa.2012.06.007

CDC (2003). *CDC Confirms Monkeypox in Rodents Interim Recommendations Aim to Curb Further Spread*. Available online at: https://www.cdc.gov/media/pressrel/r030702.htm (accessed August 14, 2022).

CDC. (2022). *Monkeypox and Smallpox Vaccine Guidance*. Available online at: https://www.cdc.gov/poxvirus/monkeypox/clinicians/smallpox-vaccine.html (accessed August 14, 2022).

Costello, V., Sowash, M., Gaur, A., Cardis, M., Pasieka, H., Wortmann, G., et al. (2022). Imported Monkeypox from International Traveler, Maryland, USA, 2021. *Emerg. Infect. Dis.* 28, 1002–1005. doi: 10.3201/eid2805.220292

Earl, P. L., Americo, J. L., and Moss, B. (2012). Lethal monkeypox virus infection of CAST/EiJ mice is associated with a deficient gamma interferon response. *J. Virol.* 86, 9105–9112. doi: 10.1128/JVI.00162-12

Earl, P. L., Americo, J. L., and Moss, B. (2015). Genetic studies of the susceptibility of classical and wild-derived inbred mouse strains to monkeypox virus. *Virology* 481, 161–165. doi: 10.1016/j.virol.2015.02.048

Earl, P. L., Americo, J. L., and Moss, B. (2020). Natural killer cells expanded in vivo or ex vivo with IL-15 overcomes the inherent susceptibility of CAST mice to lethal infection with orthopoxviruses. *PLoS Pathog.* 16, e1008505. doi: 10.1371/journal.ppat.1008505

Khanna, U., Bishnoi, A., and Vinay, K. (2022). Current outbreak of monkeypox-essentials for the dermatologist. *J. Am. Acad. Dermatol.* S0190-9622(22)02218-6. doi: 10.1016/j.jaad.2022.06.1170. [Epub ahead of print].

Kozlov, M. (2022). Monkeypox outbreaks: 4 key questions researchers have. *Nature* 606, 238–239. doi: 10.1038/d41586-022-01493-6

Kraemer, M. U. G., Tegally, H., Pigott, D. M., Dasgupta, A., Sheldon, J., Wilkinson, E., et al. (2022). Tracking the 2022 monkeypox outbreak with epidemiological data in real-time. *Lancet Infect. Dis.* 22, 941–942. doi: 10.1016/S1473-3099(22)00359-0

Kupferschmidt, K. (2022). Why monkeypox is mostly hitting men who have sex with men. *Science* 376, 1364–1365. doi: 10.1126/science.add5966

Ligon, B. L. (2004). Monkeypox: a review of the history and emergence in the Western hemisphere. *Semin. Pediatr. Infect. Dis.* 15, 280–287. doi: 10.1053/j.spid.2004.09.001

Minhaj, F. S., Ogale, Y. P., Whitehill, F., Schultz, J., Foote, M., Davidson, W., et al. (2022). Monkeypox Outbreak - Nine States, May 2022. *MMWR Morb. Mortal. Wkly. Rep.* 71, 764–769. doi: 10.15585/mmwr.mm7123e1

Mohapatra, R. K., Tuli, H. S., Sarangi, A. K., Chakraborty, S., Chandran, D., Chakraborty, C., et al. (2022). Unexpected sudden rise of human monkeypox cases in multiple non-endemic countries amid COVID-19 pandemic and salient counteracting strategies: another potential global threat? *Int. J. Surg.* 103, 106705. doi: 10.1016/j.ijsu.2022.106705

Nakoune, E., and Olliaro, P. (2022). Waking up to monkeypox. *BMJ* 377, o1321. doi: 10.1136/bmj.o1321

Peter, O. J., Kumar, S., Kumari, N., Oguntolu, F. A., Oshinubi, K., and Musa, R. (2021). Transmission dynamics of Monkeypox virus: a mathematical modelling approach. *Model Earth Syst. Environ.* 8, 3423–3434. doi: 10.1007/s40808-021-01313-2

Realegeno, S., Priyamvada, L., Kumar, A., Blackburn, J. B., Hartloge, C., Puschnik, A. S., et al. (2020). Conserved Oligomeric Golgi (COG) complex proteins facilitate orthopoxvirus entry, fusion and spread. *Viruses* 12, E707. doi: 10.3390/v12070707

Satheshkumar, P. S., and Moss, B. (2012). Sequence-divergent chordopoxvirus homologs of the o3 protein maintain functional interactions with components of the vaccinia virus entry-fusion complex. *J. Virol.* 86, 1696–1705. doi: 10.1128/JVI.06069-11

Saxena, S. K., Ansari, S., Maurya, V. K., Kumar, S., Jain, A., Paweska, J. T., et al. (2022). Re-emerging human monkeypox: a major public-health debacle. *J. Med. Virol*. doi: 10.1002/jmv.27902. [Epub ahead of print].

Sivan, G., Weisberg, A. S., Americo, J. L., and Moss, B. (2016). Retrograde transport from early endosomes to the trans-golgi network enables membrane wrapping and egress of vaccinia virus virions. *J. Virol.* 90, 8891–8905. doi: 10.1128/JVI.01114-16

Von Magnus, P., Andersen, E., Petersen, K., and Birch-Andersen, A. (1959). A pox-like disease in cynomolgus monkeys. *Acta Pathol. Microbiol. Immunol. Scand.* 46, 156–176. doi: 10.1111/j.1699-0463.1959.tb00328.x

Weaver, J. R., and Isaacs, S. N. (2008). Monkeypox virus and insights into its immunomodulatory proteins. *Immunol. Rev.* 225, 96–113. doi: 10.1111/j.1600-065X.2008.00691.x

WHO (2022a). *Monkeypox*. Available online at: https://www.who.int/news-room/fact-sheets/detail/monkeypox (accessed August 14, 2022).

WHO (2022b). *Multi-Country Monkeypox Outbreak in Non-endemic Countries*. Available online at: https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON385 (accessed August 14, 2022).

WHO (2022c). *WHO Coronavirus (COVID-19) Dashboard*. Available online at: https://covid19.who.int (accessed August 14, 2022).

Zhang, Y., Zhang, J. Y., and Wang, F.-S. (2022). Monkeypox outbreak: a novel threat after COVID-19? *Mil. Med. Res.* 9, 29. doi: 10.1186/s40779-022-00395-y

frontiers | Frontiers in Microbiology

TYPE Mini Review
PUBLISHED 23 September 2022
DOI 10.3389/fmicb.2022.1011049



OPEN ACCESS

EDITED BY
Wenn-Chyau Lee,
University of Malaya, Malaysia

REVIEWED BY
Ali Ahmed,
Monash University Malaysia, Malaysia
Sarah Guagliardo,
Centers for Disease Control and Prevention (CDC), United States
Caleb McEntire,
Department of Neurology, Massachusetts General Hospital, Harvard Medical School, United States

*CORRESPONDENCE
Nicola Luigi Bragazzi
bragazzi@yorku.ca

SPECIALTY SECTION
This article was submitted to Infectious Agents and Disease, a section of the journal Frontiers in Microbiology

RECEIVED 03 August 2022
ACCEPTED 06 September 2022
PUBLISHED 23 September 2022

CITATION
Bragazzi NL, Woldegerima WA, Iyaniwura SA, Han Q, Wang X, Shausan A, Badu K, Okwen P, Prescod C, Westin M, Omame A, Converti M, Mellado B, Wu J and Kong JD (2022) Knowing the unknown: The underestimation of monkeypox cases. Insights and implications from an integrative review of the literature. *Front. Microbiol.* 13:1011049. doi: 10.3389/fmicb.2022.1011049



# Knowing the unknown: The underestimation of monkeypox cases. Insights and implications from an integrative review of the literature

Nicola Luigi Bragazzi[1]*, Woldegebriel Assefa Woldegerima[1], Sarafa Adewale Iyaniwura[2], Qing Han[1], Xiaoying Wang[3], Aminath Shausan[4], Kingsley Badu[5], Patrick Okwen[6], Cheryl Prescod[7], Michelle Westin[7], Andrew Omame[8,9], Manlio Converti[10], Bruce Mellado[11,12], Jianhong Wu[1] and Jude Dzevela Kong[1]

[1]Laboratory for Industrial and Applied Mathematics (LIAM), Department of Mathematics and Statistics, York University, Toronto, ON, Canada, [2]Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM, United States, [3]Department of Mathematics, Trent University, Peterborough, ON, Canada, [4]School of Mathematics and Physics, University of Queensland, Saint Lucia, QLD, Australia, [5]Vector-borne Infectious Disease Group, Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [6]Effective Basic Services (eBASE), Bamenda, Cameroon, [7]Black Creek Community Health Centre, Toronto, ON, Canada, [8]Department of Mathematics, Federal University of Technology, Owerri, Nigeria, [9]Abdus Salam School of Mathematical Sciences, Government College University, Lahore, Pakistan, [10]ASL Napoli 2 Nord, Naples, Italy, [11]School of Physics and Institute for Collider Particle Physics, University of the Witwatersrand, Johannesburg, South Africa, [12]Subatomic Physics, iThemba Laboratory for Accelerator Based Sciences, Somerset West, South Africa

Monkeypox is an emerging zoonotic disease caused by the monkeypox virus, which is an infectious agent belonging to the *genus Orthopoxvirus*. Currently, commencing from the end of April 2022, an outbreak of monkeypox is ongoing, with more than 43,000 cases reported as of 23 August 2022, involving 99 countries and territories across all the six World Health Organization (WHO) regions. On 23 July 2022, the Director-General of the WHO declared monkeypox a global public health emergency of international concern (PHEIC), since the outbreak represents an extraordinary, unusual, and unexpected event that poses a significant risk for international spread, requiring an immediate, coordinated international response. However, the real magnitude of the burden of disease could be masked by failures in ascertainment and under-detection. As such, underestimation affects the efficiency and reliability of surveillance and notification systems and compromises the possibility of making informed and evidence-based policy decisions in terms of the adoption and implementation of *ad hoc* adequate preventive measures. In this review, synthesizing 53 papers, we summarize the determinants of the underestimation of sexually transmitted diseases, in general, and, in particular, monkeypox, in terms of all their various components and dimensions (under-ascertainment, underreporting,

under-detection, under-diagnosis, misdiagnosis/misclassification, and under-notification).

KEYWORDS

monkeypox, zoonotic disease, emerging and re-emerging infectious disease, underestimation, underreporting, under-detection, under-diagnosis, under-ascertainment

## Introduction

Monkeypox is an emerging zoonotic disease caused by the monkeypox virus, which is an infectious agent belonging to the family of Poxviruses (*Poxviridae*), *Chordopoxvirinae* subfamily, and *Orthopoxvirus genus* (Hughes et al., 2010; Bunge et al., 2022). These viruses are large, brick-shaped, enveloped, double-stranded DNA viruses (Diven, 2001; Alakunle et al., 2020). Monkeypox virus is related to the *Variola* virus (VARV), the causative agent of smallpox, a life-threatening infectious disease fully eradicated in 1980, and another *Orthopoxvirus* (Barquet and Domingo, 1997; Riedel, 2005; Kmiec and Kirchhoff, 2022). Monkeypox has been endemic in some African countries, since 1970, when the first human case was reported in a 9-month-old child admitted to the Basankusu Hospital in the Democratic Republic of the Congo (DRC; Durski et al., 2018).

Currently, commencing from the end of April 2022, an outbreak of monkeypox is ongoing, with more than 43,000 cases reported as of 23 August 2022, involving 99 countries and territories across all the six World Health Organization (WHO) regions (Table 1). The most impacted WHO regions are the Region of the Americas (AMR; 52.0%) and the European Region (EUR; 47.5% of cases), followed by the Western Pacific Region (WPR; 0.3%), the African Region (AFR; 0.1%), the Eastern Mediterranean Region (EMR; 0.1%), and the South-East Asian Region (SEAR; 0.04%). On 23 July 2022, the Director-General of the WHO declared monkeypox a global public health emergency of international concern (PHEIC; Nuzzo et al., 2022), since the outbreak represents an extraordinary, unusual, and unexpected event that poses a significant risk for international spread, requiring an immediate, coordinated international response.

The epidemiological and clinical features of the ongoing monkeypox outbreak are different from those established for monkeypox since its initial isolation and identification and during the previous outbreaks, with sexual transmission suspected as the major transmission route and with the community of men having sex with men (MSM) disproportionately impacted (Liu et al., 2022; Thornhill et al., 2022). According to a large-scale study, out of 528 monkeypox infections diagnosed and reported from 16 countries, between April 27 and June 24, 2022, the transmission was hypothesized to have occurred more likely *via* sexual intercourse in 95% of the cases during the current outbreak (Thornhill et al., 2022). Other transmission routes include contact with infected animals and travel to endemic countries, occupational exposure, and social and household contacts (Liu et al., 2022). As such, monkeypox is not an exclusively sexually transmitted disease (STD), but its transmission has been hypothesized to be associated with sexual contact. This is an important distinction because we are still not sure that transmission is occurring through body fluids exchanged during sex, but rather it could be *via* contact with mucosal surfaces, scarification, or even respiratory exposures.

The real magnitude of the burden of disease could be masked by failures in ascertainment and under-detection. As such, underestimation affects the efficiency and reliability of surveillance and notification systems and compromises the possibility of making informed and evidence-based policy decisions in terms of the adoption and implementation of *ad hoc* adequate preventive measures. For example, another infectious outbreak, the still ongoing "Coronavirus Disease 2019" (COVID-19) pandemic was initially underestimated and this, along with the high degree of contagiosity of the virus, contributed to its quick global spread (Wu et al., 2020; Nakamoto and Zhang, 2021).

According to the working definitions of the "BCoDE-project" (Kretzschmar et al., 2012), underestimation can be due to various factors, including under-ascertainment. This can occur when infected subjects do not seek general practitioners or specialized health services, in that they perceive their illness as mild and/or self-limiting, do not have adequate health literacy and risk/disease perception, or they are asymptomatic and unaware of their disease status. Minority groups (including migrants, the lesbian/gay/bisexual/transgender/transsexual/queer/intersex, LGBTQI+, community, and other marginalized or difficult-to-reach communities) generally do not consult general practitioners or other healthcare workers (Kretzschmar et al., 2012). Cultural, religious, legal, administrative, economic, and financial factors can influence health-seeking behaviors. Underreporting, another component of underestimation, occurs when symptomatic cases in the community refer to health services but have their disease status not properly diagnosed or misclassified (under-diagnosis), or correctly diagnosed and classified but not effectively transmitted to the public health surveillance and monitoring bodies (under-notification). The reader is referred to Table 2 for further details.

The topic of underestimation of monkeypox cases is of crucial importance in the field of public and global health. However, to the best of our knowledge, there exists no comprehensive review

10.3389/fmicb.2022.1011049

TABLE 1 Monkeypox cases (confirmed and suspected cases, deaths, and grand total) broken down according to the World Health Organization (WHO) region, and country.

| Country | Confirmed | Death | Suspected | Grand total |
|---|---|---|---|---|
| **African Region (AFR)** | **54** | **1** | **7** | **62** |
| Benin | 3 | 0 | 0 | 3 |
| Ghana | 46 | 1 | 0 | 47 |
| South Africa | 5 | 0 | 0 | 5 |
| Uganda | 0 | 0 | 6 | 6 |
| Zambia | 0 | 0 | 1 | 1 |
| **Eastern Mediterranean Region (EMR)** | **35** | **0** | **8** | **43** |
| Iran | 1 | 0 | 3 | 4 |
| Lebanon | 6 | 0 | 0 | 6 |
| Morocco | 1 | 0 | 0 | 1 |
| Pakistan | 0 | 0 | 1 | 1 |
| Qatar | 3 | 0 | 0 | 3 |
| Saudi Arabia | 6 | 0 | 0 | 6 |
| Somalia | 0 | 0 | 3 | 3 |
| Sudan | 2 | 0 | 1 | 3 |
| United Arab Emirates | 16 | 0 | 0 | 16 |
| **European Region (EUR)** | **20,606** | **2** | **1** | **20,609** |
| Andorra | 4 | 0 | 0 | 4 |
| Austria | 218 | 0 | 0 | 218 |
| Belgium | 624 | 0 | 0 | 624 |
| Bosnia And Herzegovina | 3 | 0 | 0 | 3 |
| Bulgaria | 4 | 0 | 0 | 4 |
| Croatia | 22 | 0 | 0 | 22 |
| Cyprus | 4 | 0 | 0 | 4 |
| Czech Republic | 39 | 0 | 0 | 39 |
| Denmark | 169 | 0 | 0 | 169 |
| England | 3,050 | 0 | 0 | 3,050 |
| Estonia | 9 | 0 | 0 | 9 |
| Finland | 22 | 0 | 0 | 22 |
| France | 2,873 | 0 | 0 | 2,873 |
| Georgia | 2 | 0 | 0 | 2 |
| Germany | 3,295 | 0 | 0 | 3,295 |
| Gibraltar | 6 | 0 | 0 | 6 |
| Greece | 50 | 0 | 0 | 50 |
| Hungary | 63 | 0 | 0 | 63 |
| Iceland | 12 | 0 | 0 | 12 |
| Ireland | 113 | 0 | 0 | 113 |
| Israel | 208 | 0 | 0 | 208 |
| Italy | 689 | 0 | 1 | 690 |
| Latvia | 4 | 0 | 0 | 4 |
| Lithuania | 5 | 0 | 0 | 5 |
| Luxembourg | 45 | 0 | 0 | 45 |
| Malta | 31 | 0 | 0 | 31 |
| Moldova | 2 | 0 | 0 | 2 |
| Monaco | 3 | 0 | 0 | 3 |
| Montenegro | 2 | 0 | 0 | 2 |
| Netherlands | 1,090 | 0 | 0 | 1,090 |
| Northern Ireland | 27 | 0 | 0 | 27 |
| Norway | 76 | 0 | 0 | 76 |

*(Continued)*

10.3389/fmicb.2022.1011049

TABLE 1 (Continued)

| Country | Confirmed | Death | Suspected | Grand total |
|---|---|---|---|---|
| Poland | 114 | 0 | 0 | 114 |
| Portugal | 810 | 0 | 0 | 810 |
| Romania | 34 | 0 | 0 | 34 |
| Russia | 1 | 0 | 0 | 1 |
| Scotland | 75 | 0 | 0 | 75 |
| Serbia | 31 | 0 | 0 | 31 |
| Slovakia | 12 | 0 | 0 | 12 |
| Slovenia | 43 | 0 | 0 | 43 |
| Spain | 6,117 | 2 | 0 | 6,119 |
| Sweden | 141 | 0 | 0 | 141 |
| Switzerland | 416 | 0 | 0 | 416 |
| Turkey | 5 | 0 | 0 | 5 |
| Wales | 43 | 0 | 0 | 43 |
| **Region of the Americas (AMR)** | **22,531** | **4** | **28** | **22,563** |
| Argentina | 72 | 0 | 0 | 72 |
| Bahamas | 2 | 0 | 0 | 2 |
| Barbados | 1 | 0 | 0 | 1 |
| Bermuda | 1 | 0 | 0 | 1 |
| Bolivia | 43 | 0 | 1 | 44 |
| Brazil | 3,895 | 1 | 7 | 3,903 |
| Canada | 1,168 | 0 | 11 | 1,179 |
| Cayman Islands | 0 | 0 | 1 | 1 |
| Chile | 207 | 0 | 2 | 209 |
| Colombia | 273 | 0 | 0 | 273 |
| Costa Rica | 3 | 0 | 2 | 5 |
| Curaçao | 1 | 0 | 0 | 1 |
| Dominican Republic | 6 | 0 | 0 | 6 |
| Ecuador | 19 | 1 | 1 | 21 |
| Greenland | 2 | 0 | 0 | 2 |
| Guadeloupe | 1 | 0 | 0 | 1 |
| Guatemala | 4 | 0 | 0 | 4 |
| Haiti | 0 | 0 | 1 | 1 |
| Honduras | 3 | 0 | 0 | 3 |
| Jamaica | 4 | 0 | 0 | 4 |
| Martinique | 2 | 0 | 0 | 2 |
| Mexico | 251 | 1 | 0 | 252 |
| Panama | 7 | 0 | 0 | 7 |
| Peru | 1,127 | 1 | 1 | 1,129 |
| Puerto Rico | 77 | 0 | 0 | 77 |
| Saint Martin (French part) | 1 | 0 | 0 | 1 |
| United States | 15,358 | 0 | 0 | 15,358 |
| Uruguay | 2 | 0 | 1 | 3 |
| Venezuela | 1 | 0 | 0 | 1 |
| **South-East Asian Region (SEAR)** | **15** | **1** | **0** | **16** |
| India | 9 | 1 | 0 | 10 |
| Indonesia | 1 | 0 | 0 | 1 |
| Thailand | 5 | 0 | 0 | 5 |
| **Western Pacific Region (WPR)** | **122** | **0** | **0** | **122** |
| Australia | 90 | 0 | 0 | 90 |

*(Continued)*

TABLE 1 (Continued)

| Country | Confirmed | Death | Suspected | Grand total |
|---|---|---|---|---|
| Japan | 4 | 0 | 0 | 4 |
| New Caledonia | 1 | 0 | 0 | 1 |
| New Zealand | 4 | 0 | 0 | 4 |
| Philippines | 4 | 0 | 0 | 4 |
| Singapore | 15 | 0 | 0 | 15 |
| South Korea | 1 | 0 | 0 | 1 |
| Taiwan | 3 | 0 | 0 | 3 |
| **Grand Total** | **43,363** | **8** | **44** | **43,415** |

Data are extracted and collected from the Global Health Initiative (https://www.global.health/).

TABLE 2 Underestimation, its components/dimensions with definitions and determinants.

| Failure to capture all cases | | | Definition | Determinants |
|---|---|---|---|---|
| Underestimation | Under-ascertainment | | Infected subjects do not seek health care | Health literacy, disease perception, perceived health needs, cultural and religious factors, legal, administrative, and financial barriers |
| | Underreporting | Under-diagnosis/under-detection | Disease status not diagnosed/ misclassified | Measurement error, lack of knowledge concerning testing and/or interpretation of tests |
| | | Under-notification | Diagnosis not transmitted to the surveillance and notification system | Reporting/notification policies |

addressing the determinants underlying the underestimation of STDs, in general, and, in particular, monkeypox. Therefore, the present study was undertaken to fill this gap in knowledge.

## Materials and methods

An integrative review was conducted. Even though this technique dates back to the eighties, it is emerging as an innovative tool to synthesize and appraise the existing body of scholarly literature on the designated research problem/concept, enabling the combination of a heterogeneous array of sources, from empirical to conceptual/theoretical investigations, from quantitative to qualitative and mixed-method studies, and from observational to pilot, feasibility, and interventional studies (Broome, 2000). We employed this technique since we were able to identify and formulate a broad-scope research problem/concept/phenomenon of interest, particularly complex and articulated.

An integrative review enables to (i) overview and appraise theories and practices, (ii) to build bridges across diverse study fields, disciplines, and sectors, (iii) to generate and/or refine new knowledge and novel hypotheses, and (iv) to formulate and propose an actionable framework, being, as such, particularly suited for developing and informing healthcare policies and practices in an evidence-based fashion. More specifically, an integrative review study can be defined as "a review method that summarizes past empirical or theoretical literature to provide a more comprehensive understanding of a particular phenomenon or healthcare problem" (Broome, 2000).

To achieve the ambitious objectives of generating new knowledge and/or theories, an integrative review results in one or more of the following research synthesis forms: (i) a research agenda, (ii) a taxonomy or other conceptual classifications of constructs, (iii) alternative models or conceptual frameworks, and (iv) a metatheory/an array of metatheories.

Within the so-called "evidence synthesis ecosystem," a systematic literature review and a meta-analysis have a highly focused, narrow research scope, whereas a scoping review has a broad research question and the objective of mapping, synthesizing, and combining the existing body of scholarly literature on the designated topic/research question.

We searched a major scholarly electronic database, PubMed/MEDLINE, for papers without language filters, using a search string consisting of several components. First, these components were related to (i) health-seeking behaviors (awareness, knowledge, attitudes, practices, health-literacy, and health-seeking behavior), (ii) underestimation (under-ascertainment, underreporting, under-diagnosis, misdiagnosis/misclassification, under-detection, or under-notification), and, (iii) STDs (sexual transmission, sexually transmitted disease, or sexually transmitted infection). We wanted, indeed, to study determinants of underestimation of STDs, including healthcare-seeking behaviors. During a second round of literature search, we added a fourth component related to the LGBTQI+ community, since it is being particularly impacted by the current monkeypox outbreak (see

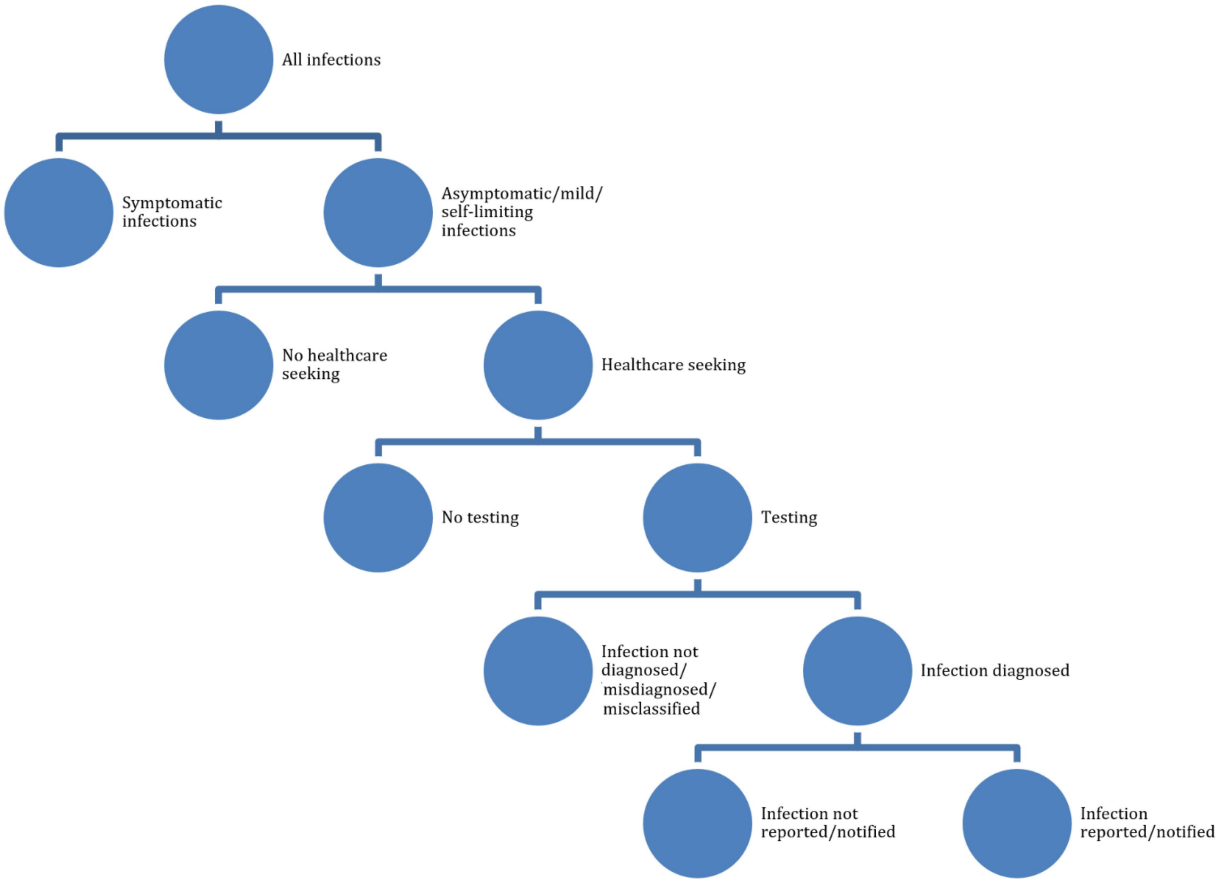


FIGURE 1
Pictorial flowchart of underestimation.

Figure 1 and Tables 2, 3 for further details). Google Scholar was searched too, looking for resources and items not indexed yet at the time of the literature search and for ensuring a broader relevant coverage of the literature.

# Results

## Underestimation of sexually transmitted diseases

Out of 230 items returned by searching PubMed/MEDLINE, 53 articles related to STDs (Franco, 1991; Koutsky et al., 1992; Lin et al., 1992; Schulte et al., 1992; Ashley et al., 1993; Webster et al., 1993; Brookmeyer et al., 1995; Maher and Hoffman, 1995; Petersen et al., 1995; Schachter and Chow, 1995; Wahdan, 1995; Schwebke et al., 1996; Agacfidan et al., 1997; Rompalo et al., 1997; Borisenko, 1998; Paget et al., 2002; Niccolai et al., 2005; Dhawan et al., 2006; Liu et al., 2006; Munson et al., 2008; Nguyen et al., 2008; Lusk et al., 2010; Andrea and Chapin, 2011; Hong et al., 2011; Roth et al., 2011; Wolfers et al., 2011; Koper et al., 2013; Krivochenitser et al., 2013; Oliffe et al., 2013; Gratzer et al., 2014; Mirzazadeh et al., 2014; Brown et al., 2015; Corbeto et al., 2015; Fakoya et al., 2015; Jenkins, 2015; Tomas et al., 2015; Johnson and Geffen, 2016; Kustec et al., 2016; Mlakar and Ramšak, 2016; Ni et al., 2016; Allard et al., 2017; Denison et al., 2017; Lee and Nishiura, 2017; Syme et al., 2017; Hall et al., 2018; Mangine et al., 2018; Shahesmaeili et al., 2018; Timsit et al., 2018; Steen et al., 2019; Knight et al., 2020; Moriña et al., 2021; Niekamp et al., 2021; Geba et al., 2022) were deemed eligible for inclusion in the present integrative review. More specifically, our comprehensive literature search enabled us to identify the following determinants of the underestimation of STDs: asymptomatic course (Wahdan, 1995; Shahesmaeili et al., 2018; Moriña et al., 2021); atypical clinical and epidemiological features (Ni et al., 2016), including atypical/unusual transmission routes (Allard et al., 2017; Lee and Nishiura, 2017; Timsit et al., 2018); differences in case definition (Schulte et al., 1992; Rompalo et al., 1997), and in regional/national testing rates (Koper et al., 2013; Kustec et al., 2016); underestimation among specific age groups, like the youth and the elderly, and populations, such as minority communities and visible racialized groups (Webster et al., 1993), migrant workers (Fakoya et al., 2015; Steen et al., 2019), sex workers (Agacfidan

TABLE 3 Search strategy adopted in the present integrative review.

| Search strategy items | Details |
|---|---|
| Keywords used in the search string | ("Health-seeking behavior" OR "health-literacy" OR "disease knowledge" OR "disease awareness" OR "disease perception" OR "risk perception")<br>(Underestimated OR underestimation OR underreporting OR underreported OR misreporting OR misreported OR under-diagnosis OR under-diagnosed OR under-ascertainment OR under-ascertained OR under-notification OR under-notified OR under-detection OR under-detected OR misclassification OR misclassified OR under-recognized OR under-recognition)<br>("Sexually transmitted infection" OR "sexually transmitted disease" OR "sexual transmission")<br>(LGBT OR LGBT+ OR LGBTQ OR LGBTQ+ OR LGBTQI OR LGBTQI+ OR "men having sex with men" OR "men who have sex with men" OR lesbian OR homosexual OR homosexuality OR bisexual OR bisexuality OR "sex and gender minorities" OR "sexual orientation" OR "gender identity") |
| Time filter | From the onset for STDs and from the beginning of the monkeypox outbreak |
| Language filter | None applied |

STD, sexually transmitted diseases.

et al., 1997; Brown et al., 2015; Hong et al., 2011; Mirzazadeh et al., 2014; Shahesmaeili et al., 2018), or swingers (Niekamp et al., 2021); use of low-sensitivity and/or low-specificity diagnostic assays (Koutsky et al., 1992; Schulte et al., 1992; Ashley et al., 1993; Petersen et al., 1995; Schachter and Chow, 1995; Schwebke et al., 1996; Paget et al., 2002; Dhawan et al., 2006; Munson et al., 2008; Lusk et al., 2010; Andrea and Chapin, 2011; Gratzer et al., 2014), or inadequate clinical and microbe-isolation procedures (Koutsky et al., 1992; Lin et al., 1992); inadequate STD screening policies/protocols (Wahdan, 1995; Lusk et al., 2010; Roth et al., 2011; Corbeto et al., 2015; Geba et al., 2022); measurement error/misclassification (Franco, 1991; Krivochenitser et al., 2013; Tomas et al., 2015); barriers to accessing STD testing and management services (Mlakar and Ramšak, 2016; Denison et al., 2017), including psychological issues (Oliffe et al., 2013), or lack of available facilities and infrastructures in resource-limited contexts (Maher and Hoffman, 1995); self-treatment (Borisenko, 1998); disease perception/health literacy (Liu et al., 2006; Nguyen et al., 2008; Wolfers et al., 2011; Hall et al., 2018), including risk perception (Syme et al., 2017), that is to say, the subjective assessment about the characteristics and severity of a given risk; and limited/strained testing and diagnostic capacity (Schulte et al., 1992).

These studies concerned the following sexually transmitted pathogens/STDs: herpetic diseases (Koutsky et al., 1992; Ashley et al., 1993), human papillomavirus or HPV (Franco, 1991; Brown et al., 2015; Shahesmaeili et al., 2018; Moriña et al., 2021), chancroid (Schulte et al., 1992), *Chlamydia trachomatis* (Lin et al., 1992; Maher and Hoffman, 1995; Schachter and Chow, 1995; Agacfidan et al., 1997; Paget et al., 2002; Krivochenitser et al., 2013; Corbeto et al., 2015; Tomas et al., 2015; Kustec et al., 2016; Mlakar and Ramšak, 2016), syphilis (Webster et al., 1993; Gratzer et al., 2014; Shahesmaeili et al., 2018) and genital ulcer disease (GUD; Rompalo et al., 1997), gonorrhea (Webster et al., 1993; Maher and Hoffman, 1995; Borisenko, 1998; Krivochenitser et al., 2013; Tomas et al., 2015; Shahesmaeili et al., 2018), trichomoniasis (Maher and Hoffman, 1995; Petersen et al., 1995; Munson et al., 2008; Lusk et al., 2010; Andrea and Chapin, 2011; Roth et al., 2011; Tomas et al., 2015; Shahesmaeili et al., 2018), bacterial vaginosis (Schwebke et al., 1996), *Ureaplasma urealyticum* (Dhawan et al., 2006), Zika virus (Allard et al., 2017; Lee and Nishiura, 2017), amebiasis (Timsit et al., 2018), and human immunodeficiency virus, or HIV (Wahdan, 1995; Liu et al., 2006; Nguyen et al., 2008; Mirzazadeh et al., 2014; Fakoya et al., 2015; Ni et al., 2016; Hall et al., 2018; Steen et al., 2019).

Three articles (Niccolai et al., 2005; Jenkins, 2015; Mangine et al., 2018) contained recommendations to overcome these shortcomings: namely, (i) to use sensitive and specific assays, (ii) to accurately collect sexual history, including data related to sexual orientation, and identify high-risk sexual behaviors (Jenkins, 2015), (iii) to strengthen sentinel surveillance and establish further sites, to improve the quality of collected data, (iv) to deploy and link multiple data sources, such as self-reports, medical record reviews, and regional/state health department reports, harmonizing, when appropriate, the various and different reporting systems and case definitions (Niccolai et al., 2005), and, (v) to exploit the web, including social media and social networks to recruit high-risk populations, like the MSM community (Mangine et al., 2018).

Three other studies (Brookmeyer et al., 1995;Johnson and Geffen, 2016; Knight et al., 2020) focused on mathematical modeling, suggesting that the underestimation of STDs can occur when one fails to properly model high-risk sexual behaviors (such as unprotected, condomless sexual intercourse, use of recreational drugs or chemsex, sex with commercial partners, or with individuals the HIV status is unknown; Johnson and Geffen, 2016; Knight et al., 2020) or does not adjust for the follow-up bias (potential losses during the follow-up; Brookmeyer et al., 1995).

Specifically concerning behavioral determinants of STDs (i.e., healthcare-seeking behaviors), a series of qualitative in-depth interviews carried out among 24 university students, exhibiting risky sexual behaviors (Denison et al., 2017), identified three main types of barriers to STD testing: (i) personal (underestimation of risk, perception of STD as a not serious disease, fear of invasive procedures, self-consciousness in genital examination, and/or being too busy); (ii) structural (economic-financial cost of testing, environment–clinician attributes and attitudes); and, (iii) social (concern/fear of stigmatization).

Finally, seven of the 53 retrieved articles focused on the MSM community (Liu et al., 2006; Koper et al., 2013; Brown et al., 2015; Mlakar and Ramšak, 2016; Hall et al., 2018; Mangine et al., 2018; Knight et al., 2020).

## Underestimation of monkeypox cases

So far, the only attempt to test the hypothesis of the impact of stigmatization on monkeypox case reporting in European countries has been done by Kenyon (Kenyon, 2022), employing Spearman's correlation test to quantitatively explore whether the monkeypox national cumulative incidence was negatively associated with the intensity of screening for STIs and a composite indicator of LGBTQI+ rights (the "Rainbow Index"). The author found, instead, a positive correlation between the monkeypox epidemiological trend and the intensity of chlamydia/gonorrhea (rho 0.68, $p < 0.0001$), and syphilis (rho 0.62, $p < 0.0001$) screening, and the Rainbow Index (rho 0.65, $p < 0.0001$), suggesting that in several Eastern European countries, the real burden of monkeypox is underestimated.

Besides stigmatization and related issues, a few monkeypox infections are asymptomatic (Fleischauer et al., 2005; Karem et al., 2007; Guagliardo et al., 2020) and, when present, symptoms are atypical, in that this outbreak differs from previous outbreaks, in terms of a shift in mean age and the most affected age group, affected sex/gender, risk factors, clinical course, signs/symptoms, and, above all, the sexual transmission route (Bragazzi et al., 2022a). As such, physicians may not recognize the infection as monkeypox. A recent "knowledge, attitudes, and practices" (KAP) survey among Italian physicians showed unsatisfying monkeypox-related knowledge and attitude levels (Riccò et al., 2022). For example, systemic complications of monkeypox, especially among children, were generally largely overlooked. Of note, Italian physicians who took part in the survey showed substantial uncertainties and knowledge gaps related to monkeypox, in terms of clinical presentation and main features, risk factors, and preventative measures, with less than one-fifth of them confident in properly recognizing incident monkeypox cases during their clinical activities. Another survey conducted in Jordan (Sallam et al., 2022), among 615 university students in health schools/faculties (medicine, nursing, dentistry, pharmacy, medical laboratory sciences, and rehabilitation), identified serious gaps in knowledge, with only three out of 11 monkeypox-related knowledge items identified correctly by >70% of the respondents. Only 26.2% of the participants knew that monkeypox is a vaccine-preventable disease. However, information about knowledge of monkeypox among physicians and allied health professionals is scarce.

Also, the monkeypox case definition has only recently been revised to be adapted to the ongoing outbreak, in order to reflect the new findings and clinical and laboratory features (Bragazzi et al., 2022a; Centers for Disease Control and Prevention (CDC), 2022).

Another factor that could result in monkeypox underestimation is testing and diagnostic capacity, with a general lack of point-of-care tests currently available and, in some countries, overall testing (Nuzzo et al., 2022). Diagnostic/testing capacity for monkeypox varies substantially worldwide—some countries like the United States are able to process up to several thousand specimens *per* week (Cohen, 2022), while others have no diagnostic capacity at all; moreover, testing and diagnostic capacity are further strained by the still ongoing COVID-19 pandemic. Testing includes non-*variola Orthopoxvirus* (NVO) generic real-time polymerase chain reaction (PCR) test, monkeypox-specific PCR, and sequencing (Jiang et al., 2022).

Further, services and healthcare provisions offered by sexual health clinics in some countries, like the United Kingdom, are being significantly impacted and disrupted. This could result in a significant delay in the diagnosis, treatment, and reporting of cases.

Finally, in most cases, contact tracing (also known as partner notification) is unfeasible or presents particular challenges in the MSM community, given that contacts of infected individuals are casual sexual partners (Bell and Potterat, 2011; Bragazzi et al., 2022a).

# Discussion

Sexually transmitted diseases are generally overlooked and underestimated (Sartorius, 2007; Bragazzi et al., 2022b). Based on our integrative review of the literature, monkeypox case underestimation could be significant. This has important implications for public and global health providers as well as policy- and decision-makers, epidemiologists, and mathematical modelers.

According to Andersen's "Behavioral Model of Health Services Use," health-seeking behaviors are complex and multidimensional, depending on an array of factors, including "predisposing factors" (such as age, sex/gender, ethnicity, or cultural and social variables), "enabling factors" (like financial variables—insurance coverage—or healthcare accessibility/availability), and "need factors" (health, risk, and disease perceptions, health literacy, medical conditions, or underlying co-morbidities; Babitsch et al., 2012). Symptoms of some STDs can be mild and individuals may not seek healthcare. Moreover, in the LGBTQI+ community, STDs are usually perceived as a "part of the way of life" and as inconvenient consequences of being sexually active. In the pre-HIV pre-exposure prophylaxis (PrEP) era, HIV was considered the most anxiety-provoking STD, followed by viral, recurring STDs, and bacterial STDs, which were conceived as trivial and treatable. On the other hand, while not generating particular concerns in terms of disease perception, a diagnosis of STDs was associated with feelings of being "dirty and ashamed" (Holt et al., 2010). Risk and disease perceptions regarding HIV have changed after PrEP introduction, but the general thought that STD is an untoward consequence of sexual activities has remained practically unchallenged. Intended and actual utilization of healthcare provisions has been found to be related to the endorsement of stigmatization of certain sexual practices, such as anal sexual intercourse (Kutner et al., 2022). Awareness and attitudes toward STDs are highly heterogenous among MSM, with some infections considered scarier and others less, depending on their

transmission mechanisms, epidemiology (prevalence), visibility of symptoms, and impact on health, as well as the availability of vaccines and treatment options, based on both personal or friends' experiences (Datta et al., 2019).

Sexual health clinics are usually the first point of access in the case of STDs. However, some sex and gender minorities (SGMs), despite being at higher risk for STDs, including monkeypox, could be underrepresented. Holmes and Beach (2020) found that individuals self-identifying as bisexuals were approximately one-quarter of sexual health clinic users, while they represent more than half of SGMs populations. The so-called "bisexual erasure" or "bisexual invisibility" may be one of the factors explaining the potential underestimation of monkeypox cases, with the number of cases reported among men having sex with men and women (MSMW) being tremendously underestimated.

All these behaviors can be explained utilizing the "minority stress theory" (MST), according to which some marginalized communities subjected to stigmatization and discrimination experience more stressors than the general population, resulting in increased stress-linked coping behaviors, substance use, encounters with random/casual sex partners, and poorer health outcomes and health-related inequalities. Health disparities could be due to lower access to healthcare services, including preventative and STD screening/testing ones (Holmes and Beach, 2020).

There are different interests and actors at stake and a holistic approach is required to address STDs, in general, and monkeypox, specifically. To really advance the field of STD- and sexual health-related research, institutional and governmental bodies should facilitate "sex-at-birth, sexual orientation, and gender identity" (SSOGI)-related data collection, dissemination, and utilization, to favor a more "inclusive STD reporting" (Baptiste-Roberts et al., 2017). Currently, SSOGI data collection is not routinely implemented, with the risk of invisibilizing individuals with bi/bi+ umbrella labels, such as bisexual, queer, and pansexual individuals (Baptiste-Roberts et al., 2017). Several LGBTQI+ organizations have been collecting SSOGI data, but current public health surveillance systems are not updated to incorporate such information (Baptiste-Roberts et al., 2017). Of note, a major shortcoming of the investigation by Kenyon (Bragazzi et al., 2022b) is that the incidence of monkeypox cases was computed utilizing the entire (general) population, rather than the MSM/SGM/LGBTQI+ population. The latter point reflects the challenges that can be encountered in measuring and collecting data related to the sexual orientation/gender identity of a patient, given that there exist several socio-cultural, historical, as well as political implications underlying these issues. Data collected by healthcare providers are affected by the patient's willingness to disclose personal, sensitive information and their degree of openness, while self-report data suffer from selection/self-selection biases. As such, the real size of the MSM/SGM/LGBTQI+ population remains unknown and discrepancies among studies and differences among countries point to the influence of societal variables as well as the precise definition of what the MSM/SGM/LGBTQI+ population is (Marcus et al., 2013).

Specifically, concerning monkeypox cases, even though in a few cases, systemic prodromal symptoms (like fever, headache, lymphadenopathy, etc.) typical of the invasion period may be missing, with visible symptoms appearing during the skin eruption stage and a few asymptomatic individuals described in the current as in previous outbreaks, there are good reasons to suspect underestimation just by looking at data, since, as noted by Nuzzo et al. (2022), the United States, despite having a larger population size, have reported fewer cases than the United Kingdom.

The engagement of the LGBTQI+ community, and especially of bisexual/pansexual (bi/bi+) populations, with community-based sexual health providers is of paramount importance (Baptiste-Roberts et al., 2017) to offer LGBTQI+-tailored sexual health services. Scaling up community outreach and recruitment of LGBTQI+ members, including bi/bi+ people to engage in sexual health services represent challenges that need to be prioritized (Baptiste-Roberts et al., 2017). Adopting an intersectional lens, with a focus on populations reporting multiple stigmatization and discrimination, such as non-White communities, is crucial to address unmet needs. Educating staff to be culturally sensitive and competent, fighting against systemic and institutional stigmatization, and homo-bi-trans-phobia, and creating an inclusive environment represent another societal onus. Institutional bodies should conduct awareness campaigns to enhance health literacy, minimize structural or perceived barriers to STD testing, develop effective and innovative strategies aimed at addressing personal beliefs and improving STD testing rates, and favor the adoption of healthy sexual practices and behaviors (Pitts, 2020).

Social media, including news outlets, should also play their role in changing societal views of STDs (Pitts, 2020), combating disinformation and infodemic (Ennab et al., 2022), and creating awareness that monkeypox can infect all humans regardless of their age, sex/gender, sexual orientation, or gender identity. Moreover, there are various factors that may increase the potential risk for exposure, including close, sexual, and/or intimate contact with someone who has monkeypox and symptoms, such as rash, soreness, or scabs. Potentially, any sexually active individual could contract the infection, even if the focus is mainly on the MSM community. This could lead to a (further) underestimation of infectious transmission among other populations, as previously mentioned.

## Conclusion and future prospects

Monkeypox is an emerging sexually transmitted infection, which is representing a global public health concern. Mathematical modeling of monkeypox should adjust for the underestimation of cases and public and global health policy- and decision-makers should consider the "hidden burden" of monkeypox when designing and implementing packages of interventions. Studies

are urgently needed to quantify the degree of underestimation of monkeypox cases to better inform the responses to the outbreak.

## Author contributions

JW and JDK conceived and drafted the paper. WAW, SAI, QH, XW, AS, KB, PO, CP, MW, AO, MC, and BM critically revised it. All authors contributed to the article and approved the submitted version.

## Funding

This research is funded by Canada's International Development Research Centre (IDRC) and the Swedish International Development Cooperation Agency (SIDA; Grant No. 109559–001). NLB and JDK acknowledge support from IDRC (Grant No. 109981), New Frontier in Research Fund-Exploratory (Grant No. NFRFE-2021-00879), and NSERC Discovery Grant (Grant No. RGPIN-2022-04559). AS would like to thank The University of Queensland's AI4PAN research group. Portions of this work were performed at the Los Alamos National Laboratory under the auspices of the US Department of Energy contract 89233218CNA000001 and supported by NIH grant R01-OD011095 (SAI).

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## References

Agacfidan, A., Chow, J. M., Pashazade, H., Ozarmagan, G., and Badur, S. (1997). Screening of sex workers in Turkey for chlamydia trachomatis. *Sex. Transm. Dis.* 24, 573–575. doi: 10.1097/00007435-199711000-00004

Alakunle, E., Moens, U., Nchinda, G., and Okeke, M. I. (2020). Monkeypox virus in Nigeria: infection biology, epidemiology, and evolution. *Viruses* 12:1257. doi: 10.3390/v12111257

Allard, A., Althouse, B. M., Hébert-Dufresne, L., and Scarpino, S. V. (2017). The risk of sustained sexual transmission of Zika is underestimated. *PLoS Pathog.* 13:e1006633. doi: 10.1371/journal.ppat.1006633

Andrea, S. B., and Chapin, K. C. (2011). Comparison of Aptima Trichomonas vaginalis transcription-mediated amplification assay and BD affirm VPIII for detection of T. vaginalis in symptomatic women: performance parameters and epidemiological implications. *J. Clin. Microbiol.* 49, 866–869. doi: 10.1128/JCM.02367-10

Ashley, R. L., Dalessio, J., Dragavon, J., Koutsky, L. A., Lee, F. K., Nahmias, A. J., et al. (1993). Underestimation of HSV-2 seroprevalence in a high-risk population by microneutralization assay. *Sex. Transm. Dis.* 20, 230–235. doi: 10.1097/00007435-199307000-00009

Babitsch, B., Gohl, D., and von Lengerke, T. (2012). Re-revisiting Andersen's behavioral model of health services use: a systematic review of studies from 1998-2011. *Psychosoc. Med.* 9. doi: 10.3205/psm000089

Baptiste-Roberts, K., Oranuba, E., Werts, N., and Edwards, L. V. (2017). Addressing health care disparities among sexual minorities. *Obstet. Gynecol. Clin. N. Am.* 44, 71–80. doi: 10.1016/j.ogc.2016.11.003

Barquet, N., and Domingo, P. (1997). Smallpox: the triumph over the most terrible of the ministers of death. *Ann. Intern. Med.* 127, 635–642. doi: 10.7326/0003-4819-127-8_part_1-199710150-00010

Bell, G., and Potterat, J. (2011). Partner notification for sexually transmitted infections in the modern world: a practitioner perspective on challenges and opportunities. *Sex. Transm. Infect.* 87, 34–36. doi: 10.1136/sextrans-2011-050229

Borisenko, K. (1998). STD surveillance in the Russian Federation. *Int. J. STD AIDS* 9:15.

Bragazzi, NL, Khamisy-Farah, R, Tsigalou, C, Mahroum, N, and Converti, M. (2022a). Attaching a stigma to the LGBTQI+ community should be avoided during the monkeypox epidemic. *J. Med. Virol.* doi: 10.1002/jmv.27913 [Epub ahead of print].

Bragazzi, NL, Kong, JD, Mahroum, N, Tsigalou, C, Khamisy-Farah, R, Converti, M., et al. (2022b). Epidemiological trends and clinical features of the ongoing monkeypox epidemic: a preliminary pooled data analysis and literature review. *J. Med. Virol.* doi: 10.1002/jmv.27931 [Epub ahead of print].

Brookmeyer, R., Quinn, T., Shepherd, M., Mehendale, S., Rodrigues, J., and Bollinger, R. (1995). The AIDS epidemic in India: a new method for estimating current human immunodeficiency virus (HIV) incidence rates. *Am. J. Epidemiol.* 142, 709–713. doi: 10.1093/oxfordjournals.aje.a117700

Broome, M. E. (2000). "Integrative literature reviews for the development of concepts," in *Concept Development in Nursing: Foundations, Techniques and Applications*. eds. B. L. Rodgers and K. A. Knafl (Philadelphia: W. B. Saunders Company), 231–250.

Brown, B., Monsour, E., Klausner, J. D., and Galea, J. T. (2015). Sociodemographic and behavioral correlates of anogenital warts and human papillomavirus-related knowledge among men who have sex with men and transwomen in Lima. *Peru. Sex Transm. Dis.* 42, 198–201. doi: 10.1097/OLQ.0000000000000258

Bunge, E. M., Hoet, B., Chen, L., Lienert, F., Weidenthaler, H., Baer, L. R., et al. (2022). The changing epidemiology of human monkeypox-a potential threat? A systematic review. *PLoS Negl. Trop. Dis.* 16:e0010141. doi: 10.1371/journal.pntd.0010141

Centers for Disease Control and Prevention (CDC) (2022). National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), division of high-consequence pathogens and pathology (DHCPP). Case definitions for use in the 2022 Monkeypox Response. Available at https://www.cdc.gov/poxvirus/monkeypox/clinicians/case-definition.html

Cohen, E. (2022). Testing is crucial to getting monkeypox under control, but there's a 'shocking' lack of demand. CNN Health. Available at: https://edition.cnn.com/2022/07/28/health/monkeypox-testing-demand/index.html

Corbeto, E. L., Gonzalez, V., Lugo, R., Almirall, M. R., Espelt, R., Avecilla, A., et al. (2015). CT/NG study group. Discordant prevalence of chlamydia trachomatis in asymptomatic couples screened by two screening approaches. *Int. J. STD AIDS* 26, 27–32. doi: 10.1177/0956462414528686

Datta, J., Reid, D., Hughes, G., Mercer, C. H., Wayal, S., and Weatherburn, P. (2019). Awareness of and attitudes to sexually transmissible infections among gay men and other men who have sex with men in England: a qualitative study. *Sex. Health* 16, 18–24. doi: 10.1071/SH18025

Denison, H. J., Bromhead, C., Grainger, R., Dennison, E. M., and Jutel, A. (2017). Barriers to sexually transmitted infection testing in New Zealand: a qualitative study. *Aust. N. Z. J. Public Health* 41, 432–437. doi: 10.1111/1753-6405.12680

Dhawan, B., Gupta, V., Khanna, N., Singh, M., and Chaudhry, R. (2006). Evaluation of the diagnostic efficacy of PCR for Ureaplasma urealyticum infection in Indian adults with symptoms of genital discharge. *Jpn. J. Infect. Dis.* 59, 57–58.

Diven, D. G. (2001). An overview of poxviruses. *J. Am. Acad. Dermatol.* 44, 1–16. doi: 10.1067/mjd.2001.109302

Durski, K. N., McCollum, A. M., Nakazawa, Y., Petersen, B. W., Reynolds, M. G., Briand, S., et al. (2018). Emergence of Monkeypox-west and Central Africa, 1970-2017. *MMWR Morb. Mortal. Wkly Rep.* 67, 306–310. doi: 10.15585/mmwr.mm6710a5

Ennab, F., Nawaz, F. A., Narain, K., Nchasi, G., Essar, M. Y., Head, M. G., et al. (2022). Monkeypox outbreaks in 2022: battling another "pandemic" of misinformation. *Int. J. Public Health* 14:1605149. doi: 10.3389/ijph.2022.1605149

Fakoya, I., Álvarez-del Arco, D., Woode-Owusu, M., Monge, S., Rivero-Montesdeoca, Y., Delpech, V., et al. (2015). A systematic review of post-migration acquisition of HIV among migrants from countries with generalised HIV epidemics living in Europe: mplications for effectively managing HIV prevention programmes and policy. *BMC Public Health* 19:561. doi: 10.1186/s12889-015-1852-9

Fleischauer, A. T., Kile, J. C., Davidson, M., Fischer, M., Karem, K. L., Teclaw, R., et al. (2005). Kuehnert MJ. Evaluation of human-to-human transmission of monkeypox from infected patients to health care workers. *Clin. Infect. Dis.* 40, 689–694. doi: 10.1086/427805

Franco, E. L. (1991). The sexually transmitted disease model for cervical cancer: incoherent epidemiologic findings and the role of misclassification of human papillomavirus infection. *Epidemiology* 2, 98–106. doi: 10.1097/00001648-199103000-00003

Geba, M. C., Powers, S., Williams, B., Dort, K. R., Rogawski McQuade, E. T., and McManus, K. A. (2022). A missed opportunity: Extragenital screening for gonorrhea and chlamydia sexually transmitted infections in people with HIV in a southeastern Ryan white HIV/AIDS program clinic setting. *Open Forum Infect. Dis.* 9:ofac322. doi: 10.1093/ofid/ofac322

Gratzer, B., Pohl, D., and Hotton, A. L. (2014). Evaluation of diagnostic serological results in cases of suspected primary syphilis infection. *Sex. Transm. Dis.* 41, 285–289. doi: 10.1097/OLQ.0000000000000126

Guagliardo, S. A. J., Monroe, B., Moundjoa, C., Athanase, A., Okpu, G., Burgado, J., et al. (2020). Asymptomatic Orthopoxvirus circulation in humans in the wake of a Monkeypox outbreak among chimpanzees in Cameroon. *Am. J. Trop. Med. Hyg.* 102, 206–212. doi: 10.4269/ajtmh.19-0467

Hall, G. C., Koenig, L. J., Gray, S. C., Herbst, J. H., Matheson, T., Coffin, P., et al. (2018). Accuracy of HIV risk perceptions among episodic substance-using men who have sex with men. *AIDS Behav.* 22, 1932–1943. doi: 10.1007/s10461-017-1935-y

Holmes, N., and Beach, L. (2020). Bisexual people's utilization of sexual health services at an LGBTQ Community Center in Chicago. *J. Bisex.* 20, 342–359. doi: 10.1080/15299716.2020.1825270

Holt, M., Bernard, D., and Race, K. (2010). Gay men's perceptions of sexually transmissible infections and their experiences of diagnosis: 'part of the way of life' to feeling 'dirty and ashamed. *Sex. Health* 7, 411–416. doi: 10.1071/SH09117

Hong, Y., Fang, X., Zhou, Y., Zhao, R., and Li, X. (2011). Factors associated with sexually transmitted infection underreporting among female sex workers in China. *J. Women's Health (Larchmt)* 20, 129–136. doi: 10.1089/jwh.2010.2139

Hughes, A. L., Irausquin, S., and Friedman, R. (2010). The evolutionary biology of poxviruses. *Infect. Genet. Evol.* 10, 50–59. doi: 10.1016/j.meegid.2009.10.001

Jenkins, W. D. (2015). LeVault KR. Sexual history taking in the emergency department - more specificity required. *J. Emerg. Med.* 48, 143–151. doi: 10.1016/j.jemermed.2014.06.051

Jiang, Z., Sun, J., Zhang, L., Lai, A., and Shuo, S. (2022). Laboratory diagnostics for monkeypox: an overview of sensitivities from various published tests. *Travel Med. Infect. Dis.* 49:102425. doi: 10.1016/j.tmaid.2022.102425

Johnson, L. F., and Geffen, N. (2016). A comparison of two mathematical modeling frameworks for evaluating sexually transmitted infection epidemiology. *Sex. Transm. Dis.* 43, 139–146. doi: 10.1097/OLQ.0000000000000412

Karem, K. L., Reynolds, M., Hughes, C., Braden, Z., Nigam, P., Crotty, S., et al. (2007). Monkeypox-induced immunity and failure of childhood smallpox vaccination to provide complete protection. *Clin. Vaccine Immunol.* 14, 1318–1327. doi: 10.1128/CVI.00148-07

Kenyon, C. (2022). Is Monkeypox being underdiagnosed in countries with more stigmatizing attitudes towards men who have sex with men? A simple ecological analysis. *Epidemiologia* 3, 363–368. doi: 10.3390/epidemiologia 3030028

Kmiec, D., and Kirchhoff, F. (2022). Monkeypox: a new threat? *Int. J. Mol. Sci.* 23:7866. doi: 10.3390/ijms23147866

Knight, J., Baral, S. D., Schwartz, S., Wang, L., Ma, H., Young, K., et al. (2020). Contribution of high risk groups' unmet needs may be underestimated in epidemic models without risk turnover: a mechanistic modelling analysis. *Infect. Dis. Model.* 1, 549–562. doi: 10.1016/j.idm.2020.07.004

Koper, N. E., van der Sande, M. A., Gotz, H. M., and Koedijk, F. D. (2013). Dutch STI clinics. Lymphogranuloma venereum among men who have sex with men in the Netherlands: regional differences in testing rates lead to underestimation of the incidence, 2006-2012. *Euro Surveill.* 18:20561. doi: 10.2807/1560-7917.es2013.18.34.20561

Koutsky, L. A., Stevens, C. E., Holmes, K. K., Ashley, R. L., Kiviat, N. B., Critchlow, C. W., et al. (1992). Underdiagnosis of genital herpes by current clinical and viral-isolation procedures. *N. Engl. J. Med.* 326, 1533–1539. doi: 10.1056/NEJM199206043262305

Kretzschmar, M., Mangen, M. J., Pinheiro, P., Jahn, B., Fèvre, E. M., Longhi, S., et al. (2012). BCoDE consortium. New methodology for estimating the burden of infectious diseases in Europe. *PLoS Med.* 9:e1001205. doi: 10.1371/journal.pmed.1001205

Krivochenitser, R., Jones, J. S., Whalen, D., and Gardiner, C. (2013). Underrecognition of cervical Neisseria gonorrhoeae and chlamydia trachomatis infections in pregnant patients in the ED. *Am. J. Emerg. Med.* 31, 661–663. doi: 10.1016/j.ajem.2012.11.017

Kustec, T., Keše, D., and Klavs, I. (2016). Under-reporting of sexually transmitted infection with chlamydia trachomatis - a revision of surveillance system is required. *Zdr. Varst.* 55, 174–178. doi: 10.1515/sjph-2016-0022

Kutner, B. A., Simoni, J. M., DeWitt, W., Gaisa, M. M., and Sandfort, T. G. M. (2022). Gay and bisexual men who report anal sex stigma alongside discomfort discussing anal sex with health workers are less likely to have ever received an anal examination or anal swab. *LGBT Health* 9, 103–113. doi: 10.1089/lgbt.2021.0104

Lee, H., and Nishiura, H. (2017). Recrudescence of Ebola virus disease outbreak in West Africa, 2014-2016. *Int. J. Infect. Dis.* 64, 90–92. doi: 10.1016/j.ijid.2017.09.013

Lin, J. S., Jones, W. E., Yan, L., Wirthwein, K. A., Flaherty, E. E., Haivanis, R. M., et al. (1992). Underdiagnosis of chlamydia trachomatis infection. Diagnostic limitations in patients with low-level infection. *Sex. Transm. Dis.* 19, 259–265. doi: 10.1097/00007435-199209000-00004

Liu, D, Chi, Y, Song, P, Zeng, X, Du, L, Chen, Y, et al. (2022). Risk factors, clinical manifestation, precaution, and management of monkeypox. *J. Evid. Med.* doi: 10.1111/jebm.12490 [Epub ahead of print].

Liu, H., Yang, H., Li, X., Wang, N., Liu, H., Wang, B., et al. (2006). Men who have sex with men and human immunodeficiency virus/sexually transmitted disease control in China. *Sex. Transm. Dis.* 33, 68–76. doi: 10.1097/01.olq.0000187266.29927.11

Lusk, M. J., Naing, Z., Rayner, B., Rismanto, N., McIver, C. J., Cumming, R. G., et al. (2010). Konecny P. Trichomonas vaginalis: underdiagnosis in urban Australia could facilitate re-emergence. *Sex. Transm. Infect.* 86, 227–230. doi: 10.1136/sti.2009.039362

Maher, D., and Hoffman, I. (1995). Prevalence of genital infections in medical inpatients in Blantyre, Malawi. *J. Inf. Secur.* 31, 77–78. doi: 10.1016/s0163-4453(95)91674-1

Mangine, C., Kukk, A., Noormets, H., Jänes, J., and Rüütel, K. (2018). Internet recruitment for sexually transmitted infection screening among men who have sex with men in Eastern Europe. *Int. J. STD AIDS* 29, 237–243. doi: 10.1177/0956462417722477

Marcus, U., Hickson, F., Weatherburn, P., and Schmidt, A. J.EMIS Network (2013). Estimating the size of the MSM populations for 38 European countries by calculating the survey-surveillance discrepancies (SSD) between self-reported new HIV diagnoses from the European MSM internet survey (EMIS) and surveillance-reported HIV diagnoses among MSM in 2009. *BMC Public Health* 3:919. doi: 10.1186/1471-2458-13-919

Mirzazadeh, A., Nedjat, S., Navadeh, S., Haghdoost, A., Mansournia, M. A., McFarland, W., et al. (2014). HIV and related risk behaviors among female sex workers in Iran: bias-adjusted estimates from the 2010 National bio-Behavoral Survey. *AIDS Behav.* 18, S19–S24. doi: 10.1007/s10461-013-0548-3

Mlakar, B., and Ramšak, A. (2016). A suspected case of lymphogranuloma venereum (LGV) suggests underdiagnosed LGV infection among Slovenian men who have sex with men. *Acta Dermatovenerol. Alp Pannonica Adriat.* 25, 35–37. doi: 10.15570/actaapa.2016.10

Moriña, D., Fernández-Fontelo, A., Cabaña, A., Puig, P., Monfil, L., Brotons, M., et al. (2021). Quantifying the under-reporting of uncorrelated longitudal data: the genital warts example. *BMC Med. Res. Methodol.* 21:6. doi: 10.1186/s12874-020-01188-4

Munson, E., Napierala, M., Olson, R., Endes, T., Block, T., Hryciuk, J. E., et al. (2008). Impact of Trichomonas vaginalis transcription-mediated amplification-based analyte-specific-reagent testing in a metropolitan setting of high sexually transmitted disease prevalence. *J. Clin. Microbiol.* 46, 3368–3374. doi: 10.1128/JCM.00564-08

Nakamoto, I., and Zhang, J. (2021). Modeling the underestimation of COVID-19 infection. *Results Phys.* 25:104271. doi: 10.1016/j.rinp.2021.104271

Nguyen, T. Q., Gwynn, R. C., Kellerman, S. E., Begier, E., Garg, R. K., Pfeiffer, M. R., et al. (2008). Population prevalence of reported and unreported HIV and related behaviors among the household adult population in new York City, 2004. *AIDS* 22, 281–287. doi: 10.1097/QAD.0b013e3282f2ef58

Ni, M., Chen, X., Hu, X., and Ma, Y. (2016). Misreporting rate and influencing factors regarding the routes of transmission among reported HIV patients in Yili Kazakh autonomous prefecture of Xinjiang Uygur autonomous region of China.

*Zhonghua Liu Xing Bing Xue Za Zhi* 37, 90–93. doi: 10.3760/cma.j.issn.0254-6450.2016.01.019

Niccolai, L. M., Kershaw, T. S., Lewis, J. B., Cicchetti, D. V., Ethier, K. A., and Ickovics, J. R. (2005). Data collection for sexually transmitted disease diagnoses: a comparison of self-report, medical record reviews, and state health department reports. *Ann. Epidemiol.* 15, 236–242. doi: 10.1016/j.annepidem.2004.07.093

Niekamp, A. M., Spauwen, L. W. L., Dukers-Muijrers, N. H. T. M., and Hoebe, C. J. P. A. (2021). How aware are swingers about their swing sex partners' risk behaviours, and sexually transmitted infection status? *BMC Infect. Dis.* 21:172. doi: 10.1186/s12879-021-05813-5

Nuzzo, J. B., Borio, L. L., and Gostin, L. O. (2022). The WHO Declaration of Monkeypox as a global public health emergency. *JAMA* 328, 615–617. doi: 10.1001/jama.2022.12513

Oliffe, J. L., Chabot, C., Knight, R., Davis, W., Bungay, V., and Shoveller, J. A. (2013). Women on men's sexual health and sexually transmitted infection testing: a gender relations analysis. *Sociol. Health Illn.* 35, 1–16. doi: 10.1111/j.1467-9566.2012.01470.x

Paget, W. J., Zbinden, R., Ritzler, E., Zwahlen, M., Lengeler, C., Stürchler, D., et al. (2002). Swiss sentinel surveillance Network of gynecologists. National laboratory reports of chlamydia trachomatis seriously underestimate the frequency of genital chlamydial infections among women in Switzerland. *Sex. Transm. Dis.* 29, 715–720. doi: 10.1097/00007435-200211000-00016

Petersen, C. S., Carl, L., Alnor, D., Thomsen, U., and Thomsen, H. K. (1995). Ignored trichomonal infestation diagnosed by Papanicolaou smear. *Genitourin. Med.* 71, 257–258. doi: 10.1136/sti.71.4.257

Pitts, C. J. (2020). Sexually transmitted infections: overlooked, underestimated, and undiagnosed. *Nurs. Clin. North Am.* 55, xiii–xiv. doi: 10.1016/j.cnur.2020.06.011. Epub

Riccò, M., Ferraro, P., Camisa, V., Satta, E., Zaniboni, A., Ranzieri, S., et al. (2022). When a neglected tropical disease goes global: knowledge, attitudes and practices of Italian physicians towards Monkeypox, preliminary results. *Trop. Med. Infect. Dis.* 7:135. doi: 10.3390/tropicalmed7070135

Riedel, S. (2005). Edward Jenner and the history of smallpox and vaccination. *Proc. (Baylor Univ. Med. Cent.)* 18, 21–25. doi: 10.1080/08998280.2005.11928028

Rompalo, A. M., Shepherd, M., Lawlor, J. P., Rand, S., Fox, R., Brookmeyer, R., et al. (1997). Definitions of genital ulcer disease and variation in risk for prevalent human immunodeficiency virus infection. *Sex. Transm. Dis.* 24, 436–442. doi: 10.1097/00007435-199708000-00009

Roth, A. M., Williams, J. A., Ly, R., Curd, K., Brooks, D., Arno, J., et al. (2011). Changing sexually transmitted infection screening protocol will result in improved case finding for trichomonas vaginalis among high-risk female populations. *Sex. Transm. Dis.* 38, 398–400. doi: 10.1097/OLQ.0b013e318203e3ce

Sallam, M., Al-Mahzoum, K., Dardas, L. A., Al-Tammemi, A. B., Al-Majali, L., Al-Naimat, H., et al. (2022). Knowledge of human Monkeypox and its relation to conspiracy beliefs among students in Jordanian health schools: filling the knowledge gap on emerging zoonotic viruses. *Medicina* 58:924. doi: 10.3390/medicina58070924

Sartorius, N. (2007). Stigmatized illnesses and health care. *Croat. Med. J.* 48, 396–397.

Schachter, J., and Chow, J. M. (1995). The fallibility of diagnostic tests for sexually transmitted diseases: the impact of behavioral and epidemiologic studies. *Sex. Transm. Dis.* 22, 191–196. doi: 10.1097/00007435-199505000-00010

Schulte, J. M., Martich, F. A., and Schmid, G. P. (1992). Chancroid in the United States, 1981-1990: evidence for underreporting of cases. *MMWR CDC Surveill. Summ.* 41, 57–61.

Schwebke, J. R., Hillier, S. L., Sobel, J. D., McGregor, J. A., and Sweet, R. L. (1996). Validity of the vaginal gram stain for the diagnosis of bacterial vaginosis. *Obstet. Gynecol.* 88, 573–576. doi: 10.1016/0029-7844(96)00233-5

Shahesmaeili, A., Karamouzian, M., Shokoohi, M., Kamali, K., Fahimfar, N., Nadji, S. A., et al. (2018). Symptom-based versus laboratory-based diagnosis of five sexually transmitted infections in female sex Workers in Iran. *AIDS Behav.* 22, 19–25. doi: 10.1007/s10461-018-2130-5

Steen, R., Hontelez, J. A. C., Mugurungi, O., Mpofu, A., Matthijsse, S. M., de Vlas, S. J., et al. (2019). Economy, migrant labour and sex work: interplay of HIV epidemic drivers in Zimbabwe over three decades. *AIDS* 33, 123–131. doi: 10.1097/QAD.0000000000002066

Syme, M. L., Cohn, T. J., and Barnack-Tavlaris, J. (2017). A comparison of actual and perceived sexual risk among older adults. *J. Sex Res.* 54, 149–160. doi: 10.1080/00224499.2015.1124379

Thornhill, J. P., Barkati, S., Walmsley, S., Rockstroh, J., Antinori, A., Harrison, L. B., et al. (2022). SHARE-net clinical group. Monkeypox virus infection in humans across 16 countries-April-June 2022. *N. Engl. J. Med.* 387, 679–691. doi: 10.1056/NEJMoa2207323

Timsit, B. L., Deroux, A., Lugosi, M., and Colombe, B. (2018). Bouillet L. Amibiase: au cours de rapports sexuels, un mode de transmission sous-estimé? [Amoebosis: May sexual transmission be an underestimated way of contamination?]. *Rev. Med. Interne* 39, 586–588. doi: 10.1016/j.revmed.2018.04.004

Tomas, M. E., Getman, D., Donskey, C. J., and Hecker, M. T. (2015). Overdiagnosis of urinary tract infection and Underdiagnosis of sexually transmitted infection in adult women presenting to an emergency department. *J. Clin. Microbiol.* 53, 2686–2692. doi: 10.1128/JCM.00670-15

Wahdan, M. H. (1995). The Middle East: past, present and future. *AIDS Asia* 2, 21–23.

Webster, L. A., Berman, S. M., and Greenspan, J. R. (1993). Surveillance for gonorrhea and primary and secondary syphilis among adolescents, United States--1981-1991. *MMWR CDC Surveill. Summ.* 42, 1–11.

Wolfers, M., de Zwart, O., and Kok, G. (2011). Adolescents in the Netherlands underestimate risk for sexually transmitted infections and deny the need for sexually transmitted infection testing. *AIDS Patient Care STDs* 25, 311–319. doi: 10.1089/apc.2010.0186

Wu, S. L., Mertens, A. N., Crider, Y. S., Nguyen, A., Pokpongkiat, N. N., Djajadi, S., et al. (2020). Substantial underestimation of SARS-CoV-2 infection in the United States. *Nat. Commun.* 11:4507. doi: 10.1038/s41467-020-18272-4

frontiers | Frontiers in Microbiology

TYPE Review
PUBLISHED 25 November 2022
DOI 10.3389/fmicb.2022.1020029



OPEN ACCESS

EDITED BY
Laurent Rénia,
Nanyang Technological University,
Singapore

REVIEWED BY
Jyoti Bhardwaj,
Indiana University Hospital,
United States
Panagiotis Karanis,
University of Nicosia,
Cyprus

*CORRESPONDENCE
Nikhat Parveen
Parveeni@njms.rutgers.edu

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Infectious Agents and Disease, a section of the journal Frontiers in Microbiology

RECEIVED 15 August 2022
ACCEPTED 07 November 2022
PUBLISHED 25 November 2022

CITATION
Akoolo L, Rocha SC and Parveen N (2022) Protozoan co-infections and parasite influence on the efficacy of vaccines against bacterial and viral pathogens. *Front. Microbiol.* 13:1020029. doi: 10.3389/fmicb.2022.1020029



# Protozoan co-infections and parasite influence on the efficacy of vaccines against bacterial and viral pathogens

Lavoisier Akoolo[1†], Sandra C. Rocha[2†] and Nikhat Parveen[2*]

[1]Biorepository and Tissue Research Facility, University of Virginia School of Medicine, Charlottesville, VA, United States, [2]Department of Microbiology, Biochemistry and Molecular Genetics, Rutgers New Jersey Medical School, Newark, NJ, United States

A wide range of protozoan pathogens either transmitted by vectors (*Plasmodium*, *Babesia*, *Leishmania* and *Trypanosoma*), by contaminated food or water (*Entamoeba* and *Giardia*), or by sexual contact (*Trichomonas*) invade various organs in the body and cause prominent human diseases, such as malaria, babesiosis, leishmaniasis, trypanosomiasis, diarrhea, and trichomoniasis. Humans are frequently exposed to multiple pathogens simultaneously, or sequentially in the high-incidence regions to result in co-infections. Consequently, synergistic or antagonistic pathogenic effects could occur between microbes that also influences overall host responses and severity of diseases. The co-infecting organisms can also follow independent trajectory. In either case, co-infections change host and pathogen metabolic microenvironments, compromise the host immune status, and affect microbial pathogenicity to influence tissue colonization. Immunomodulation by protozoa often adversely affects cellular and humoral immune responses against co-infecting bacterial pathogens and promotes bacterial persistence, and result in more severe disease symptoms. Although co-infections by protozoa and viruses also occur in humans, extensive studies are not yet conducted probably because of limited animal model systems available that can be used for both groups of pathogens. Immunosuppressive effects of protozoan infections can also attenuate vaccines efficacy, weaken immunological memory development, and thus attenuate protection against co-infecting pathogens. Due to increasing occurrence of parasitic infections, roles of acute to chronic protozoan infection on immunological changes need extensive investigations to improve understanding of the mechanistic details of specific immune responses alteration. In fact, this phenomenon should be seriously considered as one cause of breakthrough infections after vaccination against both bacterial and viral pathogens, and for the emergence of drug-resistant bacterial strains. Such studies would facilitate development and implementation of effective vaccination and treatment regimens to prevent or significantly reduce breakthrough infections.

KEYWORDS

**protozoa, bacterial infection, co-infection, immune response, vaccine efficacy**

## Introduction

Co-infections of protozoa-bacteria-viruses are an emerging phenomenon due to overlapping epidemiological niches or shared transmission routes. Co-infections can adversely affect host immune responses, pathogenicity of microbes and success of chemotherapy and vaccinations. Co-infection of *Plasmodium* species with multiple bacterial species have been reported including with *Mycobacterium* (Chukwuanukwu et al., 2017), *Salmonella* (Cunnington et al., 2011), non-typhoid (NT) Salmonella (Takem et al., 2014), in addition to viruses such as HIV (Alemu et al., 2013), SARS-CoV-2 (Anyanwu, 2021), and hepatitis viruses (Helegbe et al., 2018). Such protozoan co-infections with bacterial and viral infections have high prevalence in same endemic regions especially in Sub-Saharan Africa. Malaria patients have been shown to be susceptible to other infections, influencing the pathogenesis and prognosis of the disease. Other interactions include *Babesia-Borrelia* (Dunn et al., 2014) as well as les common *Entamoeba* spp.-*Escherichia coli* have also been reported (Fernandez-Lopez et al., 2019). Alteration in the host, for instance, due to HIV infection that compromises host immune system can render humans more susceptible to co-infection by other opportunistic pathogens (Ayoade and Joel Chandranesan, 2022). Co-infecting pathogens may also have epidemiological implications and alter the mortality or morbidity due to diseases they cause (Anyanwu, 2021). Increase in DNA uptake and genetic recombination includes transfer of antimicrobial resistance genes between the co-infecting agents (Marti et al., 2017) resulting in emergence of multi-drug resistant pathogens. Apart from the effect on the pathogens, co-infections may also impact the efficacy of vaccines and success of chemotherapeutic agents.

Bacterial-protozoan co-infections are a commonly occurring phenomenon due to overlapping ecological niches and inadequate disease control infrastructures and thus, requires an all-inclusive approach to develop preventative vaccines and effective chemotherapeutic agents (Cox, 2001). Unexplained decline in efficacy of vaccines that are routinely used, and emergence of breakthrough infections is a concerning trend in disease endemic regions and poses a threat to control of infections around the world. The success of antimicrobials and vaccinations in diseases control depends heavily on a vibrant immune system. The immunodynamics of co-infections with protozoa need considered scrutiny during development of vaccines and antibiotics because the changes have the potential to lead to emergence of antigenic variations, breakthrough infections and antibiotic resistance (Wait et al., 2020).

The rise in immunocompromised individual numbers and accompanying vaccination failures has created a favorable microenvironment for emergence of more virulent pathogens (Laufer et al., 2007). This has further increased the urgency to investigate the causes of reduced effectiveness of vaccines against bacterial and viral pathogens to facilitate formulation of more inclusive and rational corrective measures. Inequitable resource distribution and marginalization of the developing world coupled with poor nutrition and disease control infrastructure complicates the control of global disease burden as more resistant strains emerge. These pathogens are then carried throughout the globe by traveling populations in now highly interconnected world (Walsh, 1989). This scenario creates an existential threat to disease control and a paradigm shift in approaches to vaccine and drug development. Therefore, it is critically important that acute to chronic protozoan infections and their presence during co-infections are considered seriously when more severe disease manifestations are noticed, or reduced vaccines effectiveness are observed.

Based upon a comprehensive review of literature, we have summarized the impact of protozoan infections on pathogenesis of co-infecting bacterial and viral pathogens (Figure 1). We have also depicted the consequence of acute to chronic parasitic infection on emergence of drug-resistance in co-infecting pathogens and influence of protozoa on vaccines efficacy that could affect protection from infectious bacteria and viruses.

## The epidemiology of protozoa-bacteria co-infections

Protozoa-bacteria co-infections are an emerging healthcare problem especially in the developing world due to geographical overlap between different pathogens. For example, the overlapping existence of *Plasmodium* spp. and *Mycobacterium tuberculosis* that cause malaria and tuberculosis (TB), respectively particularly in countries with poor healthcare infrastructure creates a perfect setting for co-infections (Page et al., 2005). Co-infections involving gastrointestinal pathogens also commonly occur in low-income countries with poor water-sewer infrastructure and hygienic environments and thus, allow a common pathway of transmission (fecal-oral) as reported for *Campylobacter jejunum* and intestinal protozoa (Bronowski et al., 2014). Co-infections with sexually transmittable pathogens also occur frequently in women with abnormal vaginal bacteriome. Bacterial vaginosis (BV), a common syndrome when quantity and quality of vaginal microbiota is perturbed, often involves *Trichomonas vaginalis* infection (Onderdonk et al., 2016). *Clostridium perfringens* and *T. fetus* are also a frequent occurrence in bacterial-protozoan vaginosis. *Toxoplasma gondii* infections have also been observed together with *Clostridium perfringens* during endometritis (Alsammani et al., 2012). The presence of infected companion feline hosts with susceptible humans serves as the most ardent predisposing factor for *T. gondii* infection cycle. For respiratory and gastrointestinal co-infections, other common predisposing conditions in humans is underlying immunocompromised status, such as during HIV infection/AIDS or the presence of other enteric pathogens like entero-viruses.

Common vector and reservoir host(s) harboring multiple pathogens have been observed frequently. *Babesia* species, which are protozoans transmitted by *Ixodes scapularis* ticks, are hemoparasites with life cycle and pathology similar to *Plasmodium* spp. and trigger comparable impact on mammalian hosts immune responses and show several overlapping diseases manifestations (Djokic et al., 2021). Lyme disease causing spirochetal bacteria

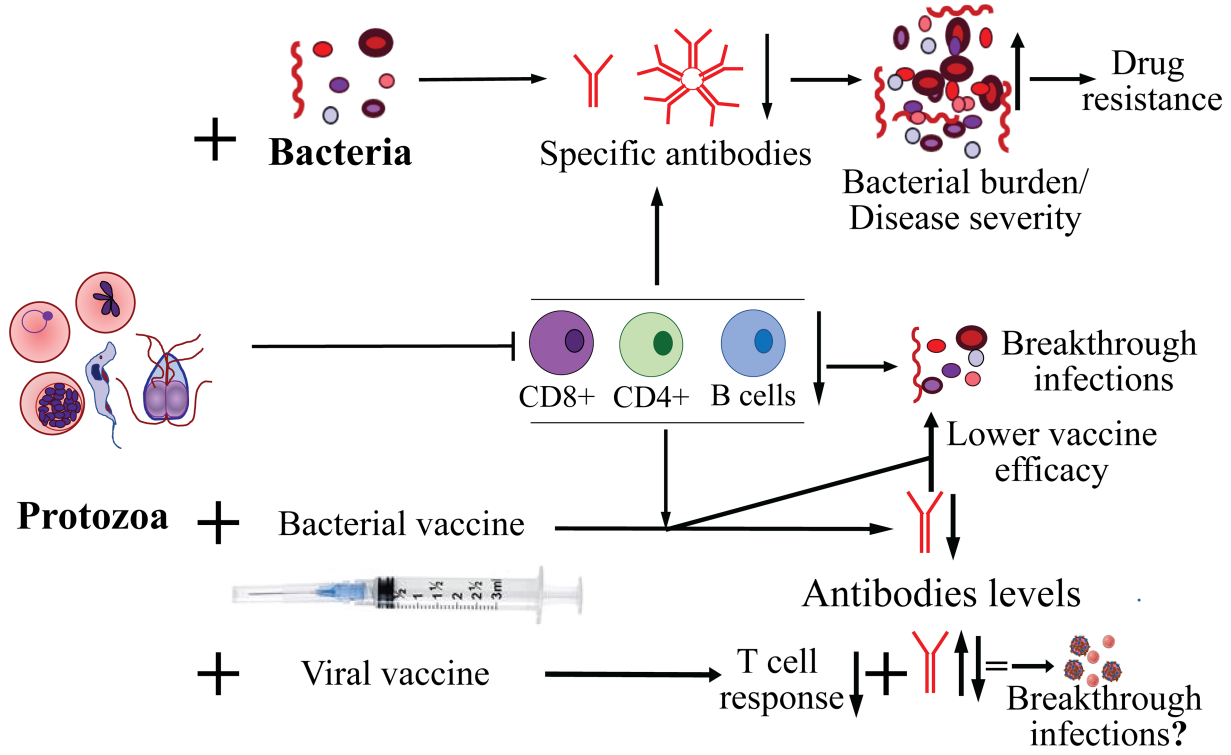


FIGURE 1
Effect of protozoan infection on adaptive immune response and co-infecting bacteria, and on effectiveness of bacterial and viral vaccines in protection from infection. The major pathogenic protozoa, such as *Plasmodium*, *Babesia*, *Trichomonas and Trypanosoma*, often cause suppression of adaptive immune response affecting both B and T cells. As a consequence, decrease in the specific antibody levels against co-infecting bacteria resulting in increase in burden of bacterial pathogens and exacerbation of severity of diseases they cause. Increased bacterial burden also enhances probability of emergence of resistance to antimicrobials. Diminished cellular and humoral immune response caused by acute to chronic infection by protozoa could also result in reduced efficacy/failure of vaccines against bacterial pathogens and may lead to breakthrough infections. Highly variable effects of protozoan infections with respect to antibody response to vaccines against different viral pathogens has been reported but their impact on breakthrough infections by the respective viruses remains to be investigated more thoroughly.

belonging to *Borrelia burgdorferi sensu* lato are also transmitted to different hosts by ticks (Stewart and Bloom, 2020). Increasing rate of co-infections by these two pathogens have been reported in the endemic regions of the United States (Swanson et al., 2006; Diuk-Wasser et al., 2016; Wormser et al., 2019) and Europe (Olsthoorn et al., 2021). Thus, in addition to *B. burgdorferi-Babesia* co-infections, simultaneous *Babesia-Anaplasma phagocytophilum* infections are also reported because tick vector and white footed mouse, *Peromyscus leucopus* (and other reservoir hosts) harbor several pathogens together. While some protozoa have established a mutual relationship with bacteria in which each microbe benefits from the infected host, other examples may involve cases where the pathogen modulates the immune status to benefit itself at the expense of the host (Schmid-Hempel, 2009).

## Protozoan infection and adaptive immune response

Following infection, protozoan parasites may either induce antibody, cell mediated immunity or stimulate both types of immune responses. These changes depend on the type of infection, localization of pathogen in the body, and the development stage of the organism. Various protozoan pathogens colonize different organs of the body, such as gastrointestinal tract (*Amoeba*), blood stream (*Trypanosoma*), within erythrocytes (*Plasmodium* and *Babesia*) and inside the macrophages (*Leishmania* spp., and *T. gondii)*. Extracellular parasites generally induce and can often be controlled by antibody mediated killing by opsonophagocytosis while intracellular protozoan pathogens control requires cell mediated immune responses (Bretscher, 1992). *Leishmania* spp. are obligate intracellular protozoan, such that defense mechanisms against this parasite depend upon CD4+ T-lymphocytes stimulation that could also activate macrophages and induce Th1 cytokines production (Gupta et al., 2013). Some parasites can induce both humoral and cell mediated immunity depending on the developmental stage of the pathogen, for, e.g., in *Plasmodium* spp. (Beck et al., 1995). In addition, they may also deploy immune evasion mechanisms for survival in the hosts, such as escape from antibodies by changing their surface antigenic coats, i.e., antigen variation observed in *Trypanosoma brucei* (Márquez-Contreras, 2018). *Trypanosoma brucei gambiense* induces humoral immune

response because of its extra-cellular existence; however, antigenic variation of the parasites is hallmark of its long-term persistence in the host because it enables the protozoan to evade the immune system-mediated elimination in many cases (Márquez-Contreras, 2018).

## The pathogenesis of protozoan-bacterial co-infections

Protozoan-bacterial co-infections affects hosts in many ways. They can either lead to an antagonistic/deleterious or synergistic/advantageous pathogenic effect on the infected hosts. Antagonisms may be caused by resource competition or stimulation/suppression of innate or adaptive immune response that could negatively affect the co-infecting pathogen (Rigaud et al., 2010). During synergistic interactions, pathogens could suppress immune response of hosts resulting in high rates of replication of one or both pathogens. For example, protozoan infections can lead to apoptotic clearance of cells of the immune system creating a favorable environment for their own multiplication and potentially for proliferation of the co-infecting bacteria (Gigley et al., 2012). In addition, co-infecting pathogens can also modulate the gene expression in each other. Furthermore, synergism may also involve direct or indirect resources sharing, such that one microbe assists the co-infecting pathogen with regards to acquisition of nutrients (Birger et al., 2015). In this case, one pathogen may create favorable environment for colonization and growth of another pathogen, for, e.g., the presence of bacterial biofilm may promote proliferation of protozoa to stimulate synergism (Denoncourt et al., 2014). Alternatively, each pathogen takes on independent mechanisms of existence with minimal effect on diseases they cause. Several examples listed below demonstrate different effects of protozoan-bacterial co-infections on each other and on host(s).

### *Plasmodium* and *mycobacterium tuberculosis*

Two major diseases, TB and malaria commonly occur together in patients due to overlapping geographical regions of infection for the two causative organisms (Baluku et al., 2019). Due to some similar initial subjective manifestations: flu-like illness, chills, fever, and fatigue, diagnostic approaches can miss some infections that leads to delayed therapeutic intervention. Thus, the lack of treatment for asymptomatic parasitic infection can apply selection pressure on TB by facilitating the emergence of drug-resistant strains (Murray et al., 2014). Interestingly, *P. falciparum* and *P. vivax* infections showed significant reduction in B and T (both CD4+ and CD8+) cells in patients (Kassa et al., 2006). *M. tuberculosis* infection has also been demonstrated to modulate the immune responses and confer immunological protection against severe malaria while weakened responses occur against bacteria. As a result, *Plasmodium-M. tuberculosis* co-infected patients show reduced/mild symptoms of malaria and a more severe symptoms of tuberculosis (Chukwuanukwu et al., 2017). In a murine model of infection, *M. tuberculosis*-induced potentiation of type 1 immune responses has been associated with protection against lethal malaria, which also appears to be related to induced production of IFN-γ and TNF-α in C57/BL6 mice (Page et al., 2005). Furthermore, mice sequentially infected with *M. tuberculosis* followed by *P. yoelii* were less capable in containing bacterial growth in lungs, spleen, and liver and resulted in increased mortality of mice (Scott et al., 2004). Also, increased *M. tuberculosis* burden were observed in lungs of mice co-infected with *P. berghei* (Mueller et al., 2014) or *P. yoelii* (Blank et al., 2016). While this co-existence exacerbates disease by *M. tuberculosis*, it is antagonistic to *Plasmodium*-induced illness. Interestingly, heat shock protein 70 (HSP70) from *M. tuberculosis* has been associated with the induction of a strong humoral and cellular response directed against *P. falciparum* (Page et al., 2005). During malaria, a marked increase in the production of the anti-inflammatory cytokines IL-10 and IL-4 (Chukwuanukwu et al., 2017) occurs and is thought to exacerbate TB pathology by reducing the protective Th1 bias and tilting immunity towards Th2 response. Additionally, co-infection with the non-lethal *P. yoelii* also resulted in more severe tuberculosis pathology with increased immune cells infiltration, and increased pro-and anti-inflammatory mediators, mainly IFN-γ, TNF-α, IL-6, IL-10, and IL-17. Moreover, higher TNF-α levels positively correlated with increased *M. tuberculosis* burden in lungs of co-infected mice (Blank et al., 2016).

### *Plasmodium* and non-typhoid salmonella

Co-infections between Non-Typhoid Salmonella (NTS) and highly pathogenic *Plasmodium* species have also been reported (Mtove et al., 2011). *Plasmodium* infection causes extensive hemolysis and release of cellular heme, which can be toxic for organisms. The protozoan converts it to hemozoin, a novel non-DNA ligand for Toll-like receptor (TLR)9, which can be captured by cells of the reticuloendothelial system (RES) and can activate innate immune responses (Simoes et al., 2015). In response, mammalian host produces heme oxygenase-1 to degrade the heme and mitigate malaria pathology by limiting production of reactive oxygen species (ROS; Gozzelino et al., 2010); however, reduced ROS has counterproductive effect due to decrease in beneficial granulocyte oxidative burst function in clearing infections, and thus allows multiplication of co-infecting *Salmonella* in neutrophils (Cunnington et al., 2012; Harding et al., 2020). Activation of TLR9 also results in the production of pro-inflammatory cytokines, certain chemokines, and causes up-regulation of costimulatory molecules (Coban et al., 2005).

### *Plasmodium* and other bacterial infections

Bacterial infections offer major complication during *Plasmodium* co-infections. During rodent *P. yoelii* infection, host immunity is impaired against diverse bacteria, including *Streptococcus pneumoniae* due to effects on innate immune response (Harding et al., 2020). A parasite-specific factor (haemozoin and bound bioactive molecules) directly contributes to *Plasmodium*-induced suppression of innate immune response

against bacteria. *P. yoelii* infections also suppresses immune responses against *Listeria monocytogenes* by causing increased apoptosis of *Listeria*-specific T cells resulting in slower induction of cellular immune responses. Interaction between different strains of *S. pneumoniae* and the rodent malaria parasite *P. chabaudi* have also been shown to promote an antagonistic sequelae (Moens et al., 2012; Fairlie-Clarke et al., 2013). Studies have also shown that complement components, C1q and C3, interact with *P. falciparum* infected RBCs to initiate the complement cascade that leads to complement depletion (Nyakoe et al., 2009), which has been attributed to increased burden of *S. pneumoniae* during *Plasmodium*-*S. pneumoniae* co-infections (Harding et al., 2020).

### *Babesia* spp. and *Borrelia burgdorferi*

*Babesia*-*B. burgdorferi* co-infections are a common occurrence due to shared reservoir host(s) and vector (Benach et al., 1985; Krause et al., 1996; Wormser et al., 2019). Studies in mice have demonstrated that when infected with *B. burgdorferi*, mice have been found to exhibit features similar to those of human Lyme disease (Barthold et al., 2010). During experimental co-infection of susceptible mice, *B. microti* infection causes splenomegaly and splenic dysfunction that results in a reduction in the levels of functional B and T cells. As a result, the production of specific antibodies against both pathogens are reduced causing poor control of *B. burgdorferi* infection (Djokic et al., 2019). Diminished adaptive immunity then exacerbates Lyme disease severity that is indicated by both; increased burden of *B. burgdorferi* in different organs of co-infected mice and more severe Lyme arthritis compared to those in mice infected with Lyme spirochetes alone (Parveen and Bhanot, 2019). These results agree with previous investigation showing that *B. burgdorferi* infection increased Lyme arthritis severity in co-infected Balb/c mice compared to singly infected mice (Moro et al., 2002). Limited human epidemiological studies have been conducted to determine outcomes of *Babesia*-*Borrelia* co-infection [reviewed (Knapp and Rice, 2015)] but overall, more diverse and persistent manifestations associated with *B. burgdorferi* infection were observed in co-infected patients (Krause et al., 1996). Thus, limited clinical studies have shown some overlapping features with those observed in mice; however, more thorough investigations are needed to fully determine the impact of co-infections on each disease severity.

### *Trypanosoma brucei* and *Brucella*

During infection with *T. brucei*, phagocytosis of the protozoan has been found to be associated with an extensive production of cytokines. Cytokines IFN-γ and TNF-α were shown to be involved in exacerbation of anemia as mice lacking the respective genes exhibited protection from anemia, while anti-inflammatory cytokine, IL-10 counteracted the effects of *Trypanosoma*-induced anemia (Tabel et al., 2008; Musaya et al., 2015). Mice infected with *T. brucei* exhibit the characteristic parasitemia waves concurrently with the host expression of elevated levels of IFN-γ. Trypanosomes overcome host innate immune response and then cause significant immunosuppression allowing proliferation of this pathogen. The induction of pro-inflammatory IFN-γ by host in response to *T. brucei* infection has been shown to reduce splenic bacteria burdens in mice infected with either *Brucella melitensis*, *B. abortus*, or *B. suis* (Machelart et al., 2017). *T. brucei*-*Brucella* co-infection is therefore antagonistic for *Brucella*. In other cases, induction of IFN-γ is not sufficient to control selected bacterial infections, for, e.g., *M. tuberculosis* (Vilaplana et al., 2014; Musaya et al., 2015).

### *Trichomonas vaginalis*, *Mycoplasma hominis*, *Atopobium* spp. and *Gardnerella* spp.

Trichomoniasis, a prevalent sexually transmitted infection (STI) is caused by the protozoan parasite *T. vaginalis*, which can establish a symbiotic relationship with *M. hominis*, a species implicated in bacterial vaginosis (Rappelli et al., 1998). *M. hominis* synergistically upregulates human monocytes pro-inflammatory response to *T. vaginalis* resulting in enhanced inflammation during trichomoniasis (Fiori et al., 2013). Due to influence of *Trichomonas* on change in vaginal pH, increase in infections in the urogenital tract also include other bacterial vaginosis associated bacteria. For example, co-existence of *Atopobium* and *Gardnerella* have also been found to cause synergistic enhancement of *T. vaginalis* induced production of chemokines (Onderdonk et al., 2016).

## Protozoa-bacteria co-infection of gastrointestinal tract

Bacterial-protozoan co-infections also affect the gastrointestinal system with significant implications to the ensuing pathology. *E. histolytica* is a pathogenic protozoan related to intestinal and extraintestinal infections. In the large intestine, it co-exists with many resident microbiotas and results in asymptomatic infection or diarrhea (Stanley, 2003). The protozoan must compete with indigenous bacteria and may breach the mucus barrier. After binding to host cells, protozoan induces cell death, which causes amebic colitis and facilitates dissemination into extraintestinal organs. The interaction between *E. histolytica* and *E. coli* O55 causes substantial changes in their genes' expression (Fernandez-Lopez et al., 2019). *E. coli* offers nutritional support for amoebic growth and helps the parasite to boost defenses against $H_2O_2$ induced oxidative stress to facilitate establishment of parasitic persistence in intestinal mucosa. As an example, oxaloacetate produced by *E. coli* protects *E. histolytica* against $H_2O_2$ induced oxidative stress while epithelial monolayers exposed to enteropathogenic bacteria are more susceptible to additional damage *by E. histolytica*. Phagocytosis of pathogenic/non-pathogenic bacteria promoted by amoebae further increased epithelial cells layer damage and exacerbated colitis severity (Galvan-Moroyoqui et al., 2008).

Murine studies have shown that *Giardia intestinalis*-enteroaggregative *E. coli* (EAEC) co-infection promotes bacterial growth impairment, microbiota-dependent delayed parasite clearance, microbial metabolic perturbations in the gut, and an alteration of localized host immune responses against EAEC

(Bartelt et al., 2017). In contrast, *G. muris* reduces the symptoms of *Citrobacter rodentium*-induced colitis, by enhancing the production of mucosal antimicrobial peptides such as mouse β-defensin 3 and Trefoil factor 3 (Manko et al., 2017).

*Helicobacter pylori* (*H. pylori*) and *Cryptosporidium* spp. are well-known for their high prevalence in immunocompromised pediatric patients worldwide especially in developing countries (Ibrahim et al., 2019). *H. pylori* may support the colonization by *Cryptosporidium* spp. and vice versa. The interaction between *H. pylori* and intestinal parasites may have serious health consequences because Cryptosporidiosis results in increased intestinal permeability while *H. pylori* causes atrophic changes in the stomach. Together they may have a serious impact on tissue integrity and the balance of gut microbiome. Further investigation is warranted to unravel how this interaction affects the gut microbiome.

## The epidemiology of protozoan parasite-viral co-infections

The interactions between viruses-protozoan co-infections and their complexities remain unexplored. These pathogens can reciprocally alter their epidemiology and/or host response to vaccines and therapies (Karp and Auwaerter, 2007). Virus-protozoan co-infections such as those caused by HIV and *Plasmodium* have been documented particularly in sub-Saharan Africa. Co-existence of these pathogens represents an emerging healthcare problem that has been causing significant morbidity and mortality, with more than 2 million deaths occurring annually (World Health Organization, 2017). Several other studies demonstrated that people living with HIV have more frequent and severe malaria manifestations (World Health Organization, 2015). HIV infected individuals are also at higher risk of exposure to leishmaniasis (Okwor and Uzonna, 2013; Diro et al., 2014) and have more efficient *T. gondii* infection with increased risks of deaths (Pott Jr. and Castelo, 2013). In addition, people with HIV have up to 21% more seroprevalences against amebiasis (Hung et al., 2012) and have significantly higher rates of trichomoniasis than HIV-negative individuals (36.4% vs. 21.3%) (Davis et al., 2016).

Other *Plasmodium*-viral co-infections are also frequently observed in sub-Saharan Africa. A study from Anastos et al., 2010 in Rwanda (East Africa) showed an association between *Plasmodium* infection and increases risk of cervical precancer in Human Papilloma Virus (HPV) infected patients (Anastos et al., 2010). In a recent study from Nigeria (West Africa), authors reported that *Plasmodium* infection coexists with Measles virus in 32.5% febrile children analyzed and they were under risk of serious consequences or even death (Aminu et al., 2021). Nigeria also has high rates of malaria-influenza co-existence among people refusing flu vaccinations. Influenza A and B were found in 54% of unvaccinated pregnant women having *Plasmodium* parasitemia (Anjorin and Nwammadu, 2020). *Plasmodium* spp. and hepatitis B Virus (HBV) infections are prone to co-exist in individuals living in the same regions. In a systematic review and meta-analysis conducted, 22 studies were analyzed and showed that overall co-infection prevalence between *Plasmodium* spp. and HBV is 6% worldwide with the highest prevalence rate (10%) in Gambia (Kotepui and Kotepui, 2020). Strong positive association was also found between seropositivity for *Plasmodium* and Ebola virus in residents from Gabon, Central Africa with co-infection prevalence of 10.2% (Abbate et al., 2020).

*Cryptosporidium* is one of the most important parasitic diarrheal agents affecting children in the developing countries (Tamomh et al., 2021). *C. hominis*, *C. parvum* and *C. meleagridis* have been implicated in diarrhoea. This protozoan has also emerged as a global opportunistic threat causing severe diarrhea (Ahmadpour et al., 2020). *Cryptosporidium* infection is common among HIV/AIDS patients (prevalence of 8,69%) worsening the protozoan infection associated symptoms causing severe diarrhea and eventually death because of low CD4+ T-cells counts (Wang et al., 2013; Fregonesi et al., 2015; Hailu et al., 2015; Yang et al., 2017; Wang et al., 2018). As a consequence of such co-infection and severe disease (Fregonesi et al., 2015; Alemu et al., 2018) parasitemia as high as 90% was observed (Ojurongbe et al., 2011).

Due to more recent emergence of infection by severe acute respiratory syndrome coronavirus 2 (SAR-CoV2) in humans, co-infections with different protozoan are not fully explored yet. Moreover, most of the available reports about SARS-CoV2 co-infections describe concomitant bacteria, fungus and other viral infections, especially associated with respiratory infections and pneumonia. In depth studies have not been conducted for SARS-CoV2 and protozoan co-infections; however a few reports are available showing co-infections occur with *Toxoplasma* (Montazeri et al., 2022), *Plasmodium* (Raham, 2021; Boonyarangka et al., 2022), *Babesia* (Jacobs and Siddon, 2021), *Leishmania* (Pikoulas et al., 2022) and *Trypanosoma* (Alberca et al., 2020). In the first year of the COVID-19 pandemic, worldwide malaria cases increased from 227 million in 2019 to 241 million in 2020 (World Health Organization, 2021) and a high rate of latent *T. gondii* infection was also found among COVID-19 patients with severe manifestations reported in the Middle East region (Montazeri et al., 2022). Therefore, more research is needed to fully understand the impact of SARS-CoV-2 infections on co-infecting protozoa, and vice versa.

## The pathogenesis of protozoan-viral co-infections

### *Plasmodium* and HIV

Due to the geographical overlap between *Plasmodium* and HIV, both pathogens can often infect humans with synergistic and adverse impact (Alemu et al., 2013). HIV infection increases the *Plasmodium* burden in patients, facilitating the increase in protozoan transmission. Conversely, infection with *Plasmodium* results in increase in number and activation state of CD4 + T cells,

creating an ideal environment for HIV replication, increasing viremia. Other mechanisms of *Plasmodium*-infection induced HIV replication include the secretion of TNF-α that can act directly stimulate HIV replication (Ayouba et al., 2008). Additionally, infection with *Plasmodium* causes pro-inflammatory (T helper 1- type) immune response with activation of CD4+, CD4 + 5RO + T cells. These T cells are preferred target for HIV replication (Spina et al., 1997). CD14+ macrophages activated during acute malaria are also a source of migratory reservoirs of HIV-1 facilitating dissemination of virus to lymphocytes during cell–cell interactions promoting disease dissemination (Pantaleo and Koup, 2004). *P. falciparum* has also been shown to stimulate HIV-1 replication through the production of cytokines (IL-6 and TNF-α) that activate lymphocytes (Inion et al., 2003). Other studies have shown that exposure to soluble *Plasmodium* antigens and hemozoin induced HIV replication or reactivation *via* CD4 T-cell stimulation together with the production of pro-inflammatory cytokines, for, e.g., IL-1β, IL-6, and TNF-α (Froebel et al., 2004).

## Co-infections with SARS-CoV2

It has been hypothesized that malaria may reduce the COVID-19 severity in endemic regions of sub-Saharan Africa (Gutman et al., 2020; Ssebambulidde et al., 2020; Osei et al., 2022). An inverse correlation between the incidence of COVID-19 and malaria with less probability of COVID-19 cases was found in malaria-endemic countries (Ssebambulidde et al., 2020). One possible explanation for this phenomenon is that malaria patients generate anti-GPI antibodies which eventually identify SARS-CoV-2 glycoproteins developing a protective response against COVID-19 improving the disease prognostic (Hussein et al., 2020). Conflicting results from a Malian longitudinal cohort study showed no association between malaria and COVID-19 seroconversion or effect on the symptoms reported for COVID-19 (Woodford et al., 2022). The identification of immunomodulatory effects provoked by malaria and helminth infections could lead us to better understanding of the factors involved in improvement of vaccine efficacy. It still remains unclear how efficacy of COVID-19 vaccines is affected by these parasites.

There are several reports showing association between Neglected Infectious Diseases (NTDs) and SARS-CoV2, in terms of how they affect the severity of COVID-19 clinical outcomes, vice versa and the development of trained immunity as occurs for helminth infections and malaria (Ssebambulidde et al., 2020; Anyanwu, 2021; Gluchowska et al., 2021; Wilairatana et al., 2021; Achan et al., 2022; Hussein et al., 2022). Our review of literature indicate that the immunomodulatory effects of COVID-19 and parasitic co-infections brought insights not by direct investigations but based upon lessons learned from other co-infections systems (Fonte et al., 2020; Gluchowska et al., 2021; Akelew et al., 2022; Woodford et al., 2022). Briefly, helminth co-infection was suggested to cause immunomodulation in COVID-19 patients to result in reduction of disease severity (Bradbury et al., 2020). This immunomodulation could be due parasite specific innate response and Th2 immune response with CD4+ T cells, eosinophils, and production of IL-4, IL-5, and IL-10, thereby reducing hyperinflammation in patients with severe COVID-19 (Rodriguez, 2020; Akelew et al., 2022). Reinforcing these observations, a recent study showed that patients co-infected with SARS-CoV2 and helminths had less severe COVID-19 due to reduced hyper-inflammation response (Wolday et al., 2021). In fact, an inverse correlation between COVID-19 existence and severity was observed in countries endemic for soil-transmitted helminths (Ssebambulidde et al., 2020).

## *Plasmodium* and SARS-CoV-2

*Plasmodium* and SARS-CoV2 co-infections have been reported to occur across the endemic and non-endemic regions (Junaedi et al., 2020). Despite the rising incidence of COVID-19 disease in the world, an unremarkably lower prevalence has been observed in malaria endemic regions (Osei et al., 2022) suggesting that *Plasmodium* presence may offer some protection against SARS-CoV-2 infection. SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) receptor to enter the host cells. However, a D-allele variant of ACEI/D polymorph has been described in a mild form of malaria. This ACE I/D polymorphism occurs in intron 16 reduces ACE2 expression. Reduced expression of ACE2 receptor in populations with this polymorphism may play a protective role against severe COVID-19. An increase of its substrate Ang II in plasma of these individuals have been demonstrated in people with African genetic background (Delanghe et al., 2020).

At the height of the COVID-19 outbreaks, relatively low prevalence rates were observed in areas known to have high malaria endemicity, prompting some interest on the role of malaria immunity in protecting COVID-19 infections (Vilaplana et al., 2014). Infections with *Plasmodium* induces both innate and adaptive immunity. Recent studies have shown that in addition to inducing adaptive B and T cells memory response, innate immune response to *Plasmodium* infection may also induce memory, a phenomenon known as trained immunity, which is capable of mounting a faster and more robust recall response and may provide cross protections against unrelated pathogens (Netea et al., 2011, 2020). Cross protection induced by trained immunity is a widely acknowledged phenomena and has been demonstrated by BCG vaccinations against *M. tuberculosis* because it provides cross protection against unrelated pathogens (Sohrabi et al., 2020). The major factors involved in innate immune response to malaria include natural killer (NK) cells, monocytes, macrophages, and pro- and anti-inflammatory cytokines (Hansen et al., 2007; Doolan et al., 2009). These responses can develop nonspecific trained immunity that can be effective against other pathogens like SARS-CoV-2, producing a faster and more effective protective response (Raham, 2021). Trained immunity against *Plasmodium* could also produce tolerance that tapers down the inflammatory response from innate immune cells, such as monocytes (Boutlis et al., 2006). Tolerance has the beneficial effect of reducing the harmful effect of excessive infection and disease (Nahrendorf

et al., 2021), and cross-protection could occur by reduction of the inflammatory progression to unrelated disease, including SARS-CoV2. Such a response may explain the reduced COVID-19 severity in malaria community (Guha et al., 2020). Further research is needed to examine these concepts as relevant to COVID-19 in malaria endemic areas. For example, the innate immune factors (NK cells, Type 1 IFN, IgG) need to be evaluated in COVID-19 asymptomatic and symptomatic patients in malaria endemic areas.

## *Plasmodium* and other viral co-infections

Co-infections by *Plasmodium* and hepatitis B and C (HBV and HCV) viruses often occur due to shared needles and blood transfusions etc. (World Health Statistics, 2022). A high incidence rate of malaria and hepatitis infection in the sub-Saharan population has been reported (Helegbe et al., 2018; Sevede et al., 2019). Co-infections with *Plasmodium* and hepatitis often results in changes in burdens of both pathogens such that individuals co-infected with *Plasmodium* spp. and HBV display lower parasitemia and higher viremia (Andrade et al., 2011). Both of these pathogens also have an antagonistic effect on anemia, while *P. falciparum* causes hemolytic anemia, HBV increases hemoglobin levels by releasing erythropoietin from regenerating hepatic tissues (Simon et al., 1982; Klassen and Spivak, 1990; Ifudu and Fowler, 2001). Conversely, during chronic HBV infection, cytokines released in response to *P. falciparum* infection could further activate the apoptosis of HBV-infected hepatocytes, and exacerbated liver damage as evidenced by the increase in pro-inflammatory cytokines, TNF-α, IL-1β, and IL-6 that are increased during *Plasmodium* and chronic HBV infection and reduction in anti-inflammatory cytokines IL-10 and IL-4 in pregnant women (Brown et al., 1992; Azizieh et al., 2018).

Historically, Ebola outbreaks have occurred in Western Africa, and it overlaps with malaria prevalence. Studies have shown that *P. falciparum* infection prior to infection with Ebola virus could induce an antiviral activity and a protective role against Ebola. Acute *Plasmodium* infection has been shown to promote IFN-γ-dependent resistance to Ebola virus infection (Rogers et al., 2020).

Outbreaks of measles have been reported in malaria endemic areas of sub-Saharan Africa generating some interest on the impact of one pathogen over the other albeit studies to-date are limited. One study has shown significantly lower parasitic prevalence and mean densities of malaria parasites were found in children up to 9 years of age who had measles or influenza than in asymptomatic control children (Rooth and Bjorkman, 1992).

## *Cryptosporidium* and HIV

Pathogens belonging genus *Cryptosporidium* are transmitted by fecal-oral route causing gastrointestinal infection in various vertebrate species, including humans (Xiao et al., 2004; Wang et al., 2011) and has been associated to chronic to life-threatening diarrhea in immunocompromised individuals (Conner et al., 2019). Both innate and adaptive immune responses play a role in protection from cryptosporidiosis and resolution of infection; however, cell-mediated immunity is crucial for clearance of cryptosporidiosis (Borad and Ward, 2010). HIV/AIDS patients with lower CD4 counts are more susceptible to cryptosporidiosis and have greater severity of disease (Hunter and Nichols, 2002). In HIV/AIDS patients with active cryptosporidiosis, infected epithelial cells express high levels of the chemokine, CXCL10, and expression levels correlate with the parasite burden (Wang et al., 2007). Since CXCL10 increases the rate of HIV replication *in vitro*, elevated CXCL10 in cryptosporidiosis may contribute to enhanced destruction of CD4+ T cells due to HIV infection (Ahmadpour et al., 2020). Humoral immune responses have also been reported to play an important role in protection against Cryptosporidiosis since studies have suggested that that antibody responses to specific antigens were associated with protection from diarrhea in *Cryptosporidium*-infected HIV/AIDS patients (Ahmadpour et al., 2020). Specific serum IgG, IgM, and IgA production were evaluated in *Cryptosporidium*-HIV co-infection showing no difference among patients with or without diarrhea (Kaushik et al., 2009). The occurrence of diarrhea in HIV-positive individuals was not always observed during *Cryptosporidium* co-infections probably because antiretroviral therapy improved the immune system functionality (Irisarri-Gutierrez et al., 2017). Conversely, *Cryptosporidium* has been shown to stimulate periductal inflammation in the biliary tree, induces biliary epithelial cell apoptosis, and thus could contribute to the pathogenesis of AIDS-cholangiopathy (Chen and LaRusso, 2002).

## *Toxoplasma* and HIV

*Toxoplasma gondii*, a coccidian protozoan obligate intracellular parasite, is the causative agent of toxoplasmosis which affects approximately 30% population worldwide. *T. gondii* infection results in malformation and life-threatening disease in developing fetuses and is one of the most prevalent causative agents of opportunistic infections in HIV/AIDS patients causing central nervous system toxoplasmosis (Montoya and Liesenfeld, 2004; Ayoade and Joel Chandranesan, 2022). According to a systematic review, toxoplasmosis co-infection prevalence in HIV patients varies greatly among different countries showing the highest numbers in Thailand (53.7%), North Sudan (75.0), Ethiopia (87.4%), Brazil (80.0%) and Iran (96.3%). These authors also observed that high prevalence of *T. gondii*-HIV co-infections in low-income countries (Wang et al., 2017). It has been shown that *T. gondii*-HIV co-infections lead to toxoplasmosis severity and increase mortality in patients who developed AIDS and were not properly treated (Da Cunha et al., 1994; Pott and Castelo, 2013; Agrawal et al., 2014). During HIV infection, depletion of CD4 cells, decreased production of cytokines and IFNγ, and impaired cytotoxic T-lymphocyte activity result in reactivation of latent *Toxoplasma* infection (Basavaraju, 2016), and is associated with persistence of IgG antibodies against *T gondii* (Robert-Gangneux and Darde, 2012). Additionally, low CD4 T lymphocyte count was associated with high frequency of encephalitis caused by toxoplasmosis in patients who developed AIDS (Lejeune et al., 2011). On the other hand, *T. gondii*

co-infection also alters the immune response, clinical manifestation and transmission of the HIV infection (Welker et al., 1993; Bala et al., 1994). In individuals singly infected with HIV, for example, both numbers of plasmacytoid dendritic cells and IFN-α production are impaired (Feldman et al., 2001), which can be exacerbated during opportunistic *Toxoplasma* co-infection. Additionally, the exposure to *T. gondii* has been shown to potentiate CD4 positive T-cells and possibly monocytes to be more permissive for HIV replication (Subauste et al., 2004), suggesting that HIV/*T. gondii* co-infected individuals potentially exhibit more severe diseases.

### Other protozoan-viral co-infections

Preliminary studies have shown that toxoplasmosis is a risk factor for acquiring SARS-CoV-2 infection and severe manifestations of COVID-19 (Fiori et al., 2013). *T. gondii* induces the shedding of mitochondrial outer membrane to promote its own growth. Intriguingly, the hijacking of host mitochondria has been shown to play a critical role in the pathogenesis of COVID-19 (Lo et al., 2014).

The relationship between HIV-1 infection and amoebiasis (Lowther et al., 2000; Hung et al., 2005, 2008) was demonstrated by the observation that HIV-infected men having sex with men (MSM) were at significantly higher risk of amebiasis than patients from other risk groups (Hung et al., 2008). One study has identified Amebic Liver Abscess (ALA) as the most common extraintestinal manifestation of invasive infection as an important condition in HIV-1-infected individuals and has been attributed to the ability of HIV-1 to suppress activity of regulatory T cells. This in turn suppresses *E. histolytica*-specific T-cell reactivity and cause increased susceptibility to invasive amebiasis in persons with early stage of HIV-1 infection (Hsieh et al., 2007).

*Trichomoniasis* is a highly prevalent STI among HIV-1-infected patients (Cu-Uvin et al., 2002). Previous investigation has demonstrated that *T. vaginalis* infection enhances HIV-1 transmission (Schwebke, 2005). Proposed mechanisms by which *T. vaginalis* infection may increase HIV-1 infection include: induction of inflammatory response in vaginal, exocervix, and urethral epithelia; disruption of mucosal barrier function; recruitment of CD4 lymphocytes and macrophages; development of microhemorrhages; degradation of secretory leukocyte protease inhibitors; and enhancement of susceptibility to bacterial vaginosis or other abnormal vaginal flora that all may increase the risk of HIV-1 acquisition (Sorvillo et al., 2001).

Rotavirus often contribute to the *Cryptosporidium* co-infection in farm animals and humans (Izzo et al., 2011; Mokomane et al., 2018; Praharaj et al., 2019). Differences in clinical manifestation between lambs infected with *Cryptosporidium* alone or together with rotavirus have been detected; however, some reports showed no differences during these two situations. More research is needed to reveal the influence of simultaneous occurrence of different pathogens, including *C. parvum*, which may either facilitate or antagonize concurrent infections. Some co-infections with *Cryptosporidium* species may not exert any response but needs to be investigated thoroughly.

## The impact of protozoan-bacterial co-infections on chemotherapeutic interventions

The emergence of co-infections could have a modulating effect the on the success or failure of chemotherapy by playing a role in the emergence of antibiotic resistant strains (Birger et al., 2015). Co-infecting pathogens are often misdiagnosed due to overlapping symptomatology, delay in treatment to allow excessive proliferation of the microbe(s) that can render the host immune response insufficient to clear infection. Increase burden can facilitate development of drug resistance in one or both pathogens (U.S. Department of Health and Human Services, 2018). Targeted treatment against one pathogen may also remove a competitor and could lead to active growth of the remaining pathogen increasing the probability of evolution of drug resistant variants as seen in malaria and TB (Colombatti et al., 2011). During synergistic infections, reduced immune system-mediated killing of pathogens may allow their replication, increasing the possibility of the emergence of *de novo* resistance.

Rifampicin is an antitubercular drug that also exhibits potent anti-malarial activity against *P. vivax* in humans (Pukrittayakamee et al., 1994). Continual usage of rifampicin against TB may have resulted in discontinuing its use against malaria parasite since the therapeutic doses for the two pathogens are different. Impaired immunological control of tuberculosis due to the presence of the co-infecting *Plasmodium* spp. may also increase the danger of a recrudescence of partially resistant pathogen populations after therapy has ended (Okeke, 2003). An indirect effect of antimicrobials to bioavailability of drugs, can be due to a physical hindrance provided by one pathogen, such as by forming a biofilm, leading to suboptimal drug concentration at the colonization site. The presence of co-infection may also increase the abundance of antibiotic-target pathogen, and thus aiding the focal infection.

Development of antimicrobials targeting common metabolic pathways of co-infecting pathogens is a promising field of research that could limit the establishment of multidrug resistance. For example, improvement in design of sulfonamide drugs to target the *de novo* folate synthesis pathway, which is used by both *Plasmodium* spp. and *M. tuberculosis* is an attractive idea for consideration in drug design. In fact, sulfonamide class of antibiotics, initially developed as antibacterial agents, have been central in the development of antifolate-based combinational drugs against malaria. Co-trimoxazole as an antibacterial prophylactic agent can also prevent the incidence of malaria. The long safety history of co-trimoxazole when used in pregnancy (to treat bacterial infections) and its antimalarial prophylactic properties have led to the evaluation of this combination to also prevent malaria during pregnancy.

## The impact of protozoan infections on the efficacy of bacterial/viral vaccines

Vaccines, like infections, involve participation of the innate and adaptive immune system which encompasses, phagocytosis, cytokine/chemokine secretion and activation of the antigen-specific adaptive immune response with subsequent immunological memory development. An effective adaptive immunity development involves activation of specific subsets of T lymphocytes, and stimulation of B lymphocytes to differentiate into antibody-secreting plasma cells followed by creation of protective immunological memory (Vetter et al., 2018). The critical starting point in the development of an effective vaccine requires identification of potent antigens that are appropriately presentable by professional antigen-presenting cells. Changes in the target antigens can impair development of an effective immune response. These changes may result from genetic variations arising as a consequence of co-infections resulting in failure of recognition by the adaptive immune response and occurrence of breakthrough infections (Bretsche et al., 2001). A previous meta-analysis study showed that parasitic infections such as those caused by helminths, protozoa and viruses at the time of vaccination were associated with worse immunological responses, tending to overcome infection less efficiently after post vaccination challenge. Multiple factors determine how parasitic infections impact the outcome of immunizations. These include: the type of parasite involved, immune response induced, vaccine formulation, route of administration, the target antigen, vaccine type (e.g., live attenuated, inactivated organism), study design and the timing of infection relative to vaccination (Wait et al., 2020).

The association between parasitic infections and impaired immune responses to vaccine antigens has been demonstrated for a diverse group of pathogens [(Wait et al., 2020) and Table 1]. For example, immune response induced by vaccines against *Haemophilus influenzae* and diphtheria (Malhotra et al., 2015), Bacille Calmette-Guerin (BCG) and tetanus toxoid (McGregor, 1962; Greenwood et al., 1972; Elliott et al., 2010; Alvarez-Larrotta et al., 2019), *S. typhi* (Williamson and Greenwood, 1978), acellular diphtheria-tetanus and pertussis vaccine (DTPa; Radwanska et al., 2008), HIV (Robinson et al., 2004), and potentially against SARS-CoV-2 too could be affected (Fonte et al., 2020; Gluchowska et al., 2021; Akelew et al., 2022). The impact of helminths on different vaccines outcomes has also been reported previously against pneumococcus (Apiwattanakul et al., 2014), BCG (Elias et al., 2008) and HIV-1C (Da'dara and Harn, 2010). Additionally, reduced vaccine efficacy has been associated with chronic helminth infections when compared to acute infections (Wait et al., 2020). In this review, we have focused on the impact of protozoan infections on the efficacy of vaccines against bacterial and viral infections.

### Protozoan parasites and vaccines against bacterial pathogens

Immunosuppression by protozoan pathogens could interfere with the immune response generated by vaccines, creating a negative correlation between parasitic infections and efficacy of vaccine in protection. Conversely, there is also evidence that vaccines may induce trained immunity and non-specific response against protozoa (Welsh and Selin, 2002; Selin et al., 2006; Agrawal, 2019) including those causing malaria and babesiosis (Clark et al., 1976, 1977; Garly et al., 2003; Walk et al., 2019). In fact, BCG vaccination, which can enhance non-specific protection to unrelated infections especially by activation of NK cells with non-specific memory by production of pro-inflammatory cytokines is provides an example (Kleinnijenhuis et al., 2014); however, parasitic infections often lead to lower antibody and IFN-γ levels, which represent a decrease in the quality of the humoral and cellular immune responses (Rowe et al., 2000).

Malaria is a highly prevalent disease in settings where poor responses to unrelated vaccines has been reported to occur (Natukunda, 2020). Several studies have shown that *Plasmodium* infection might impair vaccine induced protective immunity against other pathogens (Dietz et al., 1997; Cunnington and Riley, 2010). Diminished vaccines efficacy has been attributed to different factors, such as human and bacterial genotypes, exposure to environmental microbes, climate and geographical location and prevalence of co-infections (Tangie et al., 2022). A decreased protection against murine typhoid in *P. yoelii*-infected mice vaccinated with *S. typhimurium* antigens correlated with suppression of CD4 and CD8 T cell effector responses and increased anti-inflammatory IL-10 cytokine production (Mooney et al., 2015). *Plasmodium* spp. induced immunosuppression could be an important factor responsible for weak response to routine immunizations in malaria-endemic communities (McGregor, 1962; Gilles et al., 1983). For example, in a study in south coast of Kenya showed an impaired IgG antibody response to *H. influenza* b and diphtheria among infants of mothers infected with malaria and/or helminths during pregnancy compared to infants of uninfected mothers. The authors discussed that transplacental exposure of the fetus to parasite antigens in prenatal maternal malaria or helminth infections could lead an *in-utero* alteration of fetal immune responses affecting the response to vaccines (Malhotra et al., 2015). These observations were also confirmed in another study where Gambian children with malaria also had low antibodies titers to a *H. influenza* b conjugate vaccines than healthy controls, suggesting that the child cytokine profile at the time of antigen presentation likely modifies the immune response (Usen et al., 2000). Immune responses to both *Salmonella typhi* and meningococcal vaccines were also evaluated in Nigerian children infected with *Plasmodium*. Thus, *S. typhi* vaccination given on the first day of malaria infection had the immune responsiveness depressed, which rapidly recovered following malaria treatment while immune response to meningococcal vaccine was still impaired after a month of infection (Williamson and Greenwood, 1978). In contrast to all these findings, malaria and some helminth diseases during pregnancy

TABLE 1 The most common protozoa co-infections and its effects on bacterial/viral vaccines efficacy.

| Vaccine | Protozoan infection | Immunological response against vaccine | Effect on vaccine efficacy | References |
|---|---|---|---|---|
| BCG | *Plasmodium* spp. | B-cell depletion, loss of central memory CD4+ T cells | Vaccine efficacy not significantly altered | Tangie et al. (2022) |
| Different TB vaccines | *P. yoelii* | CD4 and CD8 T cells suppressed in mice at 2 weeks | *M. tuberculosis* CFU levels not affected | Parra et al. (2011) |
| Tetanus toxoid | *P. vivax* and *P. falciparum* | Lower expression of cytotoxic T lymphocyte antigen 4 and anti-toxoid IgG levels | Tetanus cases not evaluated | Alvarez-Larrotta et al. (2019) |
| Tetanus toxoid | *Plasmodium* spp. | Lower antibody response to tetanus toxoid in children with malaria | Tetanus cases not evaluated | McGregor (1962) |
| Tetanus toxoid and *Salmonella typhi* | *Plasmodium* spp. | Diminished antibody response to tetanus toxoid and *S. typhi* O antigen in children with acute malaria | Tetanus or *S. Typhi* cases not evaluated | Greenwood et al. (1972) |
| *S. typhi* and meningococcal vaccines | *Plasmodium* spp. | Antibody levels to both vaccines was significantly reduced when the vaccines were given on the first day of illness | Meningococcal disease or typhoid cases not evaluated | Williamson and Greenwood (1978) |
| *H. influenzae b* and diphtheria toxoid | *P. falciparum* | Impaired IgG antibody responses to *H. influenza* b and diphtheria among infants of mothers infected with malaria and/or helminths during pregnancy | *H. influenzae b* and diphtheria cases not evaluated | Malhotra et al. (2015) |
| *H. influenzae b* | *Plasmodium* spp. | 11% of infected children with malaria did not have protective titers | *H. influenzae b* cases not evaluated | Usen et al. (2000) |
| *S. pneumoniae* and diphtheria CRM197 antigens | Malaria and some helminth diseases | Higher anti-vaccine antibody levels against *S. pneumoniae* and diphtheria CRM197 antigens during pregnancy | Throat infection, pneumonia and diphtheria cases not evaluated | McKittrick et al. (2019) |
| NTS serovars | *P. yoelii* | Marked reduction of *Salmonella*-specific CD4 and CD8 T cells immunity in mice | Reduced protection with 264-fold (liver), and 31-fold (spleen) increase in bacterial burden | Mooney et al. (2015) |
| *Brucella abortus* vaccine | *T. congolense* | Vaccinated cattle had depressed IgG1 and IgG2 levels by 80%, IgM by 90%. | Cattle not challenged with B. abortus | Rurangirwa et al. (1983) |
| *Bacillus anthracis* spore vaccine | *T. congolense* | The anti-anthrax antibody levels were severely depressed in infected goats | Goats not challenged with *B. anthracis* | Mwangi et al. (1990) |
| DTPa vaccine | *T. brucei* | Antibody response not determined against vaccine | Protection by DTPa vaccine eliminated | Radwanska et al. (2008) |
| HIV DNA vaccine | *L. major* | Significant reduction in IFN-γ-production by CD8+ T cells of Balb/c mice after *in vitro* stimulation with gag antigen | HIV vaccine efficacy not determined | Robinson et al. (2004) |
| HPV-16/18 vaccine | *Plasmodium* spp. + helminths | Association of malaria parasitemia in young girls with a higher level of anti-HPV-16/18 antibodies | HPV cases not evaluated | Brown et al. (1992) |
| Classical swine fever (CSF) vaccine | *T. evansi* | Antibody responses against CSF vaccine significantly reduced in pigs | More vaccinated animals had fever and leucopenia | Holland et al. (2003) |
| Foot-and-mouth disease vaccine | *T. congolense* | *T. congolense* infected cattle had antibody titers significantly depressed after secondary vaccination | After viral challenge, difference in protection was not significant in *T. congolense* infected group | Sharpe et al. (1982) |
| Influenza A Virus (IAV) Vaccine | *P. yoelii* | Plasma cell apoptosis and circulating BAFF increased, circulating IAV specific antibodies diminished | Higher virus load in challenged *P. yoelii* infected mice | Banga et al. (2015) |
| Tetanus, measles and hepatitis vaccines | *P. falciparum* | Declines in IgG specific for tetanus, measles and hepatitis B in 53, 19 and 33% of children, respectively | Incidence of infections not evaluated | Banga et al. (2015) |
| Measles vaccine | *P. falciparum* | Antibody titers were significantly higher in the vaccinated children positive for *P. falciparum* | Measles cases not evaluated | Smedman et al. (1986) |

*(Continued)*



10.3389/fmicb.2022.1020029

TABLE 1 (Continued)

| Vaccine | Protozoan infection | Immunological response against vaccine | Effect on vaccine efficacy | References |
|---|---|---|---|---|
| Rotavirus vaccine | *Cryptosporidium* spp. etc | Immune response not evaluated | Decrease of 11.3% in vaccine efficacy in co-infections. No statistically difference was found | Praharaj et al. (2019) |
| Rotavirus vaccine | *Cryptosporidium* spp., *Giardia* spp. etc | Immune response not evaluated | Protection by the vaccine was 59% in the rotavirus co-infection group and 51% in the rotavirus mono-infection subgroup, difference not statistically significant | Mokomane et al. (2018) |

were associated with minor, mostly enhancing effects on infant anti-vaccine antibody levels against *S. pneumoniae* and diphtheria CRM197 antigens (McKittrick et al., 2019).

Submicroscopic infection by *Plasmodium* spp. has been shown to be associated with a decrease in the levels of IgG against tetanus toxoid (McGregor, 1962; Greenwood et al., 1972; Alvarez-Larrotta et al., 2019), however, tetanus and diphtheria toxoids continue to offer protection in these individuals suggesting that functional immunity was sufficiently protective. Chronic protozoan infections can result in persistence of stimulating antigen that could lead to exhausted T cells with less robust effector functions, and alterations of the differentiation and sustenance of memory T cells (Costa-Madeira et al., 2022). Up-regulation and co-expression of multiple inhibitory receptors and failure to produce antigen-independent memory T cells are also observed in these cases (Schietinger and Greenberg, 2014).

Some studies showed that pre-existing *Plasmodium* infections did not affect the efficacy of vaccines against bacterial or viral pathogens, for instance, *P. yoelii* infection did not affect different formulations of TB vaccine ability to control pulmonary growth of an acute virulent *M. tuberculosis* infection (Parra et al., 2011). The immunological response to two doses of tetanus toxoid in groups of pregnant Kenyan women showed that the presence of *Plasmodium* parasitemia does not interfere with primary or secondary immune response during chemoprophylaxis against malaria (Dietz et al., 1997). Moreover, two doses of tetanus toxoid in 2-year-old Gambian children also showed that chloroquine or pyrimethamine chemoprophylaxis lead to more protective immune response when compared with children who were not treated for malaria (McGregor, 1962).

African trypanosomiasis caused by *T. brucei* infection inhibits protective immune responses against bacterium, *Bordetella pertussis* when immunized with a trivalent human vaccine, DTPa in mice (Radwanska et al., 2008). Other species of *Trypanosoma* can also prejudice the protective immune responses to bacterial or viral infections in animals. In an earlier study, cattle previously infected by *Trypanosoma congolense* when vaccinated against *Brucella abortus* had 80% reduction in IgG1 and IgG2 immunoglobulins (Rurangirwa et al., 1983). Furthermore, anti-anthrax antibody levels were severely depressed in *T. congolense* infected goats after immunization with *Bacillus anthracis* inactivated spore vaccine (Mwangi et al., 1990). Reduced antibody responses in infected pigs were also suggested to occur due to suppression of helper T cell caused by the concurrent *T. evansi* infection. In both mice and cattle, T cell proliferation was inhibited upon mitogenic stimulation and was mediated by macrophage-like suppressor cells, with reduction in IL-2 secretion together with impaired expression of the IL-2 receptor (Sileghem et al., 1989; Sileghem and Flynn, 1992). *T. brucei* infection also results in a rapid loss of B cells by apoptosis, reducing humoral immunity that further prevents the development of protective memory responses and thus, impair

the ability of the host to recall vaccine-induced memory responses (Radwanska et al., 2008).

### Protozoan parasites and vaccines against viral pathogens

Studies in mice suggested that *Plasmodium* infection is deleterious to pre-existing levels of heterologous antibodies. Specifically, *P. chabaudi* blood-stage infection of influenza-immune inbred and outbred mice resulted in a transient drop in influenza-specific antibodies and antibody secreting cells in the bone marrow (Ng et al., 2014). These results were confirmed by another study in which Malian children with pre-existing tetanus, measles, HBV vaccination had an accelerated decline in vaccine-specific IgG after acute malaria episodes (Banga et al., 2015) due to binding of *Plasmodium*-infected erythrocytes to bone marrow stromal cells that may disrupt the survival signals of long-lived plasma cells (Rogers et al., 2000; Kinyanjui et al., 2007; Banga et al., 2015). Additionally, BAFF receptor, which promotes survival of antibody secreting plasma cells, was found to be downregulated in splenic and bone marrow plasma cells, while circulating BAFF levels and apoptotic plasma cells increased in the bone marrow of *Plasmodium*-infected mice. These results were confirmed by the observation that *Plasmodium*-induced polyclonal B cell activation and elevated levels of immunoglobulins results in apoptosis of long-lived plasma cells through a CD32-dependent mechanism [reported in (Banga et al., 2015)]. Surprisingly, modest positive effects of pre-existing *Plasmodium* and helminths infections on vaccines efficacy of HPV vaccine was observed among young girls displaying *Plasmodium* parasitemia (Brown et al., 2014). Additionally, post-immunization measles antibody titers were significantly higher in the vaccinated children positive for *P. falciparum* infection than those without malaria parasites in the blood (Smedman et al., 1986).

*Leishmania major* is another protozoan that affects the viral vaccines-induced immune response. In BALB/c mice, infection with *L. major* reduced the CD8+ T cell-specific immune response induced by HIV-1 DNA vaccine suggesting that Th2 cell response caused by *L. major* infection can negatively affect vaccine efficacy (Robinson et al., 2004). Limited studies have been conducted to indicate contribution of Trypanosomes on viral vaccines. Foot-and-mouth disease virus vaccine was evaluated in cattle infected with *T. congolense* and despite significant depression in antibodies titers, their subsequent response to live virus challenge was not significantly different from the uninfected controls suggesting persistence of functional immunity (Sharpe et al., 1982). Antibody responses against classical swine fever virus vaccine were significantly decreased in *T. evansi*-infected pigs as compared to uninfected animals (Holland et al., 2003). Thus, *Trypanosoma* affects antibody responses against viral vaccine antigens but may or may not affect protection offered by the vaccines.

The Rotavirus vaccine efficacy was evaluated in diarrhea-associated co-infections in India, including those caused by *Cryptosporidium*. Vaccine efficacy decreased from 49.6 to 60.6% in the presence of co-infections (Praharaj et al., 2019). In another study, enteric co-infections including those by *Cryptosporidium and Giardia* did not affect the effectiveness of rotavirus vaccine. Therefore, lower vaccine effectiveness reported in low-income countries could not be explained only because of co-infection with *Cryptosporidium* (Mokomane et al., 2018).

Overall, it appears that the effect of protozoan infections on antibodies protection against vaccine antigens of different viruses is variable but the correlation of change in antibody levels with the failure or success of the vaccines in protection against the specific viral infection remains to be investigated more extensively.

## Concluding remarks

Mammalian hosts encounter protozoan and other pathogens that are either transmitted consecutively or simultaneously. The impact of immunosuppression by protozoan pathogens often increases the co-infecting bacterial burden in the affected organs, thus increasing the severity of diseases they cause. It would not be surprising if chronic protozoan infection and the resulting sustained immunosuppression can activate latent infection, such as by *M. tuberculosis* in malaria endemic regions. Therefore, efforts to manage, treat bacterial and protozoan infections or develop novel vaccines need to consider the presence of co-infections because that could have a dramatic influence on host susceptibility to disease and the design of treatment approaches. We document variable effects of protozoan infections on bacterial and viral vaccines with significant effects on reduction in efficacy of bacterial vaccines. Some viral vaccine response remains unaltered by the presence of infecting protozoan, while antibody titer is either increased or decreased in other cases albeit their impact on protection from infection remains unclear. Therefore, booster doses of vaccines may be needed when breakthrough infections start appearing in a particular geographic region, such as tetanus toxoid booster is often required under specific circumstances. In fact, vaccines are often recommended to protect from recurrence of viral diseases such as shingles in otherwise healthy and middle-aged adults who were infected with chickenpox as children. Furthermore, a more targeted treatment approach needs to be developed for specific co-infections to avoid toxicity due to excessive use of chemotherapeutics. For example, common metabolic pathways of co-infecting pathogens can maximize antimicrobials efficacy, reduce toxicity due to excessive drug use, and curb multidrug resistance emergence. Studies in Africa under World Health Organization examined antibody titer against measles in vaccinated infants with malaria parasite presence/absence. Extension of such studies in older children and adults in regions with chronic protozoan infection could reveal the full picture of their impact on viral vaccines and would lead to better understanding of vaccines efficacy, help document causes of breakthrough infections and employ approaches for improvement of protection by vaccines.

## Abbreviations and descriptions

**Bacille Calmette-Guerin (BCG)** is a vaccine against tuberculosis disease in regions where this disease is highly prevalent.

**Bacterial vaginosis (BV)** is caused by a localized inflammatory response against overgrowing natural microbiota of vagina.

**Enteroaggregative *E. coli* (EAEC)** is a pathogenic *E. coli* species that causes chronic diarrhea and is known because of its ability to form aggregates on intestinal mucosal surface.

**Cross-Reactive-Material-197 (CRM197)** is a mutant version of the diphtheria toxin rending a protein non-toxic.

**Diphtheria-tetanus-acellular pertussis (DTPa)** is a trivalent vaccine against three bacteria, *Corynebacterium diphtheriae*, *Clostridium tetani* and *Bordetella pertussis* given to children to prevent from serious diseases by these pathogens. Diphtheria causes breathing problem, tetanus results in tightening of muscles while pertussis is contagious disease that is also known as whooping cough.

**Heat shock protein 70 (HSP70)** is a universally expressed conserved protein in almost all living organisms that serves as chaperone for proteins for transport across membrane and also have been shown to function as potent stimulators of the innate immune system.

**Non-Typhoid Salmonella (NTS)** are group of Salmonella species that are not usually human pathogens unlike *Salmonella enterica* serovar Typhi that causes typhoid fever.

**Reactive oxygen species (ROS)** are oxygen containing oxygen containing reactive species produced during aerobic respiration. ROS produced by neutrophils and other phagocytes in mammalian hosts can kill organisms by causing permanent damage to DNA.

**Tuberculosis (TB)** is disease caused by respiratory bacterial pathogen, *Mycobacterium tuberculosis* that can be visualized under the microscope after acid-fast staining.

## Author contributions

LA and SR wrote the initial draft and prepared Table 1. NP prepared Figure 1. All authors edited and read the final version and approved it for submission.

## Funding

This work was supported by the National Institutes of Health (R01AI137425) grant to NP.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## References

Abbate, J. L., Becquart, P., Leroy, E., Ezenwa, V. O., and Roche, B. (2020). Exposure to Ebola virus and risk for infection with malaria parasites, rural Gabon. *Emerg. Infect. Dis.* 26, 229–237. doi: 10.3201/eid2602.181120

Achan, J., Serwanga, A., Wanzira, H., Kyagulanyi, T., Nuwa, A., Magumba, G., et al. (2022). Current malaria infection, previous malaria exposure, and clinical profiles and outcomes of COVID-19 in a setting of high malaria transmission: an exploratory cohort study in Uganda. *Lancet Microbe* 3, e62–e71. doi: 10.1016/S2666-5247(21)00240-8

Agrawal, B. (2019). Heterologous immunity: role in natural and vaccine-induced resistance to infections. *Front. Immunol.* 10:2631. doi: 10.3389/fimmu.2019.02631

Agrawal, S. R., Singh, V., Ingale, S., and Jain, A. P. (2014). Toxoplasmosis of spinal cord in acquired immunodeficiency syndrome patient presenting as paraparesis: a rare entity. *J. Glob. Infect. Dis.* 6, 178–181. doi: 10.4103/0974-777X.145248

Ahmadpour, E., Safarpour, H., Xiao, L., Zarean, M., Hatam-Nahavandi, K., Barac, A., et al. (2020). Cryptosporidiosis in HIV-positive patients and related risk factors: A systematic review and meta-analysis. *Parasite* 27:27. doi: 10.1051/parasite/2020025

Akelew, Y., Andualem, H., Ebrahim, E., Atnaf, A., and Hailemichael, W. (2022). Immunomodulation of COVID-19 severity by helminth co-infection: implications for COVID-19 vaccine efficacy. *Immun. Inflamm. Dis.* 10:e573. doi: 10.1002/iid3.573

Alberca, R. W., Yendo, T. M., Leuzzi Ramos, Y. A., Fernandes, I. G., Oliveira, L. M., Teixeira, F. M. E., et al. (2020). Case report: COVID-19 and Chagas disease in two coinfected patients. *Am. J. Trop. Med. Hyg.* 103, 2353–2356. doi: 10.4269/ajtmh.20-1185

Alemu, G., Alelign, D., and Abossie, A. (2018). Prevalence of opportunistic intestinal parasites and associated factors among HIV patients while receiving ART at Arba Minch Hospital in Southern Ethiopia: A cross-sectional study. *Ethiop. J. Health Sci.* 28, 147–156. doi: 10.4314/ejhs.v28i2.6

Alemu, A., Shiferaw, Y., Addis, Z., Mathewos, B., and Birhan, W. (2013). Effect of malaria on HIV/AIDS transmission and progression. *Parasit. Vectors* 6:18. doi: 10.1186/1756-3305-6-18

Alsammani, M. A., Ahmed, S. R., Alsheeha, M. A., Saadia, Z., and Khairi, S. A. (2012). Co-infection with toxoplasma gondii and *Clostridium perfringens* in a postpartum woman with uterine gas gangrene: a case report. *J. Obstet. Gynaecol. Res.* 38, 1024–1027. doi: 10.1111/j.1447-0756.2011.01817.x

Alvarez-Larrotta, C., Agudelo, O. M., Duque, Y., Gavina, K., Yanow, S. K., Maestre, A., et al. (2019). Submicroscopic plasmodium infection during pregnancy is associated with reduced antibody levels to tetanus toxoid. *Clin. Exp. Immunol.* 195, 96–108. doi: 10.1111/cei.13213

Aminu, M., Parason, Y. I., Abdullahi, U., Tahir, M. I., Olaniyan, F. A., and Usman, M. A. (2021). Detection of measles IgM and malaria parasite among febrile

children in Zaria, Nigeria. *J. Infect. Dis. Epidemiol.* 7:189. doi: 10.23937/2474-3658/1510189

Anastos, K., Hoover, D. R., Burk, R. D., Cajigas, A., Shi, Q., Singh, D. K., et al. (2010). Risk factors for cervical precancer and cancer in HIV-infected, HPV-positive Rwandan women. *PLoS One* 5:e13525. doi: 10.1371/journal.pone.0013525

Andrade, B. B., Santos, C. J., Camargo, L. M., Souza-Neto, S. M., Reis-Filho, A., Clarencio, J., et al. (2011). Hepatitis B infection is associated with asymptomatic malaria in the Brazilian Amazon. *PLoS One* 6:e19841. doi: 10.1371/journal.pone.0019841

Anjorin, A. A. A., and Nwammadu, J. E. (2020). Seroepidemiology of seasonal influenza virus among unvaccinated pregnant women in Lagos Nigeria. *Infez Med* 28, 407–415.

Anyanwu, M. U. (2021). The association between malaria prevalence and COVID-19 mortality. *BMC Infect. Dis.* 21:975. doi: 10.1186/s12879-021-06701-8

Apiwattanakul, N., Thomas, P. G., Iverson, A. R., and McCullers, J. A. (2014). Chronic helminth infections impair pneumococcal vaccine responses. *Vaccine* 32, 5405–5410. doi: 10.1016/j.vaccine.2014.07.107

Ayoade, F., and Joel Chandranesan, A. S. (2022). "HIV-1 associated toxoplasmosis," in *StatPearls* ( Treasure Island, FL).

Ayouba, A., Badaut, C., Kfutwah, A., Cannou, C., Juillerat, A., Gangnard, S., et al. (2008). Specific stimulation of HIV-1 replication in human placental trophoblasts by an antigen of plasmodium falciparum. *AIDS* 22, 785–787. doi: 10.1097/QAD.0b013e3282f560ee

Azizieh, F., Dingle, K., Raghupathy, R., Johnson, K., VanderPlas, J., and Ansari, A. (2018). Multivariate analysis of cytokine profiles in pregnancy complications. *Am. J. Reprod. Immunol.* 79:e12818. doi: 10.1111/aji.12818

Bala, S., Englund, G., Kovacs, J., Wahl, L., Martin, M., Sher, A., et al. (1994). *Toxoplasma gondii* soluble products induce cytokine secretion by macrophages and potentiate in vitro replication of a monotropic strain of HIV. *J. Eukaryot. Microbiol.* 41:7S.

Baluku, J. B., Nassozi, S., Gyagenda, B., Namanda, M., Andia-Biraro, I., Worodria, W., et al. (2019). Prevalence of malaria and TB coinfection at a National Tuberculosis Treatment Centre in Uganda. *J. Trop. Med.* 2019:3741294. doi: 10.1155/2019/3741294

Banga, S., Coursen, J. D., Portugal, S., Tran, T. M., Hancox, L., Ongoiba, A., et al. (2015). Impact of acute malaria on pre-existing antibodies to viral and vaccine antigens in mice and humans. *PLoS One* 10:e0125090. doi: 10.1371/journal.pone.0125090

Bartelt, L. A., Bolick, D. T., Mayneris-Perxachs, J., Kolling, G. L., Medlock, G. L., Zaenker, E. I., et al. (2017). Cross-modulation of pathogen-specific pathways enhances malnutrition during enteric co-infection with Giardia lamblia and enteroaggregative Escherichia coli. *PLoS Pathog.* 13:e1006471. doi: 10.1371/journal.ppat.1006471

Barthold, S. W., Cadavid, D., and Philipp, M. T. (2010) in *Borrelia: Molecular biology, host interaction, and pathogenesis*. eds. D. S. Samuels and J. D. Radolf (Norfolk, UK: Caister Academic), 359–411.

Basavaraju, A. (2016). Toxoplasmosis in HIV infection: an overview. *Trop. Parasitol.* 6, 129–135. doi: 10.4103/2229-5070.190817

Beck, H. P., Felger, I., Genton, B., Alexander, N., al-Yaman, F., Anders, R. F., et al. (1995). Humoral and cell-mediated immunity to the Plasmodium falciparum ring-infected erythrocyte surface antigen in an adult population exposed to highly endemic malaria. *Infect. Immun.* 63, 596–600. doi: 10.1128/iai.63.2.596-600.1995

Benach, J. L., Coleman, J. L., Habicht, G. S., MacDonald, A., Grunwaldt, E., and Giron, J. A. (1985). Serological evidence for simultaneous occurrences of Lyme disease and babesiosis. *J. Infect. Dis.* 152, 473–477. doi: 10.1093/infdis/152.3.473

Birger, R. B., Kouyos, R. D., Cohen, T., Griffiths, E. C., Huijben, S., Mina, M. J., et al. (2015). The potential impact of coinfection on antimicrobial chemotherapy and drug resistance. *Trends Microbiol.* 23, 537–544. doi: 10.1016/j.tim.2015.05.002

Blank, J., Eggers, L., Behrends, J., Jacobs, T., and Schneider, B. E. (2016). One episode of self-resolving plasmodium yoelii infection transiently exacerbates chronic mycobacterium tuberculosis infection. *Front. Microbiol.* 7:152. doi: 10.3389/fmicb.2016.00152

Boonyarangka, P., Phontham, K., Sriwichai, S., Poramathikul, K., Harncharoenkul, K., Kuntawunginn, W., et al. (2022). Co-infection with plasmodium vivax and COVID-19 in Thailand. *Trop. Med. Infect. Dis.* 7:145. doi: 10.3390/tropicalmed7080145

Borad, A., and Ward, H. (2010). Human immune responses in cryptosporidiosis. *Future Microbiol.* 5, 507–519. doi: 10.2217/fmb.09.128

Boutlis, C. S., Yeo, T. W., and Anstey, N. M. (2006). Malaria tolerance--for whom the cell tolls? *Trends Parasitol.* 22, 371–377. doi: 10.1016/j.pt.2006.06.002

Bradbury, R. S., Piedrafita, D., Greenhill, A., and Mahanty, S. (2020). Will helminth co-infection modulate COVID-19 severity in endemic regions? *Nat. Rev. Immunol.* 20:342. doi: 10.1038/s41577-020-0330-5

Bretsche, P. A., Ismail, N., Menon, J. N., Power, C. A., Uzonna, J., and Wei, G. (2001). Vaccination against and treatment of tuberculosis, the leishmaniases and AIDS: perspectives from basic immunology and immunity to chronic intracellular infections. *Cell. Mol. Life Sci.* 58, 1879–1896. doi: 10.1007/pl00000824

Bretscher, P. A. (1992). An hypothesis to explain why cell-mediated immunity alone can contain infections by certain intracellular parasites and how immune class regulation of the response against such parasites can be subverted. *Immunol. Cell Biol.* 70, 343–351. doi: 10.1038/icb.1992.44

Bronowski, C., James, C. E., and Winstanley, C. (2014). Role of environmental survival in transmission of *Campylobacter jejuni*. *FEMS Microbiol. Lett.* 356, 8–19. doi: 10.1111/1574-6968.12488

Brown, J., Baisley, K., Kavishe, B., Changalucha, J., Andreasen, A., Mayaud, P., et al. (2014). Impact of malaria and helminth infections on immunogenicity of the human papillomavirus-16/18 AS04-adjuvanted vaccine in Tanzania. *Vaccine* 32, 611–617. doi: 10.1016/j.vaccine.2013.11.061

Brown, A. E., Mongkolsirichaikul, D., Innis, B., Snitbhan, R., and Webster, H. K. (1992). Falciparum malaria modulates viremia in chronic hepatitis B virus infection. *J. Infect. Dis.* 166, 1465–1466. doi: 10.1093/infdis/166.6.1465

Chen, X. M., and LaRusso, N. F. (2002). Cryptosporidiosis and the pathogenesis of AIDS-cholangiopathy. *Semin. Liver Dis.* 22, 277–290. doi: 10.1055/s-2002-34505

Chukwuanukwu, R. C., Onyenekwe, C. C., Martinez-Pomares, L., Flynn, R., Singh, S., Amilo, G. I., et al. (2017). Modulation of the immune response to mycobacterium tuberculosis during malaria/*M. tuberculosis* co-infection. *Clin. Exp. Immunol.* 187, 259–268. doi: 10.1111/cei.12861

Clark, I. A., Allison, A. C., and Cox, F. E. (1976). Protection of mice against Babesia and plasmodium with BCG. *Nature* 259, 309–311. doi: 10.1038/259309a0

Clark, I. A., Wills, E. J., Richmond, J. E., and Allison, A. C. (1977). Suppression of babesiosis in BCG-infected mice and its correlation with tumor inhibition. *Infect. Immun.* 17, 430–438. doi: 10.1128/iai.17.2.430-438.1977

Coban, C., Ishii, K. J., Kawai, T., Hemmi, H., Sato, S., Uematsu, S., et al. (2005). Toll-like receptor 9 mediates innate immune activation by the malaria pigment hemozoin. *J. Exp. Med.* 201, 19–25. doi: 10.1084/jem.20041836

Colombatti, R., Penazzato, M., Bassani, F., Vieira, C. S., Lourenco, A. A., Vieira, F., et al. (2011). Malaria prevention reduces in-hospital mortality among severely ill tuberculosis patients: a three-step intervention in Bissau, Guinea-Bissau. *BMC Infect. Dis.* 11:57. doi: 10.1186/1471-2334-11-57

Conner, M., Joshi, T., Veerisetty, S. S., and Hutchings, J. (2019). Coinfection of cytomegalovirus and cryptosporidiosis in a patient with AIDS. *ACG Case Rep. J.* 6:e00225. doi: 10.14309/crj.0000000000000225

Costa-Madeira, J. C., Trindade, G. B., Almeida, P. H. P., Silva, J. S., and Carregaro, V. (2022). T lymphocyte exhaustion during human and experimental visceral Leishmaniasis. *Front. Immunol.* 13:835711. doi: 10.3389/fimmu.2022.835711

Cox, F. E. (2001). Concomitant infections, parasites and immune responses. *Parasitology* 122, S23–S38. doi: 10.1017/S003118200001698X

Cunnington, A. J., de Souza, J. B., Walther, M., and Riley, E. M. (2011). Malaria impairs resistance to salmonella through heme- and heme oxygenase-dependent dysfunctional granulocyte mobilization. *Nat. Med.* 18, 120–127. doi: 10.1038/nm.2601

Cunnington, A. J., Njie, M., Correa, S., Takem, E. N., Riley, E. M., and Walther, M. (2012). Prolonged neutrophil dysfunction after plasmodium falciparum malaria is related to hemolysis and heme oxygenase-1 induction. *J. Immunol.* 189, 5336–5346. doi: 10.4049/jimmunol.1201028

Cunnington, A. J., and Riley, E. M. (2010). Suppression of vaccine responses by malaria: insignificant or overlooked? *Expert Rev. Vaccines* 9, 409–429. doi: 10.1586/erv.10.16

Cu-Uvin, S., Ko, H., Jamieson, D. J., Hogan, J. W., Schuman, P., Anderson, J., et al. (2002). Prevalence, incidence, and persistence or recurrence of trichomoniasis among human immunodeficiency virus (HIV)-positive women and among HIV-negative women at high risk for HIV infection. *Clin. Infect. Dis.* 34, 1406–1411. doi: 10.1086/340264

Da Cunha, S., Ferreira, E., Ramos, I., Martins, R., De Freitas, L., Borges, J. L., et al. (1994). Cerebral toxoplasmosis after renal transplantation. Case report and review. *Acta Medica Port.* 7, S61–S66.

Da'dara, A. A., and Harn, D. A. (2010). Elimination of helminth infection restores HIV-1C vaccine-specific T cell responses independent of helminth-induced IL-10. *Vaccine* 28, 1310–1317. doi: 10.1016/j.vaccine.2009.11.019

Davis, A., Dasgupta, A., Goddard-Eckrich, D., and El-Bassel, N. (2016). Trichomonas vaginalis and human immunodeficiency virus coinfection among women under community supervision: A call for Expanded *T. vaginalis* screening. *Sex. Transm. Dis.* 43, 617–622. doi: 10.1097/OLQ.0000000000000503

Delanghe, J. R., Speeckaert, M. M., and De Buyzere, M. L. (2020). The host's angiotensin-converting enzyme polymorphism may explain epidemiological findings in COVID-19 infections. *Clin. Chim. Acta* 505, 192–193. doi: 10.1016/j.cca.2020.03.031

Denoncourt, A. M., Paquet, V. E., and Charette, S. J. (2014). Potential role of bacteria packaging by protozoa in the persistence and transmission of pathogenic bacteria. *Front. Microbiol.* 5:240. doi: 10.3389/fmicb.2014.00240

Dietz, V., Galazka, A., van Loon, F., and Cochi, S. (1997). Factors affecting the immunogenicity and potency of tetanus toxoid: implications for the elimination of neonatal and non-neonatal tetanus as public health problems. *Bull. World Health Organ.* 75, 81–93. PMID: 9141753

Diro, E., Lynen, L., Ritmeijer, K., Boelaert, M., Hailu, A., and van Griensven, J. (2014). Visceral Leishmaniasis and HIV coinfection in East Africa. *PLoS Negl. Trop. Dis.* 8:e2869. doi: 10.1371/journal.pntd.0002869

Diuk-Wasser, M. A., Vannier, E., and Krause, P. J. (2016). Coinfection by Ixodes tick-borne pathogens: ecological, epidemiological, and clinical consequences. *Trends Parasitol.* 32, 30–42. doi: 10.1016/j.pt.2015.09.008

Djokic, V., Akoolo, L., Primus, S., Schlachter, S., Kelly, K., Bhanot, P., et al. (2019). Protozoan parasite *Babesia microti* subverts adaptive immunity and enhances Lyme disease severity. *Front. Microbiol.* 10:1596. doi: 10.3389/fmicb.2019.01596

Djokic, V., Rocha, S. C., and Parveen, N. (2021). Lessons learned for pathogenesis, immunology, and disease of Erythrocytic parasites: *plasmodium* and *Babesia*. *Front. Cell. Infect. Microbiol.* 11:685239. doi: 10.3389/fcimb.2021.685239

Doolan, D. L., Dobano, C., and Baird, J. K. (2009). Acquired immunity to malaria. *Clin. Microbiol. Rev.* 22, 13–36, Table of Contents. doi: 10.1128/CMR.00025-08

Dunn, J. M., Krause, P. J., Davis, S., Vannier, E. G., Fitzpatrick, M. C., Rollend, L., et al. (2014). Borrelia burgdorferi promotes the establishment of Babesia microti in the northeastern United States. *PLoS One* 9:e115494. doi: 10.1371/journal.pone.0115494

Elias, D., Britton, S., Aseffa, A., Engers, H., and Akuffo, H. (2008). Poor immunogenicity of BCG in helminth infected population is associated with increased in vitro TGF-beta production. *Vaccine* 26, 3897–3902. doi: 10.1016/j.vaccine.2008.04.083

Elliott, A. M., Mawa, P. A., Webb, E. L., Nampijja, M., Lyadda, N., Bukusuba, J., et al. (2010). Effects of maternal and infant co-infections, and of maternal immunisation, on the infant response to BCG and tetanus immunisation. *Vaccine* 29, 247–255. doi: 10.1016/j.vaccine.2010.10.047

Fairlie-Clarke, K. J., Allen, J. E., Read, A. F., and Graham, A. L. (2013). Quantifying variation in the potential for antibody-mediated apparent competition among nine genotypes of the rodent malaria parasite plasmodium chabaudi. *Infect. Genet. Evol.* 20, 270–275. doi: 10.1016/j.meegid.2013.09.013

Feldman, S., Stein, D., Amrute, S., Denny, T., Garcia, Z., Kloser, P., et al. (2001). Decreased interferon-alpha production in HIV-infected patients correlates with numerical and functional deficiencies in circulating type 2 dendritic cell precursors. *Clin. Immunol.* 101, 201–10. doi: 10.1006/clim.2001.5111

Fernandez-Lopez, L. A., Gil-Becerril, K., Galindo-Gomez, S., Estrada-Garcia, T., Ximenez, C., Leon-Coria, A., et al. (2019). Entamoeba histolytica interaction with Enteropathogenic Escherichia coli increases parasite virulence and inflammation in Amebiasis. *Infect. Immun.* 87:e00279–19. doi: 10.1128/IAI.00279-19

Fiori, P. L., Diaz, N., Cocco, A. R., Rappelli, P., and Dessi, D. (2013). Association of Trichomonas vaginalis with its symbiont mycoplasma hominis synergistically upregulates the in vitro proinflammatory response of human monocytes. *Sex. Transm. Infect.* 89, 449–454. doi: 10.1136/sextrans-2012-051006

Fonte, L., Acosta, A., Sarmiento, M. E., Ginori, M., Garcia, G., and Norazmi, M. N. (2020). COVID-19 lethality in sub-Saharan Africa and helminth immune modulation. *Front. Immunol.* 11:574910. doi: 10.3389/fimmu.2020.574910

Fregonesi, B. M., Suzuki, M. N., Machado, C. S., Tonani, K. A., Fernandes, A. P., Monroe, A. A., et al. (2015). Emergent and re-emergent parasites in HIV-infected children: immunological and socio-environmental conditions that are involved in the transmission of giardia spp. and cryptosporidium spp. *Rev. Soc. Bras. Med. Trop.* 48, 753–758. doi: 10.1590/0037-8682-0119-2015

Froebel, K., Howard, W., Schafer, J. R., Howie, F., Whitworth, J., Kaleebu, P., et al. (2004). Activation by malaria antigens renders mononuclear cells susceptible to HIV infection and re-activates replication of endogenous HIV in cells from HIV-infected adults. *Parasite Immunol.* 26, 213–217. doi: 10.1111/j.0141-9838.2004.00701.x

Galvan-Moroyoqui, J. M., Del Carmen Dominguez-Robles, M., Franco, E., and Meza, I. (2008). The interplay between Entamoeba and enteropathogenic bacteria modulates epithelial cell damage. *PLoS Negl. Trop. Dis.* 2:e266. doi: 10.1371/journal.pntd.0000266

Garly, M. L., Martins, C. L., Bale, C., Balde, M. A., Hedegaard, K. L., Gustafson, P., et al. (2003). BCG scar and positive tuberculin reaction associated with reduced child mortality in West Africa. A non-specific beneficial effect of BCG? *Vaccine* 21, 2782–2790. doi: 10.1016/s0264-410x(03)00181-6

Gigley, J. P., Bhadra, R., Moretto, M. M., and Khan, I. A. (2012). T cell exhaustion in protozoan disease. *Trends Parasitol.* 28, 377–384. doi: 10.1016/j.pt.2012.07.001

Gilles, H. M., Greenwood, B. M., Greenwood, A. M., Bradley, A. K., Blakebrough, I., Pugh, R. N., et al. (1983). The Malumfashi project--an epidemiological, clinical and laboratory study. *Trans. R. Soc. Trop. Med. Hyg.* 77, 24–31. doi: 10.1016/0035-9203(83)90005-6

Gluchowska, K., Dzieciatkowski, T., Sedzikowska, A., Zawistowska-Deniziak, A., and Mlocicki, D. (2021). The new status of parasitic diseases in the COVID-19 pandemic-risk factors or protective agents? *J. Clin. Med.* 10. doi: 10.3390/jcm10112533

Gozzelino, R., Jeney, V., and Soares, M. P. (2010). Mechanisms of cell protection by heme oxygenase-1. *Annu. Rev. Pharmacol. Toxicol.* 50, 323–354. doi: 10.1146/annurev.pharmtox.010909.105600

Greenwood, B. M., Bradley-Moore, A. M., Bryceson, A. D., and Palit, A. (1972). Immunosuppression in children with malaria. *Lancet* 299, 169–172. doi: 10.1016/s0140-6736(72)90569-7

Guha, R., Mathioudaki, A., Doumbo, S., Doumtabe, D., Skinner, J., Arora, G., et al. (2020). Plasmodium falciparum malaria drives epigenetic reprogramming of human monocytes toward a regulatory phenotype. *PLoS Pathog.* 17:e1009430 doi: 10.1371/journal.ppat.1009430

Gupta, G., Oghumu, S., and Satoskar, A. R. (2013). Mechanisms of immune evasion in leishmaniasis. *Adv. Appl. Microbiol.* 82, 155–184. doi: 10.1016/B978-0-12-407679-2.00005-3

Gutman, J. R., Lucchi, N. W., Cantey, P. T., Steinhardt, L. C., Samuels, A. M., Kamb, M. L., et al. (2020). Malaria and parasitic neglected tropical diseases: potential Syndemics with COVID-19? *Am. J. Trop. Med. Hyg.* 103, 572–577. doi: 10.4269/ajtmh.20-0516

Hailu, A. W., S, G. S., Merid, Y., Gebru, A. A., Ayene, Y. Y., and Asefa, M. K. (2015). The case control studies of HIV and intestinal parasitic infections rate in active pulmonary tuberculosis patients in Woldia general hospital and health Center in North Wollo, Amhara region Ethiopia. *Int. J. Pharma. Sci.* 5, 1092–1099. PMID: 26998497

Hansen, D. S., D'Ombrain, M. C., and Schofield, L. (2007). The role of leukocytes bearing natural killer complex receptors and killer immunoglobulin-like receptors in the immunology of malaria. *Curr. Opin. Immunol.* 19, 416–423. doi: 10.1016/j.coi.2007.07.011

Harding, C. L., Villarino, N. F., Valente, E., Schwarzer, E., and Schmidt, N. W. (2020). Plasmodium impairs antibacterial innate immunity to systemic infections in part through Hemozoin-bound bioactive molecules. *Front. Cell. Infect. Microbiol.* 10:328. doi: 10.3389/fcimb.2020.00328

Helegbe, G. K., Aryee, P. A., Mohammed, B. S., Wemakor, A., Kolbila, D., Abubakari, A. W., et al. (2018). Seroprevalence of malaria and hepatitis B coinfection among pregnant women in tamale Metropolis of Ghana: A cross-sectional study. *Can. J. Infect. Dis. Med. Microbiol.* 2018:5610981. doi: 10.1155/2018/5610981

Holland, W. G., Do, T. T., Huong, N. T., Dung, N. T., Thanh, N. G., Vercruysse, J., et al. (2003). The effect of *Trypanosoma evansi* infection on pig performance and vaccination against classical swine fever. *Vet. Parasitol.* 111, 115–123. doi: 10.1016/s0304-4017(02)00363-1

Hsieh, S. M., Chen, M. Y., Pan, S. C., Hung, C. C., and Chang, S. C. (2007). Aberrant induction of regulatory activity of CD4+CD25+ T cells by dendritic cells in HIV-infected persons with amebic liver abscess. *J. Acquir. Immune Defic. Syndr.* 44, 6–13. doi: 10.1097/01.qai.0000242457.43392.61

Hung, C. C., Chang, S. Y., and Ji, D. D. (2012). Entamoeba histolytica infection in men who have sex with men. *Lancet Infect. Dis.* 12, 729–736. doi: 10.1016/S1473-3099(12)70147-0

Hung, C. C., Deng, H. Y., Hsiao, W. H., Hsieh, S. M., Hsiao, C. F., Chen, M. Y., et al. (2005). Invasive amebiasis as an emerging parasitic disease in patients with human immunodeficiency virus type 1 infection in Taiwan. *Arch. Intern. Med.* 165, 409–415. doi: 10.1001/archinte.165.4.409

Hung, C. C., Ji, D. D., Sun, H. Y., Lee, Y. T., Hsu, S. Y., Chang, S. Y., et al. (2008). Increased risk for Entamoeba histolytica infection and invasive amebiasis in HIV seropositive men who have sex with men in Taiwan. *PLoS Negl. Trop. Dis.* 2:e175. doi: 10.1371/journal.pntd.0000175

Hunter, P. R., and Nichols, G. (2002). Epidemiology and clinical features of cryptosporidium infection in immunocompromised patients. *Clin. Microbiol. Rev.* 15, 145–154. doi: 10.1128/CMR.15.1.145-154.2002

Hussein, M. I. H., Albashir, A. A. D., Elawad, O., and Homeida, A. (2020). Malaria and COVID-19: unmasking their ties. *Malar. J.* 19:457. doi: 10.1186/s12936-020-03541-w

Hussein, R., Guedes, M., Ibraheim, N., Ali, M. M., El-Tahir, A., Allam, N., et al. (2022). Impact of COVID-19 and malaria coinfection on clinical outcomes: a retrospective cohort study. *Clin. Microbiol. Infect.* 28, 1152.e1–1152.e6. doi: 10.1016/j.cmi.2022.03.028

Ibrahim, A., Ali, Y. B. M., Abdel-Aziz, A., and El-Badry, A. A. (2019). Helicobacter pylori and enteric parasites co-infection among diarrheic and non-diarrheic Egyptian children: seasonality, estimated risks, and predictive factors. *J. Parasit. Dis.* 43, 198–208. doi: 10.1007/s12639-018-1075-y

Ifudu, O., and Fowler, A. (2001). Hepatitis B virus infection and the response to erythropoietin in end-stage renal disease. *ASAIO J.* 47, 569–572. doi: 10.1097/00002480-200109000-00035

Inion, I., Mwanyumba, F., Gaillard, P., Chohan, V., Verhofstede, C., Claeys, P., et al. (2003). Placental malaria and perinatal transmission of human immunodeficiency virus type 1. *J. Infect. Dis.* 188, 1675–1678. doi: 10.1086/379737

Irisarri-Gutierrez, M. J., Mingo, M. H., de Lucio, A., Gil, H., Morales, L., Segui, R., et al. (2017). Association between enteric protozoan parasites and gastrointestinal illness among HIV- and tuberculosis-infected individuals in the Chowke district, southern Mozambique. *Acta Trop.* 170, 197–203. doi: 10.1016/j.actatropica.2017.03.010

Izzo, M. M., Kirkland, P. D., Mohler, V. L., Perkins, N. R., Gunn, A. A., and House, J. K. (2011). Prevalence of major enteric pathogens in Australian dairy calves with diarrhoea. *Aust. Vet. J.* 89, 167–173. doi: 10.1111/j.1751-0813.2011.00692.x

Jacobs, J. W., and Siddon, A. J. (2021). Concurrent COVID-19 and babesiosis in an older, splenectomized patient. *Blood* 138:2154. doi: 10.1182/blood.2021013947

Junaedi, M., Katu, S., Ilyas, M., Daud, N., Saleh, S., and Nurjannah Lihawa, H. (2020). Case report: Covid-19 and severe malaria co-infection. *Eur. J. Mol. Clin. Med.* 7, 961–968.

Karp, C. L., and Auwaerter, P. G. (2007). Coinfection with HIV and tropical infectious diseases protozoal pathogens. *Clin. Infect. Dis.* 45, 1208–1213. doi: 10.1086/522181

Kassa, D., Petros, B., Mesele, T., Hailu, E., and Wolday, D. (2006). Characterization of peripheral blood lymphocyte subsets in patients with acute P*lasmodium falciparum* and *P. vivax* malaria infections at Wonji sugar estate, Ethiopia. *Clin. Vaccine Immunol.* 13, 376–379. doi: 10.1128/CVI.13.3.376-379.2006

Kaushik, K., Khurana, S., Wanchu, A., and Malla, N. (2009). Serum immunoglobulin G, M and A response to *Cryptosporidium parvum* in cryptosporidium-HIV co-infected patients. *BMC Infect. Dis.* 9:179. doi: 10.1186/1471-2334-9-179

Kinyanjui, S. M., Conway, D. J., Lanar, D. E., and Marsh, K. (2007). IgG antibody responses to plasmodium falciparum merozoite antigens in Kenyan children have a short half-life. *Malar. J.* 6:82. doi: 10.1186/1475-2875-6-82

Klassen, D. K., and Spivak, J. L. (1990). Hepatitis-related hepatic erythropoietin production. *Am. J. Med.* 89, 684–686. doi: 10.1016/0002-9343(90)90190-o

Kleinnijenhuis, J., Quintin, J., Preijers, F., Joosten, L. A., Jacobs, C., Xavier, R. J., et al. (2014). BCG-induced trained immunity in NK cells: role for non-specific protection to infection. *Clin. Immunol.* 155, 213–219. doi: 10.1016/j.clim.2014.10.005

Knapp, K. L., and Rice, N. A. (2015). Human coinfection with *Borrelia burgdorferi* and *Babesia microti* in the United States. *J. Parasitol. Res.* 2015:587131. doi: 10.1155/2015/587131

Kotepui, K. U., and Kotepui, M. (2020). Prevalence of and risk factors for plasmodium spp. co-infection with hepatitis B virus: a systematic review and meta-analysis. *Malar. J.* 19:368. doi: 10.1186/s12936-020-03428-w

Krause, P. J., Telford, S. R. 3rd, Spielman, A., Sikand, V., Ryan, R., Christianson, D., et al. (1996). Concurrent Lyme disease and babesiosis: Evidence for increased severity and duration of illness. *JAMA* 275, 1657–1660. doi: 10.1001/jama.1996.03530450047031

Laufer, M. K., van Oosterhout, J. J., Thesing, P. C., Dzinjalamala, F. K., Hsi, T., Beraho, L., et al. (2007). Malaria treatment efficacy among people living with HIV: the role of host and parasite factors. *Am. J. Trop. Med. Hyg.* 77, 627–632. doi: 10.4269/ajtmh.2007.77.627

Lejeune, M., Miro, J. M., De Lazzari, E., Garcia, F., Claramonte, X., Martinez, E., et al. (2011). Restoration of T cell responses to toxoplasma gondii after successful combined antiretroviral therapy in patients with AIDS with previous toxoplasmic encephalitis. *Clin. Infect. Dis.* 52, 662–670. doi: 10.1093/cid/ciq197

Lo, Y. C., Ji, D. D., and Hung, C. C. (2014). Prevalent and incident HIV diagnoses among *Entamoeba histolytica*-infected adult males: a changing epidemiology associated with sexual transmission--Taiwan, 2006–2013. *PLoS Negl. Trop. Dis.* 8:e3222. doi: 10.1371/journal.pntd.0003222

Lowther, S. A., Dworkin, M. S., and Hanson, D. L. (2000). Entamoeba histolytica/Entamoeba dispar infections in human immunodeficiency virus-infected patients in the United States. *Clin. Infect. Dis.* 30, 955–959. doi: 10.1086/313811

Machelart, A., Van Vyve, M., Potemberg, G., Demars, A., De Trez, C., Tima, H. G., et al. (2017). Trypanosoma infection favors Brucella elimination via IL-12/IFNgamma-dependent pathways. *Front. Immunol.* 8:903. doi: 10.3389/fimmu.2017.00903

Malhotra, I., McKibben, M., Mungai, P., McKibben, E., Wang, X., Sutherland, L. J., et al. (2015). Effect of antenatal parasitic infections on anti-vaccine IgG levels in children: a prospective birth cohort study in Kenya. *PLoS Negl. Trop. Dis.* 9:e0003466. doi: 10.1371/journal.pntd.0003466

Manko, A., Motta, J. P., Cotton, J. A., Feener, T., Oyeyemi, A., Vallance, B. A., et al. (2017). Giardia co-infection promotes the secretion of antimicrobial peptides beta-defensin 2 and trefoil factor 3 and attenuates attaching and effacing bacteria-induced intestinal disease. *PLoS One* 12:e0178647. doi: 10.1371/journal.pone.0178647

Márquez-Contreras, M. E. (2018). Mechanisms of immune evasion by *Trypanosoma brucei*. *Microbiol. Curr. Res.* 02, 39–44. doi: 10.4066/2591-8036.18-303

Marti, H., Kim, H., Joseph, S. J., Dojiri, S., Read, T. D., and Dean, D. (2017). Tet(C) gene transfer between chlamydia suis strains occurs by homologous recombination after co-infection: implications for spread of tetracycline-resistance among Chlamydiaceae. *Front. Microbiol.* 8:156. doi: 10.3389/fmicb.2017.00156

McGregor, I. A. A. B. M. (1962). Antibody response to tetanus toxoid inoculation in malarious and non-malarious Gambian children. *Trans. R. Soc. Trop. Med. Hyg.* 56, 364–367. doi: 10.1016/0035-9203(62)90005-6

McKittrick, N. D., Malhotra, I. J., Vu, D. M., Boothroyd, D. B., Lee, J., Krystosik, A. R., et al. (2019). Parasitic infections during pregnancy need not affect infant antibody responses to early vaccination against Streptococcus pneumoniae, diphtheria, or Haemophilus influenzae type B. *PLoS Negl. Trop. Dis.* 13:e0007172. doi: 10.1371/journal.pntd.0007172

Moens, L., Wuyts, G., Verhaegen, J., and Bossuyt, X. (2012). Cross-protective immunity against heterologous *Streptococcus pneumoniae*. *Infect. Immun.* 80, 1944–1945; author reply 1946. doi: 10.1128/IAI.06397-11

Mokomane, M., Tate, J. E., Steenhoff, A. P., Esona, M. D., Bowen, M. D., Lechiile, K., et al. (2018). Evaluation of the influence of gastrointestinal coinfections on rotavirus vaccine effectiveness in Botswana. *Pediatr. Infect. Dis. J.* 37, e58–e62. doi: 10.1097/INF.0000000000001828

Montazeri, M., Nakhaei, M., Fakhar, M., Pazoki, H., Pagheh, A. S., Nazar, E., et al. (2022). Exploring the association between latent toxoplasma gondii infection and COVID-19 in hospitalized patients: first registry-based study. *Acta Parasitol.* 67, 1172–1179. doi: 10.1007/s11686-022-00559-9

Montoya, J. G., and Liesenfeld, O. (2004). Toxoplasmosis. *Lancet* 363, 1965–1976. doi: 10.1016/S0140-6736(04)16412-X

Mooney, J. P., Lee, S. J., Lokken, K. L., Nanton, M. R., Nuccio, S. P., McSorley, S. J., et al. (2015). Transient loss of protection afforded by a live attenuated non-typhoidal salmonella vaccine in mice co-infected with malaria. *PLoS Negl. Trop. Dis.* 9:e0004027. doi: 10.1371/journal.pntd.0004027

Moro, M. H., Zegarra-Moro, O. L., Bjornsson, J., Hofmeister, E. K., Bruinsma, E., Germer, J. J., et al. (2002). Increased arthritis severity in mice coinfected with *Borrelia burgdorferi* and *Babesia microti*. *J. Infect. Dis.* 186, 428–431. doi: 10.1086/341452

Mtove, G., Amos, B., Nadjm, B., Hendriksen, I. C., Dondorp, A. M., Mwambuli, A., et al. (2011). Decreasing incidence of severe malaria and community-acquired bacteraemia among hospitalized children in Muheza, North-Eastern Tanzania, 2006-2010. *Malar. J.* 10:320. doi: 10.1186/1475-2875-10-320

Mueller, A. K., Behrends, J., Blank, J., Schaible, U. E., and Schneider, B. E. (2014). An experimental model to study tuberculosis-malaria coinfection upon natural transmission of mycobacterium tuberculosis and plasmodium berghei. *J. Vis. Exp.* 84:e50829. doi: 10.3791/50829

Murray, C. J., Ortblad, K. F., Guinovart, C., Lim, S. S., Wolock, T. M., Roberts, D. A., et al. (2014). Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990-2013: a systematic analysis for the global burden of disease study 2013. *Lancet* 384, 1005–1070. doi: 10.1016/S0140-6736(14)60844-8

Musaya, J., Matovu, E., Nyirenda, M., and Chisi, J. (2015). Role of cytokines in *Trypanosoma brucei*-induced anaemia: A review of the literature. *Malawi Med. J.* 27, 45–50. doi: 10.4314/mmj.v27i2.3

Mwangi, D. M., Munyua, W. K., and Nyaga, P. N. (1990). Immunosuppression in caprine trypanosomiasis: effects of acute *Trypanosoma congolense* infection on antibody response to anthrax spore vaccine. *Trop. Anim. Health Prod.* 22, 95–100. doi: 10.1007/BF02239832

Nahrendorf, W., Ivens, A., and Spence, P. J. (2021). Inducible mechanisms of disease tolerance provide an alternative strategy of acquired immunity to malaria. *elife* 10:e63838. doi: 10.7554/eLife.63838

Natukunda, A. E. A. (2020). Effect of intermittent preventive treatment for malaria with dihydroartemisinin-piperaquine on immune responses to vaccines among rural Ugandan adolescents: randomized controlled trial protocol B for the "population differences in vaccine responses," (POPVAC) program. *BMJ Open* 11:e040427. doi: 10.1136/bmjopen-2020-040427

Netea, M. G., Dominguez-Andres, J., Barreiro, L. B., Chavakis, T., Divangahi, M., Fuchs, E., et al. (2020). Defining trained immunity and its role in health and disease. *Nat. Rev. Immunol.* 20, 375–388. doi: 10.1038/s41577-020-0285-6

Netea, M. G., Quintin, J., and van der Meer, J. W. (2011). Trained immunity: a memory for innate host defense. *Cell Host Microbe* 9, 355–361. doi: 10.1016/j.chom.2011.04.006

Ng, D. H., Skehel, J. J., Kassiotis, G., and Langhorne, J. (2014). Recovery of an antiviral antibody response following attrition caused by unrelated infection. *PLoS Pathog.* 10:e1003843. doi: 10.1371/journal.ppat.1003843

Nyakoe, N. K., Taylor, R. P., Makumi, J. N., and Waitumbi, J. N. (2009). Complement consumption in children with plasmodium falciparum malaria. *Malar. J.* 8:7. doi: 10.1186/1475-2875-8-7

Ojurongbe, O., Raji, O. A., Akindele, A. A., Kareem, M. I., Adefioye, O. A., and Adeyeba, A. O. (2011). Cryptosporidium and other enteric parasitic infections in HIV-seropositive individuals with and without diarrhoea in Osogbo Nigeria. *Br. J. Biomed. Sci.* 68, 75–78. doi: 10.1080/09674845.2011.11730327

Okeke, I. N. (2003). "Factors contributing to the emergence of resistance," in *The resistance phenomenon in microbes and infectious disease vectors: Implications for human health and strategies for containment: Workshop summary*. eds. L. S. K. SL and M. Najafiet al. (United States: National Acaemies Press)

Okwor, I., and Uzonna, J. E. (2013). The immunology of Leishmania/HIV co-infection. *Immunol. Res.* 56, 163–171. doi: 10.1007/s12026-013-8389-8

Olsthoorn, F., Sprong, H., Fonville, M., Rocchi, M., Medlock, J., Gilbert, L., et al. (2021). Occurrence of tick-borne pathogens in questing Ixodes ricinus ticks from wester Ross Northwest Scotland. *Parasit Vect.* 14:430. doi: 10.1186/s13071-021-04946-5

Onderdonk, A. B., Delaney, M. L., and Fichorova, R. N. (2016). The human microbiome during bacterial vaginosis. *Clin. Microbiol. Rev.* 29, 223–238. doi: 10.1128/CMR.00075-15

Osei, S. A., Biney, R. P., Anning, A. S., Nortey, L. N., and Ghartey-Kwansah, G. (2022). Low incidence of COVID-19 case severity and mortality in Africa; could malaria co-infection provide the missing link? *BMC Infect. Dis.* 22:78. doi: 10.1186/s12879-022-07064-4

Page, K. R., Jedlicka, A. E., Fakheri, B., Noland, G. S., Kesavan, A. K., Scott, A. L., et al. (2005). Mycobacterium-induced potentiation of type 1 immune responses and protection against malaria are host specific. *Infect. Immun.* 73, 8369–8380. doi: 10.1128/IAI.73.12.8369-8380.2005

Pantaleo, G., and Koup, R. A. (2004). Correlates of immune protection in HIV-1 infection: what we know, what we don't know, what we should know. *Nat. Med.* 10, 806–810. doi: 10.1038/nm0804-806

Parra, M., Derrick, S. C., Yang, A., Tian, J., Kolibab, K., Oakley, M., et al. (2011). Malaria infections do not compromise vaccine-induced immunity against tuberculosis in mice. *PLoS One* 6:e28164. doi: 10.1371/journal.pone.0028164

Parveen, N., and Bhanot, P. (2019). Babesia microti-Borrelia Burgdorferi coinfection. *Pathogens* 8:117. doi: 10.3390/pathogens8030117

Pikoulas, A., Piperaki, E. T., Spanakos, G., Kallianos, A., Mparmparousi, D., Rentziou, G., et al. (2022). Visceral leishmaniasis and COVID-19 coinfection – A case report. *IDCases* 27:e01358. doi: 10.1016/j.idcr.2021.e01358

Pott, H., and Castelo, A. (2013). Isolated cerebellar toxoplasmosis as a complication of HIV infection. *Int. J. STD AIDS* 24, 70–72. doi: 10.1258/ijsa.2012.012189

Praharaj, I., Platts-Mills, J. A., Taneja, S., Antony, K., Yuhas, K., Flores, J., et al. (2019). Diarrheal etiology and impact of coinfections on rotavirus vaccine efficacy estimates in a clinical trial of a monovalent human-bovine (116E) Oral rotavirus vaccine, Rotavac, India. *Clin. Infect. Dis.* 69, 243–250. doi: 10.1093/cid/ciy896

Pukrittayakamee, S., Viravan, C., Charoenlarp, P., Yeamput, C., Wilson, R. J., and White, N. J. (1994). Antimalarial effects of rifampin in plasmodium vivax malaria. *Antimicrob. Agents Chemother.* 38, 511–514. doi: 10.1128/AAC.38.3.511

Radwanska, M., Guirnalda, P., De Trez, C., Ryffel, B., Black, S., and Magez, S. (2008). Trypanosomiasis-induced B cell apoptosis results in loss of protective anti-parasite antibody responses and abolishment of vaccine-induced memory responses. *PLoS Pathog.* 4:e1000078. doi: 10.1371/journal.ppat.1000078

Raham, T. F. (2021). Influence of malaria endemicity and tuberculosis prevalence on COVID-19 mortality. *Public Health* 194, 33–35. doi: 10.1016/j.puhe.2021.02.018

Rappelli, P., Addis, M. F., Carta, F., and Fiori, P. L. (1998). Mycoplasma hominis parasitism of trichomonas vaginalis. *Lancet* 352:1286. doi: 10.1016/S0140-6736(98)00041-5

Rigaud, T., Perrot-Minnot, M. J., and Brown, M. J. (2010). Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. *Proc. Biol. Sci.* 277, 3693–3702. doi: 10.1098/rspb.2010.1163

Robert-Gangneux, F., and Darde, M. L. (2012). Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin. Microbiol. Rev.* 25, 264–296. doi: 10.1128/CMR.05013-11

Robinson, T. M., Nelson, R., Artis, D., Scott, P., and Boyer, J. D. (2004). Experimental Leishmania major infection suppresses HIV-1 DNA vaccine induced cellular immune response. *Cells Tissues Organs* 177, 185–188. doi: 10.1159/000079992

Rodriguez, C. (2020). The global helminth belt and Covid-19: the new eosinophilic link. *Qeios*. doi: 10.32388/IWKQH9.2

Rogers, N. J., Hall, B. S., Obiero, J., Targett, G. A., and Sutherland, C. J. (2000). A model for sequestration of the transmission stages of plasmodium falciparum: adhesion of gametocyte-infected erythrocytes to human bone marrow cells. *Infect. Immun.* 68, 3455–3462. doi: 10.1128/IAI.68.6.3455-3462.2000

Rogers, K. J., Shtanko, O., Vijay, R., Mallinger, L. N., Joyner, C. J., Galinski, M. R., et al. (2020). Acute plasmodium infection promotes interferon-gamma-dependent resistance to Ebola virus infection. *Cell Rep.* 30:e 4044, 4041–4051.e4. doi: 10.1016/j.celrep.2020.02.104

Rooth, I. B., and Bjorkman, A. (1992). Suppression of plasmodium falciparum infections during concomitant measles or influenza but not during pertussis. *Am. J. Trop. Med. Hyg.* 47, 675–681. doi: 10.4269/ajtmh.1992.47.675

Rowe, J., Macaubas, C., Monger, T. M., Holt, B. J., Harvey, J., Poolman, J. T., et al. (2000). Antigen-specific responses to diphtheria-tetanus-acellular pertussis vaccine in human infants are initially Th2 polarized. *Infect. Immun.* 68, 3873–3877. doi: 10.1128/IAI.68.7.3873-3877.2000

Rurangirwa, F. R., Musoke, A. J., Nantulya, V. M., and Tabel, H. (1983). Immune depression in bovine trypanosomiasis: effects of acute and chronic *Trypanosoma congolense* and chronic *Trypanosoma vivax* infections on antibody response to Brucella abortus vaccine. *Parasite Immunol.* 5, 267–276. doi: 10.1111/j.1365-3024.1983.tb00743.x

Schietinger, A., and Greenberg, P. D. (2014). Tolerance and exhaustion: defining mechanisms of T cell dysfunction. *Trends Immunol.* 35, 51–60. doi: 10.1016/j.it.2013.10.001

Schmid-Hempel, P. (2009). Immune defence, parasite evasion strategies and their relevance for 'macroscopic phenomena' such as virulence. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 364, 85–98. doi: 10.1098/rstb.2008.0157

Schwebke, J. R. (2005). Trichomoniasis in adolescents: a marker for the lack of a public health response to the epidemic of sexually transmitted diseases in the United States. *J. Infect. Dis.* 192, 2036–2038. doi: 10.1086/498221

Scott, C. P., Kumar, N., Bishai, W. R., and Manabe, Y. C. (2004). Short report: modulation of mycobacterium tuberculosis infection by plasmodium in the murine model. *Am. J. Trop. Med. Hyg.* 70, 144–148. doi: 10.4269/ajtmh.2004.70.144

Selin, L. K., Brehm, M. A., Naumov, Y. N., Cornberg, M., Kim, S. K., Clute, S. C., et al. (2006). Memory of mice and men: CD8+ T-cell cross-reactivity and heterologous immunity. *Immunol. Rev.* 211, 164–181. doi: 10.1111/j.0105-2896.2006.00394.x

Sevede, D., Doumbia, M., Kouakou, V., Djehiffe, V., Pineau, P., and Dosso, M. (2019). Increased liver injury in patients with chronic hepatitis and IgG directed against hepatitis E virus. *EXCLI J.* 18, 955–961. doi: 10.17179/excli2019-1827

Sharpe, R. T., Langley, A. M., Mowat, G. N., Macaskill, J. A., and Holmes, P. H. (1982). Immunosuppression in bovine trypanosomiasis: response of cattle infected with *Trypanosoma congolense* to foot-and-mouth disease vaccination and subsequent live virus challenge. *Res. Vet. Sci.* 32, 289–293. doi: 10.1016/S0034-5288(18)32382-8

Sileghem, M., Darji, A., Remels, L., Hamers, R., and De Baetselier, P. (1989). Different mechanisms account for the suppression of interleukin 2 production and the suppression of interleukin 2 receptor expression in *Trypanosoma brucei*-infected mice. *Eur. J. Immunol.* 19, 119–124. doi: 10.1002/eji.1830190119

Sileghem, M., and Flynn, J. N. (1992). Suppression of interleukin 2 secretion and interleukin 2 receptor expression during tsetse-transmitted trypanosomiasis in cattle. *Eur. J. Immunol.* 22, 767–773. doi: 10.1002/eji.1830220321

Simoes, M. L., Goncalves, L., and Silveira, H. (2015). Hemozoin activates the innate immune system and reduces plasmodium berghei infection in Anopheles gambiae. *Parasit. Vectors* 8:12. doi: 10.1186/s13071-014-0619-y

Simon, P., Boffa, G., Ang, K. S., and Menault, M. (1982). Polycythaemia in a haemodialyzed anephric patient with hepatitis. Demonstration of erythropoietin secretion (author's transl). *Nouv. Press. Med.* 11, 1401–1403. PMID: 7079159

Smedman, L., Silva, M. C., Gunnlaugsson, G., Norrby, E., and Zetterstrom, R. (1986). Augmented antibody response to live attenuated measles vaccine in children with plasmodium falciparum parasitaemia. *Ann. Trop. Paediatr.* 6, 149–153. doi: 10.1080/02724936.1986.11748428

Sohrabi, Y., Dos Santos, J. C., Dorenkamp, M., Findeisen, H., Godfrey, R., Netea, M. G., et al. (2020). Trained immunity as a novel approach against COVID-19 with a focus on bacillus Calmette-Guerin vaccine: mechanisms, challenges and perspectives. *Clin. Transl. Immunol.* 9:e 1228. doi: 10.1002/cti2.1228

Sorvillo, F., Smith, L., Kerndt, P., and Ash, L. (2001). *Trichomonas vaginalis*, HIV, and African-Americans. *Emerg. Infect. Dis.* 7, 927–932. doi: 10.3201/eid0706.010603

Spina, C. A., Prince, H. E., and Richman, D. D. (1997). Preferential replication of HIV-1 in the CD45RO memory cell subset of primary CD4 lymphocytes in vitro. *J. Clin. Invest.* 99, 1774–1785. doi: 10.1172/JCI119342

Ssebambulidde, K., Segawa, I., Abuga, K. M., Nakate, V., Kayiira, A., Ellis, J., et al. (2020). Parasites and their protection against COVID-19- ecology or immunology? *Med. Rxiv.* doi: 10.1101/2020.05.11.20098053

Stanley, S. L. (2003). Amoebiasis. *Lancet* 361, 1025–1034. doi: 10.1016/S0140-6736(03)12830-9

Stewart, P. E., and Bloom, M. E. (2020). Sharing the ride: Ixodes scapularis symbionts and their interactions. *Front. Cell. Infect. Microbiol.* 10:142. doi: 10.3389/fcimb.2020.00142

Subauste, C. S., Wessendarp, M., Portilllo, J. A., Andrade, R. M., Hinds, L. M., Gomez, F. J., et al. (2004). Pathogen-specific induction of CD154 is impaired in CD4+ T cells from human immunodeficiency virus-infected patients. *J. Infect. Dis.* 189, 61–70. doi: 10.1086/380510

Swanson, S. J., Neitzel, D., Reed, K. D., and Belongia, E. A. (2006). Coinfections acquired from ixodes ticks. *Clin. Microbiol. Rev.* 19, 708–727. doi: 10.1128/CMR.00011-06

Tabel, H., Wei, G., and Shi, M. (2008). T cells and immunopathogenesis of experimental African trypanosomiasis. *Immunol. Rev.* 225, 128–139. doi: 10.1111/j.1600-065X.2008.00675.x

Takem, E. N., Roca, A., and Cunnington, A. (2014). The association between malaria and non-typhoid salmonella bacteraemia in children in sub-Saharan Africa: a literature review. *Malar. J.* 13:400. doi: 10.1186/1475-2875-13-400

Tamomh, A. G., Agena, A. M., Elamin, E., Suliman, M. A., Elmadani, M., Omara, A. B., et al. (2021). Prevalence of cryptosporidiosis among children with diarrhoea under five years admitted to Kosti teaching hospital, Kosti City, Sudan. *BMC Infect. Dis.* 21:349. doi: 10.1186/s12879-021-06047-1

Tangie, E., Walters, A., Hsu, N. J., Fisher, M., Magez, S., Jacobs, M., et al. (2022). BCG-mediated protection against *M. tuberculosis* is sustained post-malaria infection independent of parasite virulence. *Immunology* 165, 219–233. doi: 10.1111/imm.13431

U.S. Department of Health and Human Services (2018). *Report of the other tick-borne diseases and co-infections subcommittee to the tick-borne disease working group, HHS.Gov*. U.S. Department of Health and Human Services.

Usen, S., Milligan, P., Ethevenaux, C., Greenwood, B., and Mulholland, K. (2000). Effect of fever on the serum antibody response of Gambian children to *Haemophilus influenzae* type b conjugate vaccine. *Pediatr. Infect. Dis. J.* 19, 444–449. doi: 10.1097/00006454-200005000-00010

Vetter, V., Denizer, G., Friedland, L. R., Krishnan, J., and Shapiro, M. (2018). Understanding modern-day vaccines: what you need to know. *Ann. Med.* 50, 110–120. doi: 10.1080/07853890.2017.1407035

Vilaplana, C., Prats, C., Marzo, E., Barril, C., Vegue, M., Diaz, J., et al. (2014). To achieve an earlier IFN-gamma response is not sufficient to control mycobacterium tuberculosis infection in mice. *PLoS One* 9:e100830. doi: 10.1371/journal.pone.0100830

Wait, L. F., Dobson, A. P., and Graham, A. L. (2020). Do parasite infections interfere with immunisation? A review and meta-analysis. *Vaccine* 38, 5582–5590. doi: 10.1016/j.vaccine.2020.06.064

Walk, J., de Bree, L. C. J., Graumans, W., Stoter, R., van Gemert, G. J., van de Vegte-Bolmer, M., et al. (2019). Outcomes of controlled human malaria infection after BCG vaccination. *Nat. Commun.* 10:874. doi: 10.1038/s41467-019-08659-3

Walsh, J. A. (1989). Disease problems in the third world. *Ann. N. Y. Acad. Sci.* 569, 1–16. doi: 10.1111/j.1749-6632.1989.tb27354.x

Wang, H. C., Dann, S. M., Okhuysen, P. C., Lewis, D. E., Chappell, C. L., Adler, D. G., et al. (2007). High levels of CXCL10 are produced by intestinal epithelial cells in AIDS patients with active cryptosporidiosis but not after reconstitution of immunity. *Infect. Immun.* 75, 481–487. doi: 10.1128/IAI.01237-06

Wang, R. J., Li, J. Q., Chen, Y. C., Zhang, L. X., and Xiao, L. H. (2018). Widespread occurrence of cryptosporidium infections in patients with HIV/AIDS: epidemiology, clinical feature, diagnosis, and therapy. *Acta Trop.* 187, 257–263. doi: 10.1016/j.actatropica.2018.08.018

Wang, Z. D., Wang, S. C., Liu, H. H., Ma, H. Y., Li, Z. Y., Wei, F., et al. (2017). Prevalence and burden of toxoplasma gondii infection in HIV-infected people: a systematic review and meta-analysis. *Lancet HIV* 4, e177–e188. doi: 10.1016/S2352-3018(17)30005-X

Wang, L., Zhang, H., Zhao, X., Zhang, L., Zhang, G., Guo, M., et al. (2013). Zoonotic cryptosporidium species and *Enterocytozoon bieneusi* genotypes in HIV-positive patients on antiretroviral therapy. *J. Clin. Microbiol.* 51, 557–563. doi: 10.1128/JCM.02758-12

Wang, R., Zhang, X., Zhu, H., Zhang, L., Feng, Y., Jian, F., et al. (2011). Genetic characterizations of cryptosporidium spp. and giardia duodenalis in humans in Henan, China. *Exp. Parasitol.* 127, 42–45. doi: 10.1016/j.exppara.2010.06.034

Welker, Y., Molina, J. M., Poirot, C., Ferchal, F., Decazes, J. M., Lagrange, P., et al. (1993). Interaction between human immunodeficiency virus and toxoplasma gondii replication in dually infected monocytoid cells. *Infect. Immun.* 61, 1596–1598. doi: 10.1128/iai.61.4.1596-1598.1993

Welsh, R. M., and Selin, L. K. (2002). No one is naive: the significance of heterologous T-cell immunity. *Nat. Rev. Immunol.* 2, 417–426. doi: 10.1038/nri820

Wilairatana, P., Masangkay, F. R., Kotepui, K. U., Milanez, G. J., and Kotepui, M. (2021). Prevalence and characteristics of malaria among COVID-19 individuals: A systematic review, meta-analysis, and analysis of case reports. *PLoS Negl. Trop. Dis.* 15:e0009766. doi: 10.1371/journal.pntd.0009766

Williamson, W. A., and Greenwood, B. M. (1978). Impairment of the immune response to vaccination after acute malaria. *Lancet* 1, 1328–1329. doi: 10.1016/s0140-6736(78)92403-0

Wolday, D., Gebrecherkos, T., Arefaine, Z. G., Kiros, Y. K., Gebreegzabher, A., Tasew, G., et al. (2021). Effect of co-infection with intestinal parasites on COVID-19 severity: A prospective observational cohort study. *E Clin. Med.* 39:101054. doi: 10.1016/j.eclinm.2021.101054

Woodford, J., Sagara, I., Diawara, H., Assadou, M. H., Katile, A., Attaher, O., et al. (2022). Recent malaria does not substantially impact COVID-19 antibody response or rates of symptomatic illness in communities with high malaria and COVID-19 transmission in Mali, West Africa. *Front. Immunol.* 13:959697. doi: 10.3389/fimmu.2022.959697

World Health Organization (2015). "*Guidelines for the treatment of malaria*" *3d ed.* (Geneva: World Health Organization).

World Health Organization (2017). "*World malaria report 2017*". World Health Organization: Geneva.

World Health Organization (2021). "*World malaria report 2021*". World Health Organization: Geneva.

World Health Statistics (2022). "*Monitoring health for the SDGs, sustainable development goals*". (Geneva: World Health Organization).

Wormser, G. P., McKenna, D., Scavarda, C., Cooper, D., El Khoury, M. Y., Nowakowski, J., et al. (2019). Co-infections in persons with early Lyme disease, New York, USA. *Emerg. Infect. Dis.* 25, 748–752. doi: 10.3201/eid2504.181509

Xiao, L., Fayer, R., Ryan, U., and Upton, S. J. (2004). Cryptosporidium taxonomy: recent advances and implications for public health. *Clin. Microbiol. Rev.* 17, 72–97. doi: 10.1128/CMR.17.1.72-97.2004

Yang, Y., Zhou, Y., Cheng, W., Pan, X., Xiao, P., Shi, Y., et al. (2017). Prevalence and determinants of cryptosporidium infection in an underdeveloped rural region of southwestern China. *Am. J. Trop. Med. Hyg.* 96, 595–601. doi: 10.4269/ajtmh.16-0732

frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 23 December 2022
DOI 10.3389/fmicb.2022.1089394



OPEN ACCESS

EDITED BY
Varakorn Kosaisavee,
Mahidol University,
Thailand

REVIEWED BY
Muhammad Ehsan,
Islamia University of Bahawalpur, Pakistan
Rui-Si Hu,
University of Electronic Science and Technology of China, China
Chun-Ren Wang,
Heilongjiang Bayi Agricultural University, China
Qiaocheng Chang,
Shantou University, China
Sheila Donnelly,
University of Technology Sydney, Australia
Krystyna Cwiklinski,
University of Liverpool, United Kingdom

*CORRESPONDENCE
Weiyu Zhang
✉ zweiyu@gxu.edu.cn
Wenda Di
✉ diwenda@gxu.edu.cn

SPECIALTY SECTION
This article was submitted to Infectious Agents and Disease, a section of the journal Frontiers in Microbiology

RECEIVED 04 November 2022
ACCEPTED 02 December 2022
PUBLISHED 23 December 2022

CITATION
Zheng M, Jiang X, Kong X, Guo Y, Zhang W and Di W (2022) Proteomic analysis of *Fasciola gigantica* excretory and secretory products (*Fg*ESPs) co-immunoprecipitated using a time course of infected buffalo sera. *Front. Microbiol.* 13:1089394. doi: 10.3389/fmicb.2022.1089394



# Proteomic analysis of *Fasciola gigantica* excretory and secretory products (*Fg*ESPs) co-immunoprecipitated using a time course of infected buffalo sera

Mengwei Zheng[1,2,3,4], Xuelian Jiang[1,2,3,4], Xinping Kong[1,2,3,4], Yanfeng Guo[1,2,3,4], Weiyu Zhang[1,2,3,4]* and Wenda Di[1,2,3,4]*

[1]College of Animal Science and Technology, Guangxi University, Nanning, China, [2]Guangxi Zhuang Autonomous Region Engineering Research Center of Veterinary Biologics, Nanning, China, [3]Guangxi Key Laboratory of Animal Reproduction, Breeding and Disease Control, Nanning, China, [4]Guangxi Colleges and Universities Key Laboratory of Prevention and Control for Animal Disease, Nanning, China

**Introduction:** Widespread *Fasciola gigantica* infection in buffaloes has caused great economic losses in buffalo farming. Studies on *F. gigantica* excretory and secretory products (*Fg*ESP) have highlighted their importance in *F. gigantica* parasitism and their potential in vaccine development. Identifying *Fg*ESP components involved in *F. gigantica*-buffalo interactions during different periods is important for developing effective strategies against fasciolosis.

**Methods:** Buffaloes were assigned to non-infection ($n$=3, as control group) and infection ($n$=3) groups. The infection group was orally administrated 250 metacercariae. Sera were collected at 3, 10, and 16weeks post-infection (wpi) for the non-infection group and at 0 (pre-infection), 1, 3, 6, 8, 10, 13, and 16 wpi for the infection group. *Fg*ESP components interacting with sera from the non-infection and infection groups assay were pulled down by co-IP and identified using LC–MS/MS. Interacting *Fg*ESP components in infection group were subjected to Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathway and gene ontology (GO) functional annotation to infer their potential functions.

**Results and discussion:** Proteins of *Fg*ESP components identified in the non-infection group at 3, 10, and 16 wpi accounted for 80.5%, 84.3%, and 82.1% of all proteins identified in these three time points, respectively, indicating surroundings did not affect buffalo immune response during maintenance. Four hundred and ninety proteins were identified in the infection group, of which 87 were consistently identified at 7 time points. Following GO analysis showed that most of these 87 proteins were in biological processes, while KEGG analysis showed they mainly functioned in metabolism and cellular processing, some of which were thought to functions throughout the infection process. The numbers of specific interactors identified for each week were 1 ($n$=12), 3 ($n$=5), 6 ($n$=8), 8 ($n$=15), 10 ($n$=23), 13 ($n$=22), and 16 ($n$=14) wpi, some of which were thought to functions in specific infection process. This study screened the antigenic targets in *Fg*ESP during a dense time course over a long period. These findings may enhance the

understanding of molecular *F. gigantica*-buffalo interactions and help identify new potential vaccine and drug target candidates.

KEYWORDS

***Fasciola gigantica*, co-immunoprecipitation, excretory and secretory products, interaction, LC–MS/MS, screening**

# 1. Introduction

Fasciolosis is a widespread zoonotic disease caused by *Fasciola hepatica* and *Fasciola gigantica* that primarily affects public health and economically important livestock. It is considered as one of the top 17 neglected tropical diseases (Piedrafita et al., 2010). *Fasciola hepatica* mainly infects sheep and cattle worldwide, while *F. gigantica* mainly infects buffalo in the subtropic and tropic zones (Doy and Hughes, 1984; Chen et al., 2000; Zhang et al., 2005; Aghayan et al., 2019; Niedziela et al., 2021). Since its infection of livestock leads to annual economic losses of >$3 billion worldwide (Calvani and Šlapeta, 2021). In addition, at least 2.4 million individuals are infected worldwide and 180 million are at risk of new infections (Meemon and Sobhon, 2015). Despite affecting human and livestock health in an area that represents up to 77% of the global population, research interest in *F. gigantica* consistently lags behind that of *F. hepatica* (Agatsuma et al., 2000), and little is known about the factors that contribute to the pathogenicity and virulence of *F. gigantica*.

*F. gigantica* metacercariae ingestion by the definitive host leads to excystation and the release of newly excysted juveniles (NEJs) that burrow through the duodenal wall into the peritoneum. They then move toward the liver and penetrate the liver capsule. The immature flukes migrate through the liver for 11 weeks, reaching and maturing in the bile ducts for 12–16 weeks post-infection (wpi), after which they commence egg laying (Calvani and Šlapeta, 2021). The *F. gigantica* life cycle in definitive mammalian hosts largely relies on excretory and secretory products (*Fg*ESP) since they act as antigens that stimulate humoral and cell-mediated immunity and also function in fluke survival and host–parasite interactions (El-Ghaysh et al., 1999; Zhang et al., 2006; Novobilsky et al., 2007; Hacariz et al., 2011; Wang et al., 2021). Some *FgESP* components, such as cathepsin L1, cathepsin B, saposin-like protein 2 (SAP-2), have been identified to identify potential vaccine candidates (Chantree et al., 2013; Kueakhai et al., 2013, 2015).

Previous proteomic studies have shown that the *Fg*ESP release profile varies across three developmental stages: the NEJ 24 h post-excystment, immature fluke 21 days post-infection (immature), and adult (Lalor et al., 2021). In the early infection stage, NEJs secrete a range of stage-specific peptidases and proteolytic-related proteins to break down extracellular matrix components that maintain tissue integrity and participate in fluke invasion (Di Maggio et al., 2019; Davey et al., 2022). During the liver migratory phase, immature fluke secretions are dominated by peptidases involved in blood digestion, cathepsin peptidases, and their inhibitors to support tissue penetration and blood feeding (Lalor et al., 2021). Once adults arrive at the bile duct, they feed on and detoxify bile components by expressing cathepsin L and B peptidases, enzymes, peptidase inhibitors, legumain, helminth defense molecules, and glycoproteins (Meemon et al., 2004; Ghosh et al., 2005; Adisakwattana et al., 2007; Sansri et al., 2013; Ryan et al., 2020; Cwiklinski and Dalton, 2022), some of which function in immunoregulation (Ticho et al., 2020). Therefore, it is vital to identify the *Fg*ESP components produced by *F. gigantica* at different developmental stages to understand molecular buffalo-*F. gigantica* interactions and the *F. gigantica* development process in buffalo. However, difficulties in obtaining parasites at different developmental stages *in vivo* and *in vitro* make it impossible to obtain and study *Fg*ESPs at different developmental stages. Consequently, *Fg*ESPs produced by adults, which can be easily obtained, were identified in buffalo serum during different *F. gigantica* infection periods to identify changes in them.

Huang first explored the interaction of *FgESP* with buffalo serum at three-time points (6, 10, and 14 wpi; Huang et al., 2019a). Considering the complex interaction mechanisms between *F. gigantica* and buffalo at larval and adult stages, it is still required to conduct continuous and periodic observations concerning host-pathogen interactions. Furthermore, the recent completion of genome and transcriptome sequencings (Zhang et al., 2017; Pandey et al., 2020; Luo et al., 2021) enables us to obtain more protein sequence information (UniProt *F. gigantica* database; downloaded on 2021/11/19) of *F. gigantica* in public databases. The sera of *F. gigantica*-infected buffalo were collected at seven time points (1, 3, 6, 8, 10, 13, and 16 wpi) and co-immunoprecipitated (co-IP) to pull down *Fg*ESP components that interacted with them. These components were characterized by liquid chromatography–tandem mass spectrometry (LC–MS/MS) and bioinformatics. This approach can be used to analyze specific proteins and provide a reliable basis for the screening of diagnostic antigens of *F. gigantica*.

# 2. Materials and methods

## 2.1. Preparation of buffalo serum representing different infection periods

*Fasciola gigantica* metacercariae were collected from *Galba pervia* experimentally infected with miracidia, encysted on 4 $cm^2$

polythene strips, and stored in distilled water at 4°C until required. Each metacercariae batch was examined for viability and then counted.

Six 6-month-old buffaloes of Murrah, Nili-Ravi, Mediterranean, and their crossbreds with indigenous buffaloes in Guangxi (China) were randomly assigned to non-infection (A1, A2, and A3) and infection (B1, B2, and B3) groups, with three in each group (Supplementary Table S1). They were stall-fed on a balanced diet in the dairy of the Buffalo Research Institute, Chinese Academy of Agricultural Sciences, and Guangxi Zhuang Nationality Autonomous Region. They were confirmed free from fluke infection through indirect *Fg*ESP enzyme-linked immunosorbent assays (ELISA; Supplementary Table S2) and coprological examination (Zhang et al., 2006). In week 0, the infection group was given a gelatine capsule containing 250 viable *F. gigantica* metacercariae, while the non-infection group were mock-inoculated with 0.85% sodium chloride solution without metacercariae, the mean numbers of flukes recovered were 55.5 ± 14.1 (22.2 ± 5.6 of infection dose) in infection group (Wang et al., 2022b). Whole blood was collected from the non-infection (3, 10, and 16 wpi) and infection (0, 1, 3, 6, 8, 10, 13, and 16 wpi) groups for serum preparation and stored at −80°C until needed.

## 2.2. *Fg*ESP preparation

*Fg*ESPs were prepared as previously described (Novobilský et al., 2007). Briefly, adult *F. gigantica* were collected from infected buffaloes' livers and washed three times in warm phosphate-buffered saline (PBS, pH 7.2) to remove the residual material. Next, flukes were incubated in sterile Roswell Park Memorial Institute (RPMI) 1,640 media supplemented with antibiotics and antimycotics (10,000 UI/ml penicillin G and 10 mg/ml amphotericin B) at 37°C for 2 h. Then, flukes were transferred into sterile RPMI 1640 media and incubated at 37°C for a further 5 h. After incubation, the supernatant was centrifuged at 2,500 *g* for 30 min at 4°C and then filtered through a 0.22 μm nylon filter. Finally, the supernatant was concentrated, freeze-dried into a powder, and stored at −80°C. Before use, the powder was dissolved in deionized water. Its protein concentration was determined using a Bicinchoninic Acid (BCA) Assay Kit (Beijing Solarbio Science & Technology Co., Ltd., China).

## 2.3. Co-IP of *Fg*ESP-antibody binding proteins

The Protein A/G Plus-Agarose Immunoprecipitation Kit (Santa Cruz Biotechnology, USA) was used to pull down the *Fg*ESP-serum antibody binding proteins according to the manufacturer's instructions. For the non-infection group, 5 mg of *Fg*ESPs was incubated with 1 ml of serum (A1, A2, and A3 at 3, 10, and 16 wpi) and 20 μl of Protein A/G Plus-Agarose Beads at 4°C for 2 h. Next, pellets were collected by centrifugation at 1,000 *g* and 4°C for 5 min. Then, the pellets were washed three times with 500 μl PBS and centrifugation at 1,000 *g* and 4°C for 5 min. After the final washing, the sediment was resuspended in 50 μl PBS, and 10 μl was used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The remaining 40 μl was used for LC–MS/MS identification.

For the infection group, 5 mg of *Fg*ESPs was precleared (negative serum and *FgESP* pull down non-specific interaction proteins through Co-IP) by incubation with 1 ml of negative (week 0) serum and 20 μl of Protein A/G Plus-Agarose Beads at 4°C for 2 h. After pelleting the beads by centrifugation at 1,000 *g* and 4°C for 5 min, the supernatant was transferred and divided equally into three fresh tubes. Next, 500 μl of corresponding buffalo sera (B1, B2, and B3 at 1, 3, 6, 8, 10, 13, and 16 wpi) was added to each tube with 20 μl of Protein A/G Plus-Agarose Beads and incubated at 4°C overnight. The pellet was collected by centrifugation at 1,000 *g* and 4°C for 5 min. The pellets were washed three times with 500 μl PBS and centrifugation at 1,000 *g* and 4°C for 5 min. After the final washing, sediments were resuspended in 50 μl PBS, and 10 μl was used for SDS-PAGE analysis. The remaining 40 μl was used for LC–MS/MS identification.

## 2.4. In-solution trypsin digestion

Liquid mass spectrometry (LMS) was performed by gel chromatography, and the protein solution was conducted to SDS-PAGE, then the targets band was extracted from the gel and cut into 0.5 mm cubes. Next, the decolorized gel was washed three times with acetonitrile solution until gelatinous particles were completely white. Then, 500 μl of 10 mM dithiothreitol was added and incubated at 56°C for 30 min. Next, 500 μl of a decolorizing solution was added and mixed at room temperature for 10 min. Then, the gelatinous particles were centrifuged at 3,000 *g* to remove the supernatant. Next, 500 μl of 55 mM iodoacetamide was added and incubated for a further 30 min at room temperature before being centrifuged at 3,000 *g*. Then, 500 μl of decolorizing solution was added and incubated for 10 min at room temperature before being centrifuged at 3,000 *g* to remove the supernatant. Next, 500 μl of acetonitrile was added until the micelles were completely whitened and then vacuum-dried for 5 min. Then, trypsin was added according to the gel volume and incubated in an ice bath for 30 min. Next, 25 mM ammonium bicarbonate (pH 8.0) was added and incubated at 37°C overnight. Then, 300 μl of extraction solution (60% acetonitrile and 5% formic acid) was added and sonicated for 10 min. Finally, the solution was centrifuged at 3,000 *g*, and the supernatant was collected and vacuum-dried.

## 2.5. LC–MS/MS analysis

The sample was dissolved with 20 μl of 0.2% trifluoroacetate, centrifuged at 10,000 rpm for 20 min, and dried with a vacuum

concentrator (LaboGene, SCAN SPEED 40, Denmark). Samples were then adjusted to 1 μg/μL using the machine's buffer. The sample volume was set to 5 μl, and the collection scan mode was set to 60 min. In the sample, we scanned for peptides with a mass-to-charge ratio of 350–1,200. The mass spectrometry data was collected using the Triple TOF 5600 + LC/MS system (AB SCIEX, USA). The peptide samples were dissolved in 2% acetonitrile with 0.1% formic acid and analyzed using the Triple TOF 5600 Plus mass spectrometer coupled with the Eksigent nanoLC system (AB SCIEX, USA). The peptide solution was added to the C18 capture (3 μm; 350 μm × 0.5 mm; AB Sciex, USA) and C18 analytical (3 μm; 75 μm × 150) columns with a 60 min time gradient and a 300 nl/min flow rate for gradient elution. The two mobile phases were buffers A (2% acetonitrile, 0.1% formic acid, and 98% water) and B (98% acetonitrile, 0.1% formic acid, 2% water). For information-dependent acquisition, the MS spectrum was scanned with a 250 ms ion accumulation time, and the MS spectrums of 30 precursor ions were acquired with a 50 ms ion accumulation time. The MS1 spectrum was collected in the range 350–1,200 m/z, and the MS2 spectrum was collected in the range 100–1,500 m/z. The precursor ion dynamic exclusion time was set to 15 s.

## 2.6. Data analysis

The raw MS/MS files were submitted to ProteinPilot (version 4.5,[1] SCIEX, Redwood City, CA, USA) for analysis. ProteinPilot's Paragon algorithm was used to search the UniProtKB-A1E5T4 (A1E5T4_FASGI) database (access time is 2021/11/19) and identify proteins using the following parameters: TripleTOF 5,600, cysteine modification with iodoacetamide, and biological modification as the ID focus. The identified protein results were subject to certain filtering criteria. Peptides with an unused score > 1.3 (credibility of >95%) were considered credible, and proteins containing at ≥1 unique peptide were retained.

# 3. Results

## 3.1. *Fasciola gigantica* infection confirmation

*Fasciola gigantica* infection was confirmed in the three buffaloes in the infection group based on positive indirect *Fg*ESP-based ELISA findings 2 wpi. *F. gigantica* eggs were also detected in the faeces between 12 and 14 wpi. In addition, autopsies at 16 wpi found livers from the infection group to show obvious gross pathological lesions, and adult flukes were detected and the mean numbers of flukes recovered were 55.5 ± 14.1 (22.2 ± 5.6 of infection dose), indicating established infections (Wang et al., 2022a). All buffaloes in the non-infection group had negative indirect *Fg*ESP-based ELISA findings.

## 3.2. SDS-PAGE confirmation

SDS-PAGE indicated that serum-derived antibodies could recognize and pull down specific *Fg*ESP components at different infection periods in the non-infection and infection groups (Figure 1). The molecular weights of majority of specific proteins identified and pulled down by non-infection groups ranged from 25.0 kDa to 116.0 kDa, while infection group ranged from 18.41 kDa to 116.0 kDa.

## 3.3. LC–MS/MS analysis of non-infection and infection groups

In the non-infection group, individual buffalo were applied to analyze the percent of interacting proteins in three stages of infection: early (3 wpi), middle (10 wpi) and late (16 wpi), and the effect of external environment on the experiment was evaluated by the percentage of the number of shared proteins identified in the three stages compared with the total number of proteins in the three stages. A1W3:318/395 = 80.5%; A1W10:318/426 = 74.6%; A1W16:318/416 = 76.4%, A2 and A3 were also displayed (Table 1).

Overall, 509, 533, and 519 specific proteins were identified in buffaloes A1, A2, and A3, of which 419 were identified in all three, accounting for 82.3, 78.6, and 80.7% of all proteins identified at 3, 10, and 16 wpi, respectively (Table 2; Figure 2). As total of 632 proteins were identified in all three buffaloes, 3 wpi accounting for 80.5% of all proteins identified, 10 and 16 wpi accounting for 84.3, and 82.1% of all proteins identified, respectively (Table 2; Supplementary Table S3).

In the infection group, 490 specific proteins were identified across all examined wpi. The numbers identified were 171 (1 wpi), 109 (3 wpi), 186 (6 wpi), 230 (8 wpi), 248 (10 wpi), 251 (13 wpi), and 237 (16 wpi). Overall, 87 proteins were identified consistently across all examined wpi. The numbers of specific proteins to each wpi were 12 (1 wpi), 5 (3 wpi), 8 (6 wpi), 15 (8 wpi), 23 (10 wpi), 22 (13 wpi), and 14 (16 wpi), respectively (Figure 3).

## 3.4. Analysis of consistently detected proteins in the infection group

Gene Ontology (GO) classification was used to investigate the biological function of the 87 proteins consistently identified in the infection group. They were clustered into the "biological process," "cellular component," and "molecular function" categories. Within the "biological process" category, proteins

1 https://sciex.com.cn/products/software/

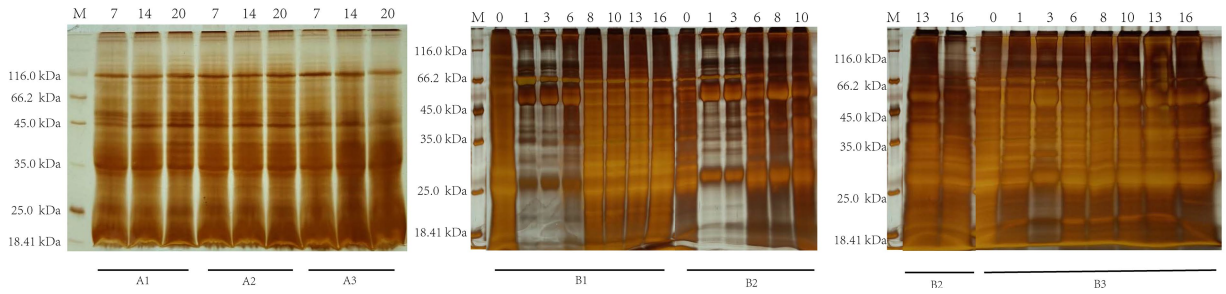


FIGURE 1
SDS-PAGE analysis of buffalo serum cocultured with *Fg*ESPs during different infection periods. The numerical value above represents the serum's wpi. The capital letter below represents the ID of buffaloes from the non-infection (A1, A2, and A3) and infection (B1, B2, and B3) groups.

**TABLE 1** The percent of shared number accounting number of specific week in the non-infection group buffaloes.

| | 3&10&16 wpi | 3 wpi | | 10 wpi | | 16 wpi | |
|---|---|---|---|---|---|---|---|
| | Shared number | Number (N3) | Percent (%) shared / N3 | Number (N10) | Percent (%) shared / N10 | Number (N16) | Percent (%) shared / N16 |
| A1 | 318 | 395 | 80.5 | 426 | 74.6 | 416 | 76.4 |
| A2 | 313 | 418 | 74.9 | 416 | 74.2 | 425 | 73.6 |
| A3 | 299 | 355 | 84.2 | 413 | 72.4 | 409 | 73.1 |

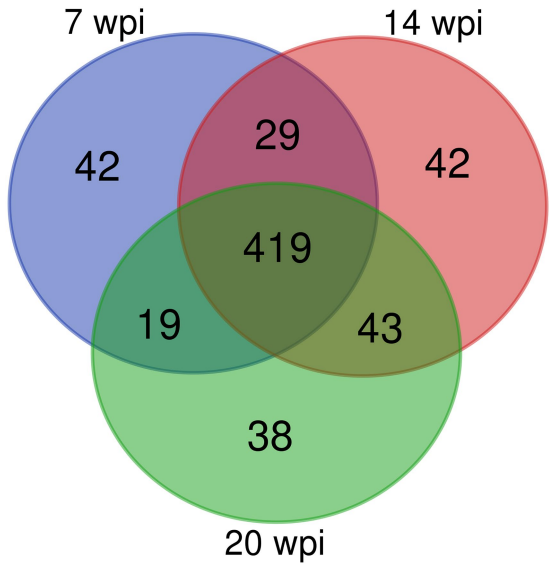


FIGURE 2
A Venn diagram showing the number of overlapping proteins pulled down by non-infection group's buffalo serum using the *Fg*ESPs.

clustered in the "cellular process" (25.8%), "metabolic process" (19.1%), "biological regulation" (11.5%), "developmental process" (9.6%), "response to stimulus" (7.7%), and "multicellular organismal process" (6.7%) subcategories. Within the "cellular component" category, the proteins clustered in the "cellular anatomical entity" (81.2%) and "protein-containing complex" (18.8%) subcategories. Within the "molecular function" category, the proteins mainly clustered in the "binding" (46.0%) and "catalytic activity" (41.6%) subcategories, with other subcategories accounting for much smaller proportions (Figure 4A; Supplementary Table S4).

Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations suggested that the most abundant pathways represented by the 87 proteins were "global and overview maps," "amino acid metabolism," and "carbohydrate metabolism" in "metabolism," followed by "cell growth and death" in "cellular processes" (Figure 4B; Table 3). Furthermore, 24 of the 87 proteins were annotated in more than one KEGG pathway (Supplementary Table S4). These included glycometabolism-related proteins, such as phosphoglucomutase, glutamate dehydrogenase, UTP-glucose-1-phosphate uridylyltransferase, fructose-bisphosphate aldolase, and malate dehydrogenase, which were annotated in ≥5 KEGG pathways. In addition, 14–3-3 proteins (Chaithirayanon et al., 2004; Tian et al., 2018), ferritin (Caban-Hernandez et al., 2012), Fh5 (Rossjohn et al., 1997), and heat shock proteins (HSPs; Moxon et al., 2010) were also annotated in KEGG pathways.

The subcellular localizations of the 87 proteins were cytoplasmic (35.8%), cytoplasm and nucleus (21.9%), and mitochondrial (14.6%; Figure 4C; Supplementary Table S4).

TABLE 2 The percent of shared number accounting the number of specific week and the number of specific weeks accounting the number of all periods in the non-infection group.

| wpi | Shared number | Number of specific week (N1) | Percent (%) shared / N1 | Number of three periods (N2) | Percent (%) N1 / N2 |
|---|---|---|---|---|---|
| 3 | 419 | 509 | 82.3 | 632 | 80.5 |
| 10 | | 533 | 78.6 | | 84.3 |
| 16 | | 519 | 80.7 | | 82.1 |

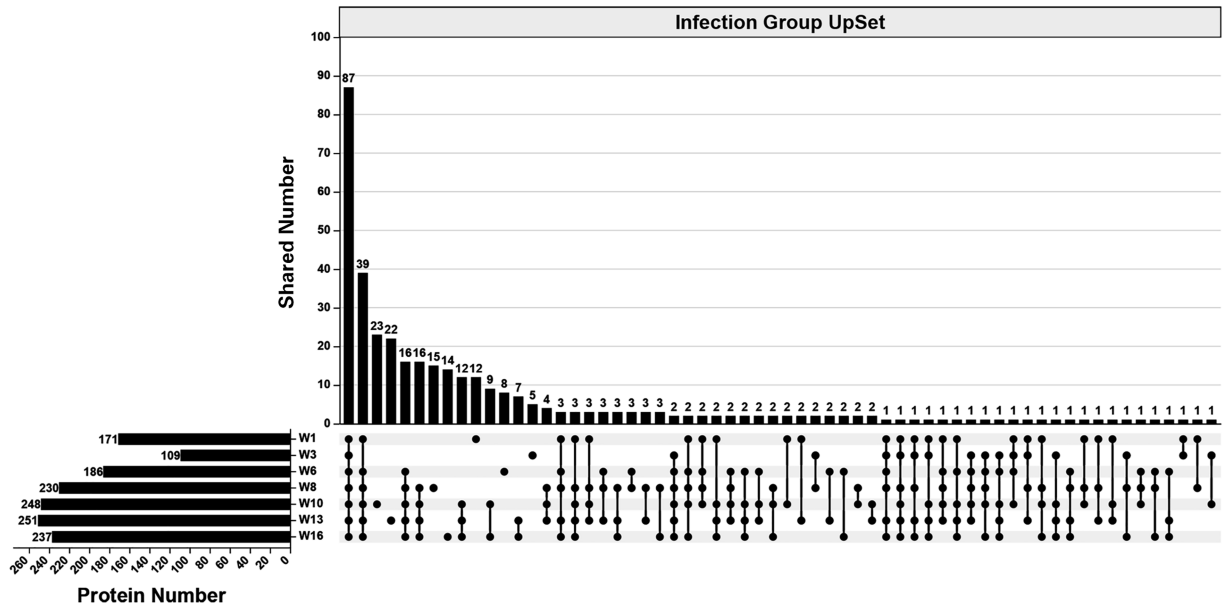


FIGURE 3
An UpSet diagram showing the number of unique and shared proteins in the infection group across 1, 3, 6, 8, 10, 13, and 16 wpi.

## 3.5. Specific proteins detected in the infection group

Partial proteins detected in *Fg*ESPs from the buffaloes' sera single and multiple wpi are shown in Supplementary Table S5. Since this study has described or clustered these proteins into specific KEGG pathways, their functions, such as calcium binding, could be inferred. Complete lists of proteins identified at a single wpi or across multiple wpis are provided in Supplementary Tables S6, S7.

# 4. Discussion

This study used SDS-PAGE to confirm the co-IP assay. The non-infection control group showed that many identified proteins were shared across time points (3, 10, and 16 wpi), suggesting that the buffaloes' surroundings did not affect their immune response during maintenance. While we identified numerous proteins in the infection group already reported with *F. gigantica*, we described some unique proteins associated with *F. gigantica* and used KEGG database and subcellular localization analyses to infer their potential functions.

KEGG analysis of the 87 proteins continuously identified in the infection group showed that some are associated with various signaling pathways (Table 3), including cytochrome-P450-related drug metabolism (Fh51, prostaglandin-H2 D-isomerase, and glutathione transferase), hippo signaling (cardiac muscle alpha-actin), estrogen signaling (HSP90 alpha [HSP90α]), interleukin (IL)-17 signaling (HSP90α), Th17 cell differentiation (HSP90α), phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT) signaling (HSP90α), nucleotide-binding oligomeric domain (NOD)-like receptor (NLR) signaling (HSP90α), forkhead box O (FOXO) signaling (phosphoenolpyruvate carboxykinase), Wnt signaling (cAMP-dependent protein kinase catalytic subunit alpha), and longevity regulation (activation protein theta polypeptide).

Anthelmintics can be neutralized or bio-transformed jointly or independently by three protein-level defense systems, termed phases I to III (Cvilink et al., 2008). In vertebrates and most invertebrates, the phase I pathway is oxidative *via* the cytochrome P450 superfamily

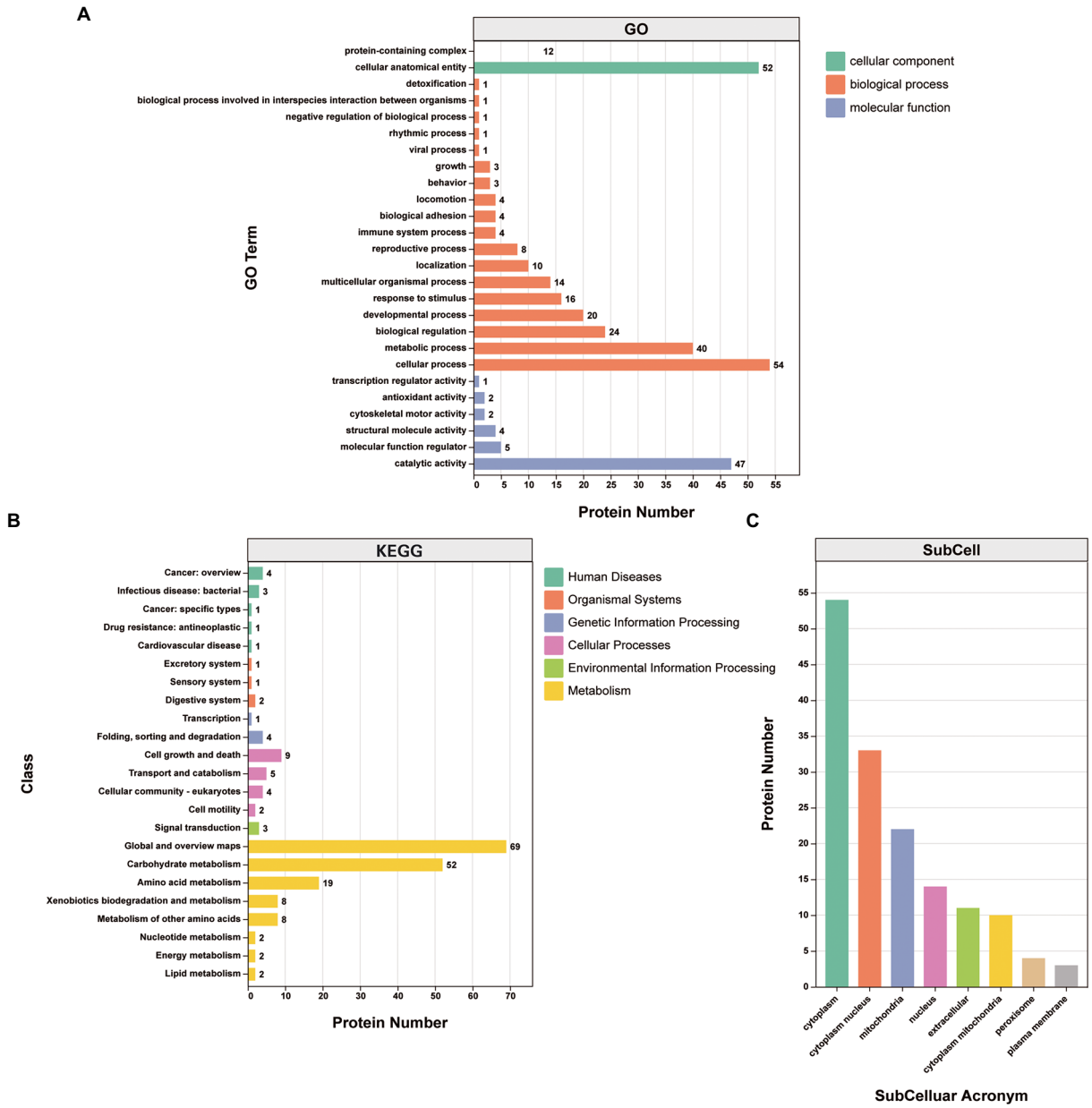


FIGURE 4
Analysis of the 87 proteins consistently identified in the infection group. **(A)** GO annotation clustered the proteins into three categories: molecular function, cellular component, and biological process. GO annotation and classifications are shown based on secondary names. While the horizontal axis represents protein numbers, the vertical axis represents GO secondary names. **(B)** KEGG pathway protein annotation and its corresponding category in the KEGG database are shown in different colors. While the horizontal axis represents protein numbers, the vertical axis represents the KEGG class names. **(C)** Protein subcellular localization. While the horizontal axis represents subcellular classification, the vertical axis represents protein numbers.

(Brophy et al., 2012). However, parasitic helminths are much less able to neutralize external toxins (xenobiotics) than their mammalian hosts (Cvilink et al., 2009), potentially reflecting their lack of important phase I cytochrome P-450-dependent detoxification components. Studies have shown that Glutathione-S-transferase (GST), ATP-binding cassette (ABC), fatty acid-binding protein and adenosine deaminase (ADA) in the excretory products of fluke functions in detoxification during the parasitic process (Morphew et al., 2007; Kumkate et al., 2008; Kalita et al., 2017; Rehman et al., 2020), and the alteration of ADA activity could induce the host immune responses switch to Th-2 type and facilitate the establishment of flukes within their host (Rehman et al., 2021). In addition to the above proteins, KEGG analysis in the present study showing that Fh51, prostaglandin-H2 D-isomerase, and glutathione transferase has been identified and clustered to cytochrome-P450-related xenobiotic (drug) metabolism, indicating these three proteins

TABLE 3 Partial proteins consistently identified in the infection group.

| Acc | Protein description | Peptide | Unique peptide | Coverage (%) | Length | Mass |
|---|---|---|---|---|---|---|
| tr\|A0A504Z0W5\|A0A504Z0W5_FASGI | Fh51 | 1 | 1 | 15.89 | 107 | 1,2544.3 |
| tr\|A0A504Z3A0\|A0A504Z3A0_FASGI | Prostaglandin-H2 D-isomerase | 14 | 14 | 66.82 | 211 | 2,4568.3 |
| tr\|A0A504YW63\|A0A504YW63_FASGI | Glutathione transferase | 10 | 10 | 29.07 | 313 | 3,5968.5 |
| tr\|A0A504YYH3\|A0A504YYH3_FASGI | Cardiac muscle alpha actin | 15 | 1 | 41.07 | 375 | 4,1696.3 |
| tr\|A0A504YG42\|A0A504YG42_FASGI | Heat shock protein heat shock protein 90 alpha | 27 | 25 | 40.3 | 722 | 8,2427.7 |
| tr\|A0A504YUP2\|A0A504YUP2_FASGI | Phosphoenolpyruvate carboxykinase (GTP) | 36 | 36 | 70 | 550 | 6,1415.2 |
| tr\|A0A504YN48\|A0A504YN48_FASGI | cAMP-dependent protein kinase catalytic subunit alpha | 3 | 3 | 14.02 | 321 | 37,344 |
| tr\|A0A504YX93\|A0A504YX93_FASGI | Tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein theta polypeptide | 15 | 13 | 66.27 | 252 | 2,8661.1 |

may also involve in detoxification during *F. gigantica* parasitism (Alirahmi et al., 2010; Kalita et al., 2019).

As a highly conserved molecular chaperone protein, HSP90 involved in signal transduction, cell cycle control, stress management and folding, degradation, and transport of proteins (Johnson, 2012; Roy et al., 2012; Gillan and Devaney, 2014; Hoter et al., 2018; Zininga et al., 2018; Biebl and Buchner, 2019; Backe et al., 2020). HSP90 also has been thought involved in host immune system modulation *via* platyhelminth secretomes (Liu et al., 2009; Xu et al., 2020). There are two cellular subtypes of HSP90, while HSP90α isoforms been secreted from cells, HSP90β isoforms (HSP90β) primarily operate intracellularly (Jayaprakash et al., 2015). In this study, HSP90α has been identified and clustered to interleukin (IL)-17 signaling, Th17 cell differentiation, phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT) signaling, nucleotide-binding oligomeric domain (NOD)-like receptor (NLR) signaling.

The IL-17 family is a cytokine subgroup that plays crucial roles in host defense against microbes and inflammatory disease development (Chen et al., 2017). IL-17E (also called IL-25) is associated with type 2 T helper cell (Th2) response, promoting Th2-related cytokine production for eosinophil recruitment and contributing to host defense against parasitic helminth infections (Pan et al., 2001; Ballantyne et al., 2007; Saenz et al., 2008; Kang et al., 2012). Recently, researchers found that peripheral blood lymphocytes (pBLs) significant upregulated Th2/Th17 type immune response at 3 and 42 dpi in buffaloes infected with *F. gigantica* (Hu et al., 2022), which was consistent with previous studies showing that the Th1-related response is inhibited early in *F. gigantica* infection, while the Th2-related response favoring parasitism is promoted (Molina, 2005; Rodriguez et al., 2017). HSP90α may regulates the IL-17 signaling pathway during early infection, enabling *F. gigantica* host parasitism. Therefore, it can infer that *F. gigantica* further participates in the Th2 / Th17 type immune response by secreting HSP90α in the IL-17 signaling pathway in the early stage of infection, thus regulating the host immune developed to a direction conducive to fluke survival.

Although HSP90α was identified in *Fg*ESP, it may also function in intracellular process. The PI3K-AKT signaling pathway regulates the number of neoblast/pluripotent cells in *Schmidtea mediterranea* (Peiris et al., 2016) and is essential for enhancing pluripotent cell survival (Hossini et al., 2016). Neoblast/pluripotent cells were produced and proliferated throughout the *F. hepatica* life cycle (McCusker et al., 2016; Cwiklinski et al., 2018), suggesting its key role in *Fasciola* growth and development. Studies have identified cell surface location-chaperone, and assign their functions to the recognition of infectious agents or their components and subsequent intracellular signaling (Henderson et al., 2006). Considering molecular chaperone characteristic of HSP90α, together with its clustering to PI3K-AKT signaling pathway, HSP90α was supposed to regulate the Neoblast/pluripotent through the PI3K-AKT signaling pathway, which ultimately regulate the growth and development of *F. gigantica*. Cytoplasmic NLRs function as innate pattern recognition receptors, the first line of defense against microbial infection

(Zhang et al., 2018) that recognize pathogens, recruit innate immune cells, and activate adaptive immune responses (Fukata et al., 2009; Cooney et al., 2010). NOD1 and NOD2 proteins can be recruited to the plasma membrane and regulate nuclear factor kappa-light chain enhancers of activated B-cell signaling and mitogen-activated protein kinase (MAPK) pathway (Philpott et al., 2014). We hypothesize that HSP90α suppresses host innate and adaptive immune responses through the NLR signaling pathway, enhancing *F. gigantica* survival.

Wnt signaling pathway including canonical Wnt β-catenin-dependent and non-canonical Wnt/Ca2+ signaling pathways (Ovchinnikov et al., 2020), which can initiate and regulate various cellular activities (including cell proliferation and calcium homeostasis), regulate the establishment of the anterior–posterior axis (AP axis) and the medial-lateral axis (Petersen and Reddien, 2009; De Robertis, 2010), also involved in the neural system formation (Adell et al., 2009). While canonical pathway can be modulated to alter glucose concentrations in the blood and surrounding tissues (Zhou et al., 2014; Chen et al., 2018), the non-canonical pathway mediates inflammatory responses, leading to suppression of host inflammatory responses by inhibiting positive feedback mechanisms (De, 2011).

Some of the 87 proteins consistently identified in the infection group were not assigned a KEGG signaling pathway, including ferritin. A recent study showed that ferritin in *Fh*ESPs separated by 2D electrophoresis did not react with infected sheep serum, suggesting that ferritin was a non-immunogenic *Fh*ESP protein (Becerro-Recio et al., 2021). However, this study found that ferritin consistently reacted with serum from *F. gigantica*-infected buffalo, indicating that ferritin in *Fg*ESPs is a complete antigen. Therefore, ferritin's function in *Fg*ESPs needs to be explored further.

Five of the 19 proteins consistently identified during the invasive infection phase (1–3 wpi) were uncharacterized (Supplementary Tables S6, S7). The microtubule-associated protein Futsch was associated with biological processes in GO taxonomic annotation. Microtubulin is a benzimidazole (BZ) target extensively studied in parasitology (von Samson-Himmelstjerna et al., 2007). A study using triclabendazole (TCBZ), a BZ derivative used to treat fascioliasis, showed that *F. hepatica*'s microtubule-mediated functions were inhibited by TCBZ exposure, suggesting that microtubule proteins may be effective TCBZ targets (Hanna, 2015).

Polyubiquitin proteins and three histones (H2A, H2B, and H3) were identified at 1 wpi. After excystation, NEJs interact with intestinal epithelial cells and inhibit the immune cell signaling cascade by downregulating intracellular signaling and the downstream ubiquitination-associated proteins required to trigger the immune response (Lammas and Duffus, 1983; Dalton et al., 2009; Lalor et al., 2021). Molecules secreted or excreted during this stage (1–3 wpi) likely play vital roles in host invasion and have the potential to be candidate vaccine/drug targets to inhibit NEJ infestation and migration.

Four of the 26 proteins identified between 6 and 8 wpi were uncharacterized (Supplementary Tables S6, S7). Programmed cell death 6-interacting protein and Thimet oligopeptidase (M03 family) were identified at both 6 and 8 wpi. GO analysis of T-complex protein 1 subunit γ, annexin, and dynein beta chain ciliary protein, which were only identified at 6 wpi, identified their localization and motility functions. Constitutive HSP70, HSP90 chaperone protein kinase-targeting subunit, glycerol-3-phosphate dehydrogenase (nicotinamide adenine dinucleotide), succinate dehydrogenase (ubiquinone) iron–sulfur subunit, mitochondria (fragment), and puromycin-sensitive aminopeptidase were only identified at 8 wpi. KEGG analysis showed a functional focus on energy metabolism, including oxidative phosphorylation, the citric acid cycle, starch and sucrose metabolism, purine metabolism, pyrimidine metabolism, and nicotinic acid and nicotinamide metabolism. Between 6 and 8 wpi, *Fasciola* migrate to the host's liver and induce high oxidative stress levels (Da Silva et al., 2017). HSP70 may function in protein folding and assembly, refolding misfolded and aggregated proteins, and transferring proteins to mediate the environmental stress and cellular homeostasis effects, which is critical for parasite survival (Polla, 1991; Mayer and Bukau, 2005; Smith et al., 2008). The active metabolic pathways provide the nutrients for *F. hepatica* growth and development between 6 to 8 wpi (Tanaka and Miyajima, 2016), and it may be similar in *F. gigantica* growth and development.

Long-term *F. gigantica* survival requires a balance between immuno-suppressive and-modulatory effects induced by *F. gigantica* and the host's innate and adaptive immune responses. Twelve of the 78 proteins identified between 10 and 16 wpi were uncharacterized (Supplementary Tables S6, S7). Legumain-like calcium-binding protein 39 and transforming growth factor-β (TGF-β)-inducible protein ig-h3 (fragment) were consistently identified during this period. KEGG pathway analysis indicated that they primarily function in metabolic pathways. Studies have shown that recombinant legumain is specifically recognized by positive sera from *F. hepatica*-infected sheep, showing good reactogenicity (Zhang et al., 2021). Subsequent studies showed it differed biologically between *Schistosoma haematobium* and *F. gigantica*, indicating its vaccine potential against *F. gigantica* (Adisakwattana et al., 2007).

Tegumental calcium-binding EF-hand protein 4 (CABP4) was identified at both 10 and 13 wpi (Supplementary Table S6). The EF-hand is an important functional protein domain in *F. gigantica* calcium-binding protein (Santiago et al., 1998). The EF-hand-containing protein CABP4 is an important *Fg*ESP component that shows an immunomodulatory effect during *F. gigantica* infection (Subpipattana et al., 2012; Huang et al., 2019b; Ehsan et al., 2021). Studies investigating *Fh*CABP1, *Fh*CABP2, and *Fh*CaBP4 have been performed (Banford et al., 2013; Thomas and Timson, 2015; Cheung et al., 2016). However, relevant studies on *F. gigantica* calcium-binding proteins are lacking. Given the calcium-binding protein family's ability to induce immunoglobulin E-mediated host immune responses (Santiago et al., 1998), there is a need to study their immunomodulatory functions in *F. gigantica*.

*Fasciola* migration in the liver triggers a wound-healing response that induces fibrosis to repair the damage (Dorey

et al., 2021), culminating in liver fibrosis and granulomas. This progress may be related to forkhead box P3 (FOXP3)$^+$ T regulatory cell (Treg) levels (Pacheco et al., 2018), regulatory cytokines (IL-10 and TGF-β), and proinflammatory cytokines (tumor necrosis factor-alpha and IL-1β; Valero et al., 2017). Here, the TGF-β-inducible protein ig-h3 (fragment) identified between 10 and 16 wpi may participate in the host tissue damage repair (Supplementary Table S5). The aldolase (fructose-bisphosphate) identified between 10 and 16 wpi is secreted by or attached to the epidermis of *Fasciola* (Morales and Espino, 2012). It mainly acts as a ligand for various host components contributing to fluke invasion (Zhang et al., 2019), host immune and hemostatic systems regulation, angiogenesis, and nutrient absorption (Gómez-Arreaza et al., 2014).

*Fg*ESPs are exposed to the host immune system and widely used as antigens in serological assays. Five of the 19 proteins identified between 1 and 3 wpi were uncharacterized (Supplementary Tables S6, S7). The specificity and sensibility of these proteins still need to be confirmed by Western blot and ELISA. Once some have been purified and shown to react well with positive serum, they can be used to develop new early-diagnosis antigen immunological diagnostic methods. However, this study did not identify well-performing early diagnosis antigens, such as cathepsin L and secreted aspartyl proteinase 2, indicating more accurate approaches may be needed to understand the precise buffalo-*F. gigantica* interaction (Cornelissen et al., 2001; Sriveny et al., 2006; Kueakhai et al., 2011; Mirzadeh et al., 2017).

During early infection stages, *F. gigantica* induces the Th2-related response and suppresses the Th1-related response in the host. Molecules functioning in this process are potential vaccine candidates (Donnelly et al., 2008; Walsh et al., 2009). Fifteen of the 96 proteins identified between 6 and 10 wpi were uncharacterized (Supplementary Tables S6, S7), including cathepsin L. Cathepsin-L peptidases have been extensively studied since they are internalized by host immune cells and degrade the pathogen recognition receptor Toll-like receptor 3, preventing Toll/IL-1R domain-containing adaptor-inducing interferon-β-containing adaptor protein-dependent signaling that is essential for the Th1 inflammatory response (Falcón et al., 2014). The mammalian target of rapamycin (mTOR), MAPK, and FOXO signaling pathways act synergistically to promote *FOXP3* expression and differentiation into Treg cells (Delgoffe et al., 2009). Treg cells secrete the regulatory cytokines TGF-β and IL-10, regulating the Th1-and Th2-related responses (Hill et al., 2007). This process may be related to immunomodulation and long-term host colonization (Maizels and Lawrence, 1991; King et al., 1992). Since calcium-binding protein 39, F-actin-capping protein subunit beta, and V-type proton ATPase subunit H clustered with the mTOR signaling pathway; constitutive HSP70 clustered with the MAPK signaling pathway; and the glucose transporter clustered with the FOXO signaling pathway, these proteins may regulate the Th1-and Th2-related responses. Studying their immunomodulatory functions may contribute to vaccine candidate identification.

## 5. Conclusion

This study performed a detailed screening of antigenic *Fg*ESP targets, as 490 proteins were identified in the infection group, of which 87 were consistently identified at 7 time points, the numbers of specific interactors identified for each week were 1 ($n = 12$), 3 ($n = 5$), 6 ($n = 8$), 8 ($n = 15$), 10 ($n = 23$), 13 ($n = 22$), and 16 ($n = 14$) wpi. These findings will lay the foundation for further studies on *F. gigantica*-host interactions and fascioliasis diagnosis and prevention.

## Data availability statement

The data presented in the study are deposited in the ProteomeXchange repository, accession number PXD038582.

## Ethics statement

This animal study, including sera collection, was approved by the Ethics Committee of the School of Animal Science and Technology at Guangxi University. The animals used in this study were handled according to good animal practices as required by the Animal Ethics Procedures and Guidelines of the People's Republic of China.

## Author contributions

WZ conceived the project. MZ performed the laboratory work and data analysis and wrote the manuscript. XJ, XK, and YG performed supporting data analyses. WD revised the manuscript and contributed to the final submission. All authors contributed to the article and approved the submitted version.

## Funding

This study was supported by the National Natural Science Foundation of China (Grant no. 31960706).

## Acknowledgments

The authors extend gratitude to Kelong Wei and Mingtang Chen for maintaining the buffalo. The authors also extend gratitude to Jinhui Wang, Zhengjiao Wu, Zhen Meng, and Xiangxiang Yuan for their preliminary work of the experiment.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1089394/full#supplementary-material

## References


Adell, T., Salo, E., Boutros, M., and Bartscherer, K. (2009). Smed-Evi/Wntless is required for beta-catenin-dependent and independent processes during planarian regeneration. *Development* 126:S291. doi: 10.1016/j.mod.2009.06.789

Adisakwattana, P., Viyanant, V., Chaicumpa, W., Vichasri-Grams, S., Hofmann, A., Korge, G., et al. (2007). Comparative molecular analysis of two asparaginyl endopeptidases and encoding genes from *Fasciola gigantica*. *Mol. Biochem. Parasitol.* 156, 102–116. doi: 10.1016/j.molbiopara.2007.07.006

Agatsuma, T., Arakawa, Y., Iwagami, M., Honzako, Y., Cahyaningsih, U., Kang, S. Y., et al. (2000). Molecular evidence of natural hybridization between *Fasciola hepatica* and *F. gigantica*. *Parasitol. Int.* 49, 231–238. doi: 10.1016/S13835769(00)00051-9

Aghayan, S., Gevorgian, H., Ebi, D., Atoyan, H. A., Addy, F., Mackenstedt, U., et al. (2019). Fasciola spp. in Armenia: genetic diversity in a global context. *Vet. Parasitol.* 268, 21–31. doi: 10.1016/j.vetpar.2019.02.009

Alirahmi, H., Farahnak, A., Golmohamadi, T., and Esharghian, M. R. (2010). Comparative assay of glutathione S-transferase (GSTs) activity of excretory-secretory materials and somatic extract of Fasciola spp parasites. *Acta Med. Iran.* 48, 367–370.

Backe, S. J., Sager, R. A., Woodford, M. R., Makedon, A. M., and Mollapour, M. (2020). Post-translational modifications of Hsp90 and translating the chaperone code. *J. Biol. Chem.* 295, 11099–11117. doi: 10.1074/jbc.REV120.011833

Ballantyne, S. J., Barlow, J. L., Jolin, H. E., Nath, P., Williams, A. S., Chung, K. F., et al. (2007). Blocking IL-25 prevents airway hyperresponsiveness in allergic asthma. *J. Allergy Clin. Immunol.* 120, 1324–1331. doi: 10.1016/j.jaci.2007.07.051

Banford, S., Drysdale, O., Hoey, E. M., Trudgett, A., and Timson, D. J. (2013). FhCaBP3: a Fasciola hepatica calcium binding protein with EF-hand and dynein light chain domains. *Biochimie* 95, 751–758. doi: 10.1016/j.biochi.2012.10.027

Becerro-Recio, D., Gonzalez-Miguel, J., Ucero, A., Sotillo, J., Martinez-Moreno, A., Perez-Arevalo, J., et al. (2021). Recognition pattern of the *Fasciola hepatica* excretome/secretome during the course of an experimental infection in sheep by 2D Immunoproteomics. *Pathogens* 10:725. doi: 10.3390/pathogens10060725

Biebl, M. M., and Buchner, J. (2019). Structure, function, and regulation of the Hsp90 machinery. *Cold Spring Harb. Perspect. Biol.* 11:a034017. doi: 10.1101/cshperspect.a034017

Brophy, P. M., Mackintosh, N., and Morphew, R. M. (2012). Anthelmintic metabolism in parasitic helminths: proteomic insights. *Parasitology* 139, 1205–1217. doi: 10.1017/S003118201200087X

Caban-Hernandez, K., Gaudier, J. F., and Espino, A. M. (2012). Characterization and differential expression of a ferritin protein from *Fasciola hepatica*. *Mol. Biochem. Parasitol.* 182, 54–61. doi: 10.1016/j.molbiopara.2011.12.005

Calvani, N. E. D., and Šlapeta, J. (2021). *Fasciola* species introgression: just a fluke or something more? *Trends Parasitol.* 37, 25–34. doi: 10.1016/j.pt.2020.09.008

Chaithirayanon, K., Korge, G., Hofmann, A., Grams, R., Schwendemann, A., and Sobhon, P. (2004). A novel gene encodes a new member of *Fasciola gigantica* 14-3-3 protein family. *Eur. J. Cell Biol.* 83:24.

Chantree, P., Phatsara, M., Meemon, K., Chaichanasak, P., Changklungmoa, N., Kueakhai, P., et al. (2013). Vaccine potential of recombinant cathepsin B against *Fasciola gigantica*. *Exp. Parasitol.* 135, 102–109. doi: 10.1016/j.exppara.2013.06.010

Chen, X., Ayala, I., Shannon, C., Fourcaudot, M., Acharya, N. K., Jenkinson, C. P., et al. (2018). The diabetes gene and Wnt pathway effector TCF7L2 regulates adipocyte development and function. *Diabetes* 67, 554–568. doi: 10.2337/db170318

Chen, L., Daugschies, A., Wang, B., and Mao, X. (2000). Blood eicosanoids and immune indices during fasciolosis in water buffaloes. *Parasitol. Int.* 49, 273–278. doi: 10.1016/S1383-5769(00)00056-8

Chen, C., Itakura, E., Nelson, G. M., Sheng, M., Laurent, P., Fenk, L. A., et al. (2017). IL-17 is a neuromodulator of *Caenorhabditis elegans* sensory responses. *Nature* 542:43-+. doi: 10.1038/nature20818

Cheung, S., Thomas, C. M., and Timson, D. J. (2016). FhCaBP1 (FH22): a Fasciola hepatica calcium-binding protein with EF-hand and dynein light chain domains. *Exp. Parasitol.* 170, 109–115. doi: 10.1016/j.exppara.2016.09.013

Cooney, R., Baker, J., Brain, O., Danis, B., Pichulik, T., Allan, P., et al. (2010). NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat. Med.* 16, 90–97. doi: 10.1038/nm.2069

Cornelissen, J., Gaasenbeek, C. P. H., Borgsteede, F. H. M., Holland, W. G., Harmsen, M. M., and Boersma, W. J. A. (2001). Early immunodiagnosis of fasciolosis in ruminants using recombinant Fasciola hepatica cathepsin L-like protease. *Int. J. Parasit.* 31, 728–737. doi: 10.1016/S0020-7519(01)00175-8

Cvilink, V., Lamka, J., and Skalova, L. (2009). Xenobiotic metabolizing enzymes and metabolism of anthelminthics in helminths. *Drug Metab. Rev.* 41, 8–26. doi: 10.1080/03602530802602880

Cvilink, V., Skalova, L., Szotakova, B., Lamka, J., Kostiainen, R., and Ketola, R. A. (2008). LC-MS-MS identification of albendazole and flubendazole metabolites formed ex vivo by *Haemonchus contortus*. *Anal. Bioanal. Chem.* 391, 337–343. doi: 10.1007/s00216-008-1863-9

Cwiklinski, K., and Dalton, J. P. (2022). Exploiting comparative Omics to understand the pathogenic and virulence-associated protease: anti-protease relationships in the zoonotic parasites *Fasciola hepatica* and *Fasciola gigantica*. *Genes (Basel)* 13:1854. doi: 10.3390/genes13101854

Cwiklinski, K., Jewhurst, H., McVeigh, P., Barbour, T., Maule, A. G., Tort, J., et al. (2018). Infection by the Helminth parasite Fasciola hepatica requires rapid regulation of metabolic, virulence, and invasive factors to adjust to its mammalian host. *Mol. Cell. Proteomics* 17, 792–809. doi: 10.1074/mcp.RA117.000445

Da Silva, A. S., Baldissera, M. D., Bottari, N. B., Gabriel, M. E., Rhoden, L. A., Piva, M. M., et al. (2017). Oxidative stress and changes in adenosine deaminase activity of cattle experimentally infected by Fasciola hepatica. *Parasitology* 144, 520–526. doi: 10.1017/S0031182016002043

Dalton, J. P., Brindley, P. J., Donnelly, S., and Robinson, M. W. (2009). The enigmatic asparaginyl endopeptidase of helminth parasites. *Trends Parasitol.* 25, 59–61. doi: 10.1016/j.pt.2008.11.002

Davey, S. D., Chalmers, I. W., Fernandez-Fuentes, N., Swain, M. T., Smith, D., Abbas Abidi, S. M., et al. (2022). In silico characterisation of the complete Ly6 protein family in *Fasciola gigantica* supported through transcriptomics of the newlyexcysted juveniles. *Mol. Omics* 18, 45–56. doi: 10.1039/d1mo00254f

De, A. (2011). Wnt/Ca2+ signaling pathway: a brief overview. *Acta Biochim. Biophys. Sin.* 43, 745–756. doi: 10.1093/abbs/gmr079

De Robertis, E. M. (2010). Wnt signaling in axial patterning and regeneration: lessons from Planaria. *Sci. Signal.* 3:pe21. doi: 10.1126/scisignal.3127pe21

Delgoffe, G. M., Kole, T. P., Zheng, Y., Zarek, P. E., Matthews, K. L., Xiao, B., et al. (2009). The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* 30, 832–844. doi: 10.1016/j.immuni.2009.04.014

Di Maggio, L. S., Tirloni, L., Pinto, A. F. M., Diedrich, J. K., Yates, J. R., Carmona, C., et al. (2019). Dataset supporting the proteomic differences found between excretion/secretion products from two isolates of Fasciola hepatica newly excysted juveniles (NEJ) derived from different snail hosts. *Data Brief* 25:104272. doi: 10.1016/j.dib.2019.104272

Donnelly, S., Stack, C. M., O'Neill, S. M., Sayed, A. A., Williams, D. L., and Dalton, J. P. (2008). Helminth 2-Cys peroxiredoxin drives Th2 responses through a mechanism involving alternatively activated macrophages. *FASEB J.* 22, 4022–4032. doi: 10.1096/fj.08-106278

Dorey, A., Cwiklinski, K., Rooney, J., De Marco Verissimo, C., López Corrales, J., Jewhurst, H., et al. (2021). Autonomous non antioxidant roles for *Fasciola hepatica* secreted Thioredoxin-1 and Peroxiredoxin-1. *Front. Cell. Infect. Microbiol.* 11:667272. doi: 10.3389/fcimb.2021.667272

Doy, T. G., and Hughes, D. L. (1984). *Fasciola hepatica*: site of resistance to reinfection in cattle. *Exp. Parasitol.* 57, 274–278. doi: 10.1016/0014-4894(84)90101-2

Ehsan, M., Hu, R.-S., Hou, J.-L., Elsheikha, H. M., Li, X.-D., Liang, P.-H., et al. (2021). *Fasciola gigantica* tegumental calcium-binding EF-hand protein 4 exerts immunomodulatory effects on goat monocytes. *Parasit. Vectors* 14:276. doi: 10.1186/s13071-021-04784-5

El-Ghaysh, A., Turner, R. J., Brophy, P. M., and Barrett, J. (1999). Effect of *Fasciola gigantica* somatic extracts and excretory/secretory products on superoxide production by activated neutrophils. *Vet. Parasitol.* 84, 91–100. doi: 10.1016/S03044017(99)00089-8

Falcón, C. R., Masih, D., Gatti, G., Sanchez, M. C., Motrán, C. C., and Cervi, L. (2014). *Fasciola hepatica* Kunitz type molecule decreases dendritic cell activation and their ability to induce inflammatory responses. *PLoS One* 9:e114505. doi: 10.1371/journal.pone.0114505

Fukata, M., Vamadevan, A. S., and Abreu, M. T. (2009). Toll-like receptors (TLRs) and nod-like receptors (NLRs) in inflammatory disorders. *Semin. Immunol.* 21, 242–253. doi: 10.1016/j.smim.2009.06.005

Ghosh, S., Rawat, P., Velusamy, R., Joseph, D., Gupta, S. C., and Singh, B. P. (2005). 27 kDa *Fasciola gigantica* glycoprotein for the diagnosis of prepatent fasciolosis in cattle. *Vet. Res. Commun.* 29, 123–135. doi: 10.1023/B:VERC.0000047497.57392.8c

Gillan, V., and Devaney, E. (2014). Nematode Hsp90: highly conserved but functionally diverse. *Parasitology* 141, 1203–1215. doi: 10.1017/S0031182014000304

Gómez-Arreaza, A., Acosta, H., Quiñones, W., Concepción, J. L., Michels, P. A. M., and Avilán, L. (2014). Extracellular functions of glycolytic enzymes of parasites: unpredicted use of ancient proteins. *Mol. Biochem. Parasitol.* 193, 75–81. doi: 10.1016/j.molbiopara.2014.02.005

Hacariz, O., Sayers, G., and Mulcahy, G. (2011). A preliminary study to understand the effect of Fasciola hepatica tegument on naive macrophages and humoral responses in an ovine model. *Vet. Immunol. Immunopathol.* 139, 245–249. doi: 10.1016/j.vetimm.2010.08.007

Hanna, R. (2015). Fasciola hepatica: histology of the reproductive organs and differential effects of Triclabendazole on drug-sensitive and drug-resistant fluke isolates and on flukes from selected field cases. *Pathogens* 4, 431–456. doi: 10.3390/pathogens4030431

Henderson, B., Allan, E., and Coates, A. R. M. (2006). Stress wars: the direct role of host and bacterial molecular chaperones in bacterial infection. *Infect. Immun.* 74, 3693–3706. doi: 10.1128/IAI.01882-05

Hill, J. A., Feuerer, M., Tash, K., Haxhinasto, S., Perez, J., Melamed, R., et al. (2007). Foxp3 transcription-factor-dependent and-independent regulation of the regulatory T cell transcriptional signature. *Immunity* 27, 786–800. doi: 10.1016/j.immuni.2007.09.010

Hossini, A. M., Quast, A. S., Plötz, M., Grauel, K., Exner, T., Küchler, J., et al. (2016). PI3K/AKT signaling pathway is essential for survival of induced pluripotent stem cells. *PLoS One* 11:e0154770. doi: 10.1371/journal.pone.0154770

Hoter, A., El-Sabban, M. E., and Naim, H. Y. (2018). The HSP90 family: structure, regulation, function, and implications in health and disease. *Int. J. Mol. Sci.* 19:2560. doi: 10.3390/ijms19092560

Hu, R.-S., Zhang, F.-K., Ma, Q.-N., Ehsan, M., Zhao, Q., and Zhu, X.-Q. (2022). Transcriptomic landscape of hepatic lymph nodes, peripheral blood lymphocytes and spleen of swamp buffaloes infected with the tropical liver fluke *Fasciola gigantica*. *PLoS Negl. Trop. Dis.* 16:e0010286. doi: 10.1371/journal.pntd.0010286

Huang, S.-Y., Yue, D.-M., Hou, J.-L., Zhang, X.-X., Zhang, F., Wang, C.-R., et al. (2019a). Proteomic analysis of *Fasciola gigantica* excretory and secretory products (FgESPs) interacting with buffalo serum of different infection periods by shotgun LCMS/MS. *Parasitol. Res.* 118, 453–460. doi: 10.1007/s00436-018-6169-z

Huang, S.-Y., Yue, D.-M., Hou, J.-L., Zhang, X.-X., Zhang, F.-K., Wang, C.-R., et al. (2019b). Proteomic analysis of *Fasciola gigantica* excretory and secretory products (FgESPs) interacting with buffalo serum of different infection periods by shotgun LCMS/MS. *Parasitol. Res.* 118, 453–460. doi: 10.1007/s00436-018-6169-z

Jayaprakash, P., Dong, H., Zou, M., Bhatia, A., O'Brien, K., Chen, M., et al. (2015). Hsp90 alpha and Hsp90 beta together operate a hypoxia and nutrient paucity stressresponse mechanism during wound healing. *J. Cell Sci.* 128, 1475–1480. doi: 10.1242/jcs.166363

Johnson, J. L. (2012). Evolution and function of diverse Hsp90 homologs and cochaperone proteins. *Biochim. Biophys. Acta-Mol. Cell Res.* 1823, 607–613. doi: 10.1016/j.bbamcr.2011.09.020

Kalita, J., Shukla, R., Shukla, H., Gadhave, K., Giri, R., and Tripathi, T. (2017). Comprehensive analysis of the catalytic and structural properties of a mu-class glutathione s-transferase from *Fasciola gigantica*. *Sci. Rep.* 7:17547. doi: 10.1038/s41598-017-17678-3

Kalita, J., Shukla, R., and Tripathi, T. (2019). Structural basis of urea-induced unfolding of *Fasciola gigantica* glutathione S-transferase. *J. Cell. Physiol.* 234, 4491–4503. doi: 10.1002/jcp.27253

Kang, Z., Swaidani, S., Yin, W., Wang, C., Barlow, J. L., Gulen, M. F., et al. (2012). Epithelial cell-specific Act1 adaptor mediates interleukin-25-dependent helminth expulsion through expansion of Lin(−)c-kit(+) innate cell population. *Immunity* 36, 821–833. doi: 10.1016/j.immuni.2012.03.021

King, C. L., Kumaraswami, V., Poindexter, R. W., Kumari, S., Jayaraman, K., Alling, D. W., et al. (1992). Immunologic tolerance in lymphatic filariasis. Diminished parasite-specific T and B lymphocyte precursor frequency in the microfilaremic state. *J. Clin. Invest.* 89, 1403–1410. doi: 10.1172/JCI115729

Kueakhai, P., Changklungmoa, N., Chaichanasak, P., Jaikua, W., Itagaki, T., and Sobhon, P. (2015). Vaccine potential of recombinant pro-and mature cathepsinL1 against fasciolosis gigantica in mice. *Acta Trop.* 150, 71–78. doi: 10.1016/j.actatropica.2015.06.020

Kueakhai, P., Changklungmoa, N., Riengrojpitak, S., Chaichanasak, P., Meemon, K., Chaithirayanon, K., et al. (2013). Vaccine potential of recombinant saposin-like protein 2 against Fasciolosis gigantica in mice. *Vaccine* 31, 5518–5523. doi: 10.1016/j.vaccine.2013.09.027

Kueakhai, P., Meemon, K., Changklungmoa, N., Chaithirayanon, K., Riengrojpitak, S., and Sobhon, P. (2011). Characterization and localization of saposin-like protein-2 (SAP-2) in *Fasciola gigantica*. *Parasitol. Res.* 108, 1493–1500. doi: 10.1007/s00436010-2201-7

Kumkate, S., Chunchob, S., and Janvilisri, T. (2008). Expression of ATP-binding cassette multidrug transporters in the giant liver fluke *Fasciola gigantica* and their possible involvement in the transport of bile salts and anthelmintics. *Mol. Cell. Biochem.* 317, 77–84. doi: 10.1007/s11010-008-9833-2

Lalor, R., Cwiklinski, K., Calvani, N. E. D., Dorey, A., Hamon, S., Corrales, J. L., et al. (2021). Pathogenicity and virulence of the liver flukes *Fasciola hepatica* and *Fasciola gigantica* that cause the zoonosis Fasciolosis. *Virulence* 12, 2839–2867. doi: 10.1080/21505594.2021.1996520

Lammas, D. A., and Duffus, W. P. (1983). The shedding of the outer glycocalyx of juvenile *Fasciola hepatica*. *Vet. Parasitol.* 12, 165–178. doi: 10.1016/03044017(83)90005-5

Liu, F., Cui, S.-J., Hu, W., Feng, Z., Wang, Z.-Q., and Han, Z.-G. (2009). Excretory/secretory proteome of the adult developmental stage of human blood fluke, *Schistosoma japonicum*. *Mol. Cell. Proteomics* 8, 1236–1251. doi: 10.1074/mcp.M800538-MCP200

Luo, X., Cui, K., Wang, Z., Li, Z., Wu, Z., Huang, W., et al. (2021). High-quality reference genome of *Fasciola gigantica*: insights into the genomic signatures of transposon-mediated evolution and specific parasitic adaption in tropical regions. *PLoS Negl. Trop. Dis.* 15:e0009750. doi: 10.1371/journal.pntd.0009750

Maizels, R. M., and Lawrence, R. A. (1991). Immunological tolerance: the key feature in human filariasis? *Parasitol. Today* 7, 271–276. doi: 10.1016/01694758(91)90093-4

Mayer, M. P., and Bukau, B. (2005). Hsp70 chaperones: cellular functions and molecular mechanism. *Cell. Mol. Life Sci.* 62, 670–684. doi: 10.1007/s00018-004-4464-6

McCusker, P., McVeigh, P., Rathinasamy, V., Toet, H., McCammick, E., O'Connor, A., et al. (2016). Stimulating Neoblast-like cell proliferation in juvenile *Fasciola hepatica* supports growth and progression towards the adult phenotype in vitro. *PLoS Negl. Trop. Dis.* 10:e0004994. doi: 10.1371/journal.pntd.0004994

Meemon, K., Grams, R., Vichasri-Grams, S., Hofmann, A., Korge, G., Viyanant, V., et al. (2004). Molecular cloning and analysis of stage and tissue-specific expression of cathepsin B encoding genes from *Fasciola gigantica*. *Mol. Biochem. Parasitol.* 136, 1–10. doi: 10.1016/j.molbiopara.2004.02.010

Meemon, K., and Sobhon, P. (2015). Juvenile-specific cathepsin proteases in *Fasciola* spp.: their characteristics and vaccine efficacies. *Parasitol. Res.* 114, 2807–2813. doi: 10.1007/s00436-015-4589-6

Mirzadeh, A., Valadkhani, Z., Yoosefy, A., Babaie, J., Golkar, M., Rastaghi, A. R. E., et al. (2017). Expression, purification and in vitro refolding of the recombinant truncated Saposin-like protein 2 antigen for development of diagnosis of human fascioliasis. *Acta Trop.* 171, 163–171. doi: 10.1016/j.actatropica.2017.03.002

Molina, E. C. (2005). Serum interferon-gamma and interleukins-6 and-8 during infection with *Fasciola gigantica* in cattle and buffaloes. *J. Vet. Sci.* 6, 135–139. doi: 10.4142/jvs.2005.6.2.135

Morales, A., and Espino, A. M. (2012). Evaluation and characterization of *Fasciola hepatica* tegument protein extract for serodiagnosis of human fascioliasis. *Clin. Vaccine Immunol.* 19, 1870–1878. doi: 10.1128/CVI.00487-12

Morphew, R. M., Wright, H. A., LaCourse, E. J., Woods, D. J., and Brophy, P. M. (2007). Comparative proteomics of excretory-secretory proteins released by the liver fluke *Fasciola hepatica* in sheep host bile and during in vitro culture ex host. *Mol. Cell. Proteomics* 6, 963–972. doi: 10.1074/mcp.M600375-MCP200

Moxon, J. V., LaCourse, E. J., Wright, H. A., Perally, S., Prescott, M. C., Gillard, J. L., et al. (2010). Proteomic analysis of embryonic *Fasciola hepatica*: characterization and antigenic potential of a developmentally regulated heat shock protein. *Vet. Parasitol.* 169, 62–75. doi: 10.1016/j.vetpar.2009.12.031

Niedziela, D. A., Naranjo-Lucena, A., Molina-Hernández, V., Browne, J. A., Martínez-Moreno, Á., Pérez, J., et al. (2021). Timing of Transcriptomic peripheral blood mononuclear cell responses of sheep to Fasciola hepatica infection differs from those of cattle, reflecting different disease phenotypes. *Front. Immunol.* 12:729217. doi: 10.3389/fimmu.2021.729217

Novobilsky, A., Kasny, M., Mikes, L., Kovarcik, K., and Koudela, B. (2007). Humoral immune responses during experimental infection with *Fascioloides magna* and *Fasciola hepatica* in goats and comparison of their excretory/secretory products. *Parasitol. Res.* 101, 357–364. doi: 10.1007/s00436-007-0463-5

Novobilský, A., Kasný, M., Mikes, L., Kovarcík, K., and Koudela, B. (2007). Humoral immune responses during experimental infection with *Fascioloides magna* and *Fasciola hepatica* in goats and comparison of their excretory/secretory products. *Parasitol. Res.* 101, 357–364. doi: 10.1007/s00436-007-0463-5

Ovchinnikov, V. Y., Kashina, E. V., Mordvinov, V. A., and Fromm, B. (2020). EVtransported microRNAs of *Schistosoma mansoni* and *Fasciola hepatica*: potential targets in definitive hosts. *Infect. Genet. Evol.* 85:104528. doi: 10.1016/j.meegid.2020.104528

Pacheco, I. L., Abril, N., Zafra, R., Molina-Hernández, V., Morales-Prieto, N., Bautista, M. J., et al. (2018). *Fasciola hepatica* induces Foxp3 T cell, proinflammatory and regulatory cytokine overexpression in liver from infected sheep during early stages of infection. *Vet. Res.* 49:56. doi: 10.1186/s13567-018-0550-x

Pan, G., French, D., Mao, W., Maruoka, M., Risser, P., Lee, J., et al. (2001). Forced expression of murine IL-17E induces growth retardation, jaundice, a Th2-biased response, and multiorgan inflammation in mice. *J. Immunol.* 167, 6559–6567. doi: 10.4049/jimmunol.167.11.6559

Pandey, T., Ghosh, A., Todur, V. N., Rajendran, V., Kalita, P., Kalita, J., et al. (2020). Draft genome of the liver fluke *Fasciola gigantica*. *ACS Omega* 5, 11084–11091. doi: 10.1021/acsomega.0c00980

Peiris, T. H., Ramirez, D., Barghouth, P. G., and Oviedo, N. J. (2016). The Akt signaling pathway is required for tissue maintenance and regeneration in planarians. *BMC Dev. Biol.* 16:7. doi: 10.1186/s12861-016-0107-z

Petersen, C. P., and Reddien, P. W. (2009). Wnt signaling and the polarity of the primary body Axis. *Cells* 139, 1056–1068. doi: 10.1016/j.cell.2009.11.035

Philpott, D. J., Sorbara, M. T., Robertson, S. J., Croitoru, K., and Girardin, S. E. (2014). NOD proteins: regulators of inflammation in health and disease (vol 14, pg 9, 2014). *Nat. Rev. Immunol.* 14:131. doi: 10.1038/nri3601

Piedrafita, D., Spithill, T. W., Smith, R. E., and Raadsma, H. W. (2010). Improving animal and human health through understanding liver fluke immunology. *Parasite Immunol.* 32, 572–581. doi: 10.1111/j.1365-3024.2010.01223.x

Polla, B. S. (1991). Heat shock proteins in host-parasite interactions. *Immunol. Today* 12, A38–A41. doi: 10.1016/S0167-5699(05)80011-8

Rehman, A., Rehman, L., Ullah, R., Beg, M. A., Khan, M. A. H., and Abidi, S. M. A. (2021). Oxidative status and changes in the adenosine deaminase activity in experimental host infected with tropical liver fluke, *Fasciola gigantica*. *Acta Trop.* 213:105753. doi: 10.1016/j.actatropica.2020.105753

Rehman, A., Ullah, R., Gupta, D., Khan, M. A. H., Rehman, L., Beg, M. A., et al. (2020). Generation of oxidative stress and induction of apoptotic like events in curcumin and thymoquinone treated adult *Fasciola gigantica* worms. *Exp. Parasitol.* 209:107810. doi: 10.1016/j.exppara.2019.107810

Rodriguez, E., Carasi, P., Frigerio, S., da Costa, V., van Vliet, S., Noya, V., et al. (2017). Fasciola hepatica immune regulates CD11c(+) cells by interacting with the macrophage gal/Galnac Lectin. *Front. Immunol.* 8:264. doi: 10.3389/fimmu.2017.00264

Rossjohn, J., Feil, S. C., Wilce, M. C. J., Sexton, J. L., Spithill, T. W., and Parker, M. W. (1997). Crystallization, structural determination and analysis of a novel parasite vaccine candidate: *Fasciola hepatica* glutathione S-transferase. *J. Mol. Biol.* 273, 857–872. doi: 10.1006/jmbi.1997.1338

Roy, N., Nageshan, R. K., Ranade, S., and Tatu, U. (2012). Heat shock protein 90 from neglected protozoan parasites. *Biochim. Biophys. Acta-Mol. Cell Res.* 1823, 707–711. doi: 10.1016/j.bbamcr.2011.12.003

Ryan, S., Shiels, J., Taggart, C. C., Dalton, J. P., and Weldon, S. (2020). Fasciola hepatica-derived molecules as regulators of the host immune response. *Front. Immunol.* 11:2182. doi: 10.3389/fimmu.2020.02182

Saenz, S. A., Taylor, B. C., and Artis, D. (2008). Welcome to the neighborhood: epithelial cell-derived cytokines license innate and adaptive immune responses at mucosal sites. *Immunol. Rev.* 226, 172–190. doi: 10.1111/j.1600-065X.2008.00713.x

Sansri, V., Changklungmoa, N., Chaichanasak, P., Sobhon, P., and Meemon, K. (2013). Molecular cloning, characterization and functional analysis of a novel juvenile-specific cathepsin L of *Fasciola gigantica*. *Acta Trop.* 128, 76–84. doi: 10.1016/j.actatropica.2013.06.013

Santiago, M. L., Hafalla, J. C. R., Kurtis, J. D., Aligui, G. L., Wiest, P. M., Olveda, R. M., et al. (1998). Identification of the Schistosoma japonicum 22.6-kDa antigen as a major target of the human IgE response: similarity of IgE-binding epitopes to allergen peptides. *Int. Arch. Allergy Immunol.* 117, 94–104. doi: 10.1159/000023995

Smith, R. E., Spithill, T. W., Pike, R. N., Meeusen, E. N. T., and Piedrafita, D. (2008). *Fasciola hepatica* and *Fasciola gigantica*: cloning and characterisation of 70 kDa heat-shock proteins reveals variation in HSP70 gene expression between parasite species recovered from sheep. *Exp. Parasitol.* 118, 536–542. doi: 10.1016/j.exppara.2007.11.012

Sriveny, D., Raina, O. K., Yadav, S. C., Chandra, D., Jayraw, A. K., Singh, M., et al. (2006). Cathepsin L cysteine proteinase in the diagnosis of bovine *Fasciola gigantica* infection. *Vet. Parasitol.* 135, 25–31. doi: 10.1016/j.vetpar.2005.10.016

Subpipattana, P., Grams, R., and Vichasri-Grams, S. (2012). Analysis of a calciumbinding EF-hand protein family in *Fasciola gigantica*. *Exp. Parasitol.* 130, 364–373. doi: 10.1016/j.exppara.2012.02.005

Tanaka, M., and Miyajima, A. (2016). Liver regeneration and fibrosis after inflammation. *Inflamm. Regen.* 36:19. doi: 10.1186/s41232-016-0025-2

Thomas, C. M., and Timson, D. J. (2015). FhCaBP2: a *Fasciola hepatica* calciumbinding protein with EF-hand and dynein light chain domains. *Parasitology* 142, 1375–1386. doi: 10.1017/S0031182015000736

Tian, A.-L., Lu, M., Calderon-Mantilla, G., Petsalaki, E., Dottorini, T., Tian, X., et al. (2018). A recombinant *Fasciola gigantica* 14-3-3 epsilon protein (rFg14-3-3e) modulates various functions of goat peripheral blood mononuclear cells. *Parasit. Vectors* 11:152. doi: 10.1186/s13071-018-2745-4

Ticho, A. L., Malhotra, P., Dudeja, P. K., Gill, R. K., and Alrefai, W. A. (2020). Intestinal absorption of bile acids in health and disease. *Compr. Physiol.* 10, 21–56. doi: 10.1002/cphy.c190007

Valero, J., Bernardino, L., Cardoso, F. L., Silva, A. P., Fontes-Ribeiro, C., Ambrósio, A. F., et al. (2017). Impact of neuroinflammation on hippocampal neurogenesis: relevance to aging and Alzheimer's disease. *J. Alzheimers Dis.* 60, S161–S168. doi: 10.3233/JAD-170239

von Samson-Himmelstjerna, G., Blackhall, W. J., McCarthy, J. S., and Skuce, P. J. (2007). Single nucleotide polymorphism (SNP) markers for benzimidazole resistance in veterinary nematodes. *Parasitology* 134, 1077–1086. doi: 10.1017/S0031182007000054

Walsh, K. P., Brady, M. T., Finlay, C. M., Boon, L., and Mills, K. H. G. (2009). Infection with a helminth parasite attenuates autoimmunity through TGF-betamediated suppression of Th17 and Th1 responses. *J. Immunol.* 183, 1577–1586. doi: 10.4049/jimmunol.0803803

Wang, S.-S., Chen, D., He, J.-J., Zheng, W.-B., Tian, A.-L., Zhao, G.-H., et al. (2021). *Fasciola gigantica*-derived excretory-secretory products alter the expression of mRNAs, miRNAs, lncRNAs, and circRNAs involved in the immune response and metabolism in goat peripheral blood mononuclear cells. *Front. Immunol.* 12:653755. doi: 10.3389/fimmu.2021.653755

Wang, J.-H., He, K.-X., Wang, Z.-D., Wen, C.-L., Han, X.-G., Meng, Z., et al. (2022b). Effect of primary and secondary *Fasciola gigantica* infection on specific IgG responses, hepatic enzyme levels and weight gain in buffaloes. *Parasitol. Res.* 121, 1709–1718. doi: 10.1007/s00436-022-07519-2

Wang, J.-H., He, K., Wu, Z., Jin, W., Wu, W., Guo, Y., et al. (2022a). Development of a colloidal gold immunochromatographic strip for the rapid detection of antibodies against *Fasciola gigantica* in buffalo. *Front. Vet. Sci.* 9:1004932. doi: 10.3389/fvets.2022.1004932

Xu, Z., Ji, M., Li, C., Du, X., Hu, W., McManus, D. P., et al. (2020). A biological and immunological characterization of Schistosoma Japonicum heat shock proteins 40 and 90 alpha. *Int. J. Mol. Sci.* 21:4034. doi: 10.3390/ijms21114034

Zhang, X.-X., Cong, W., Elsheikha, H. M., Liu, G.-H., Ma, J.-G., Huang, W.-Y., et al. (2017). De novo transcriptome sequencing and analysis of the juvenile and adult stages of *Fasciola gigantica*. *Infect. Genet. Evol.* 51, 33–40. doi: 10.1016/j.meegid.2017.03.007

Zhang, X.-X., Cwiklinski, K., Hu, R.-S., Zheng, W.-B., Sheng, Z.-A., Zhang, F.-K., et al. (2019). Complex and dynamic transcriptional changes allow the helminth *Fasciola gigantica* to adjust to its intermediate snail and definitive mammalian hosts. *BMC Genomics* 20:729. doi: 10.1186/s12864-019-6103-5

Zhang, F.-K., Hou, J.-L., Guo, A.-J., Tian, A.-L., Sheng, Z.-A., Zheng, W.-B., et al. (2018). Expression profiles of genes involved in TLRs and NLRs signaling pathways of water buffaloes infected with *Fasciola gigantica*. *Mol. Immunol.* 94, 18–26. doi: 10.1016/j.molimm.2017.12.007

Zhang, G. W., Meng, Q. L., Qiao, J., Wang, X. F., Zhang, K., Ning, C. C., et al. (2021). Molecular characterization, genetic evolution and antigenic characteristics of Legumain of *Fasciola hepatica*. *Southwest China J. Agric. Sci.* 34, 2289–2294. doi: 10.16213/j.cnki.scjas.2021.10.030

Zhang, W. Y., Moreau, E., Hope, J. C., Howard, C. J., Huang, W. Y., and Chauvin, A. (2005). *Fasciola hepatica* and *Fasciola gigantica*: comparison of cellular response to experimental infection in sheep. *Exp. Parasitol.* 111, 154–159. doi: 10.1016/j.exppara.2005.06.005

Zhang, W. Y., Moreau, E., Yang, B. Z., Li, Z. Q., Hope, J. C., Howard, C. J., et al. (2006). Humoral and cellular immune responses to *Fasciola gigantica* experimental infection in buffaloes. *Res. Vet. Sci.* 80, 299–307. doi: 10.1016/j.rvsc.2005.07.003

Zhou, Y., Park, S.-Y., Su, J., Bailey, K., Ottosson-Laakso, E., Shcherbina, L., et al. (2014). TCF7L2 is a master regulator of insulin production and processing. *Hum. Mol. Genet.* 23, 6419–6431. doi: 10.1093/hmg/ddu359

Zininga, T., Ramatsui, L., and Shonhai, A. (2018). Heat shock proteins as immunomodulants. *Molecules* 23:2846. doi: 10.3390/molecules23112846

frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 16 February 2023
DOI 10.3389/fmicb.2023.1126418



OPEN ACCESS

EDITED BY
Yang Cheng,
Harvard Medical School,
United States

REVIEWED BY
Camilo E. Khatchikian,
Dartmouth College,
United States
Angus Cook,
University of Western Australia,
Australia

*CORRESPONDENCE
Ting-Wu Chuang
✉ chtingwu@tmu.edu.tw

SPECIALTY SECTION
This article was submitted to Infectious Agents and Disease, a section of the journal Frontiers in Microbiology

RECEIVED 17 December 2022
ACCEPTED 30 January 2023
PUBLISHED 16 February 2023

CITATION
Phang WK, Hamid MHbA, Jelip J, Mudin RNb, Chuang T-W, Lau YL and Fong MY (2023) Predicting *Plasmodium knowlesi* transmission risk across Peninsular Malaysia using machine learning-based ecological niche modeling approaches.
*Front. Microbiol.* 14:1126418.
doi: 10.3389/fmicb.2023.1126418



# Predicting *Plasmodium knowlesi* transmission risk across Peninsular Malaysia using machine learning-based ecological niche modeling approaches

Wei Kit Phang[1], Mohd Hafizi bin Abdul Hamid[2], Jenarun Jelip[2], Rose Nani binti Mudin[3], Ting-Wu Chuang[4]*, Yee Ling Lau[1] and Mun Yik Fong[1]

[1]Department of Parasitology, Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia, [2]Disease Control Division, Ministry of Health Malaysia, Putrajaya, Malaysia, [3]Sabah State Health Department, Ministry of Health Malaysia, Kota Kinabalu, Sabah, Malaysia, [4]Department of Molecular Parasitology and Tropical Diseases, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

The emergence of potentially life-threatening zoonotic malaria caused by *Plasmodium knowlesi* nearly two decades ago has continued to challenge Malaysia healthcare. With a total of 376 *P. knowlesi* infections notified in 2008, the number increased to 2,609 cases in 2020 nationwide. Numerous studies have been conducted in Malaysian Borneo to determine the association between environmental factors and knowlesi malaria transmission. However, there is still a lack of understanding of the environmental influence on knowlesi malaria transmission in Peninsular Malaysia. Therefore, our study aimed to investigate the ecological distribution of human *P. knowlesi* malaria in relation to environmental factors in Peninsular Malaysia. A total of 2,873 records of human *P. knowlesi* infections in Peninsular Malaysia from 1st January 2011 to 31st December 2019 were collated from the Ministry of Health Malaysia and geolocated. Three machine learning-based models, maximum entropy (MaxEnt), extreme gradient boosting (XGBoost), and ensemble modeling approach, were applied to predict the spatial variation of *P. knowlesi* disease risk. Multiple environmental parameters including climate factors, landscape characteristics, and anthropogenic factors were included as predictors in both predictive models. Subsequently, an ensemble model was developed based on the output of both MaxEnt and XGBoost. Comparison between models indicated that the XGBoost has higher performance as compared to MaxEnt and ensemble model, with $AUC_{ROC}$ values of $0.933 \pm 0.002$ and $0.854 \pm 0.007$ for train and test datasets, respectively. Key environmental covariates affecting human *P. knowlesi* occurrence were distance to the coastline, elevation, tree cover, annual precipitation, tree loss, and distance to the forest. Our models indicated that the disease risk areas were mainly distributed in low elevation (75–345m above mean sea level) areas along the Titiwangsa mountain range and inland central-northern region of Peninsular Malaysia. The high-resolution risk map of human knowlesi malaria constructed in this study can be further utilized for multi-pronged interventions targeting community at-risk, macaque populations, and mosquito vectors.

KEYWORDS

***Plasmodium knowlesi*, Peninsular Malaysia, ecological niche modeling, XGBoost, ensemble modeling, maximum entropy**

# 1. Introduction

Environmental variations including land cover types, climate changes, anthropogenic landscapes, and host distributions have been linked to the geographical distribution and altered transmission patterns of malaria and other vector-borne diseases worldwide (Medone et al., 2015; Morand and Lajaunie, 2021; Kulkarni et al., 2022). In Malaysia, the transmission of the simian malaria species *Plasmodium knowlesi*, *via Anopheles* Leucosphyrus group mosquitoes, has been attributed to environmental changes affecting the proximity between people, macaque reservoirs (mainly *Macaca fascicularis* and *M. nemestrina*), and mosquito vectors (Cuenca et al., 2021). It is important to highlight that the incidence of human knowlesi malaria has grown significantly over the last two decades, threatening the malaria elimination efforts in Malaysia and other Southeast Asian countries (Singh et al., 2004; Shearer et al., 2016; Chin et al., 2020). It is suggested that the increasing reports of human knowlesi malaria are driven by deforestation, agricultural expansion, and spatial overlaps between the human population and wildlife hosts (Moyes et al., 2016; Fornace et al., 2019).

Malaysia is geographically divided by the South China Sea into two regions, Peninsular Malaysia and Malaysian Borneo. Heterogeneities exist in the distribution of *P. knowlesi* vectors between these regions such as *An. cracens*, *An. introlatus*, and *An. hackeri* in Peninsular Malaysia, and *An. balabacensis* and *An. latens* in Malaysian Borneo (Tan et al., 2008; Wong et al., 2015; Ang et al., 2020; Jeyaprakasam et al., 2021a). Molecular epidemiological studies have found that the geographical separation could have also driven the allopatric divergence of *P. knowlesi* into distinct subpopulations (Divis et al., 2017). Studies in Sabah, a state in Malaysian Borneo, have demonstrated the association between environmental factors and knowlesi malaria risk (Brock et al., 2019; Fornace et al., 2019; Sato et al., 2019; Hod et al., 2022). However, environmental influences on knowlesi malaria in Peninsular Malaysia are not widely studied. Therefore, it is of interest to know how environmental factors may impact knowlesi malaria transmission in Peninsular Malaysia.

As a part of the malaria intervention strategy in Malaysia, disease screening *via* active case detection, mass blood survey, and entomological surveillance were conducted mainly in localities with a history of malaria cases. This intervention strategy is not able to effectively cover other parts of the populations which are at high-risk or may be exposed to the disease without case notifications, especially among Orang Asli (i.e., indigenous people) communities in forested areas lacking accessible roads. Also, not knowing the locations of the high-risk area may affect the systematic implementation of macaque reservoir screening and entomological surveillance. Therefore, identifying the ecological niche of the disease can support plans for controlling disease transmission.

The emerging role of machine learning approaches in healthcare and spatial epidemiology is instrumental, especially in modeling the covariate contribution toward disease transmission as well as to predict the spatial distribution of the disease (Kopczewska, 2022; Temenos et al., 2022). For instance, MaxEnt (maximum entropy) algorithm enables the estimation of the geographical range of a target disease by determining the probability distribution of maximum entropy (i.e., most spread out or closest to uniform) based on the availability of case presence and ecological information within the study area (Phillips et al., 2006). Besides, decision-tree-based models such as random forest and gradient-boosted tree are popularly used in ecological niche modeling. These models have been widely applied to estimate the potential risk areas of diseases such as malaria (Bhatt et al., 2017), dengue (Liu et al., 2016), West Nile virus (Shartova et al., 2022), scrub typhus (Acharya et al., 2019), brucellosis (Jia and Joyner, 2015), and Chagas disease (Mischler et al., 2012) as well as to estimate the spatial distribution of the vectors of Lyme disease (Burrows et al., 2022), chikungunya (Richman et al., 2018), leishmaniasis (Cunze et al., 2019), and malaria (Akpan et al., 2018). Previous studies have demonstrated the use of boosted regression tree (BRT) to map the geographical distribution of natural reservoirs and vectors of *P. knowlesi* and estimated the risk of *P. knowlesi* infection throughout Southeast Asia (Moyes et al., 2016; Shearer et al., 2016). Also, several studies applied ensemble modeling techniques by integrating multiple predictive models to generate a prediction of malaria risk with higher performance (Bhatt et al., 2017; Chemison et al., 2021).

A relatively new approach known as extreme gradient boosting (XGBoost), was found to outperform various models in spatial modeling (Zhao et al., 2021). In addition to improving the model performance, understanding the influence of each parameter in the model is important for public health administration. Recently, SHAP (SHapley Additive exPlanations) tool has rendered detailed explanations to once-considered black-box machine learning models without sacrificing performance. This approach is coupled with XGBoost as a method emphasized in this study.

Understanding the transmission patterns and geographical distribution of *P. knowlesi* in Peninsular Malaysia is essential to strategize effective disease control measures and enhance understanding of how ecologies affect the risks of knowlesi malaria. To address these needs, we aimed to investigate the impacts of diverse environmental variations toward human knowlesi malaria occurrence as well as to predict potential high-risk areas for human knowlesi malaria at fine spatial resolution across Peninsular Malaysia using machine learning models of MaxEnt and XGBoost.

# 2. Materials and methods

## 2.1. Ethic statement

This study was registered with the National Medical Research Register (NMRR-16-2,109–32,928), and ethical approval was obtained from the Malaysian Research Ethical Committee (MREC) [reference no. KKM/NIHSEC/P16-1782 (11)]. For all case data, information that identifies the patient was anonymized.

## 2.2. Geography of Peninsular Malaysia

Malaysia is a country in Southeast Asia and has two regions, Peninsular Malaysia and Malaysian Borneo (Figure 1A). Our study focused on Peninsular Malaysia which extends from latitude 1°15′50.0″N to 6°43′36.0″N and from longitude 99°35′E to 104°35″E (Figure 1B). From 2010 to 2019, Peninsular Malaysia experienced a loss of 2.26 million hectares of tree cover (Global Forest Watch, 2021; Figure 1C). Within this period, at least 90% of the tree loss was attributable to deforestation activities (Global Forest Watch, 2021). Previous studies suggested that landscape changes driven by deforestation would increase the likelihood of spillover of the macaque population into the human population, thus, increasing the risk of knowlesi malaria exposure (Fornace et al., 2016).

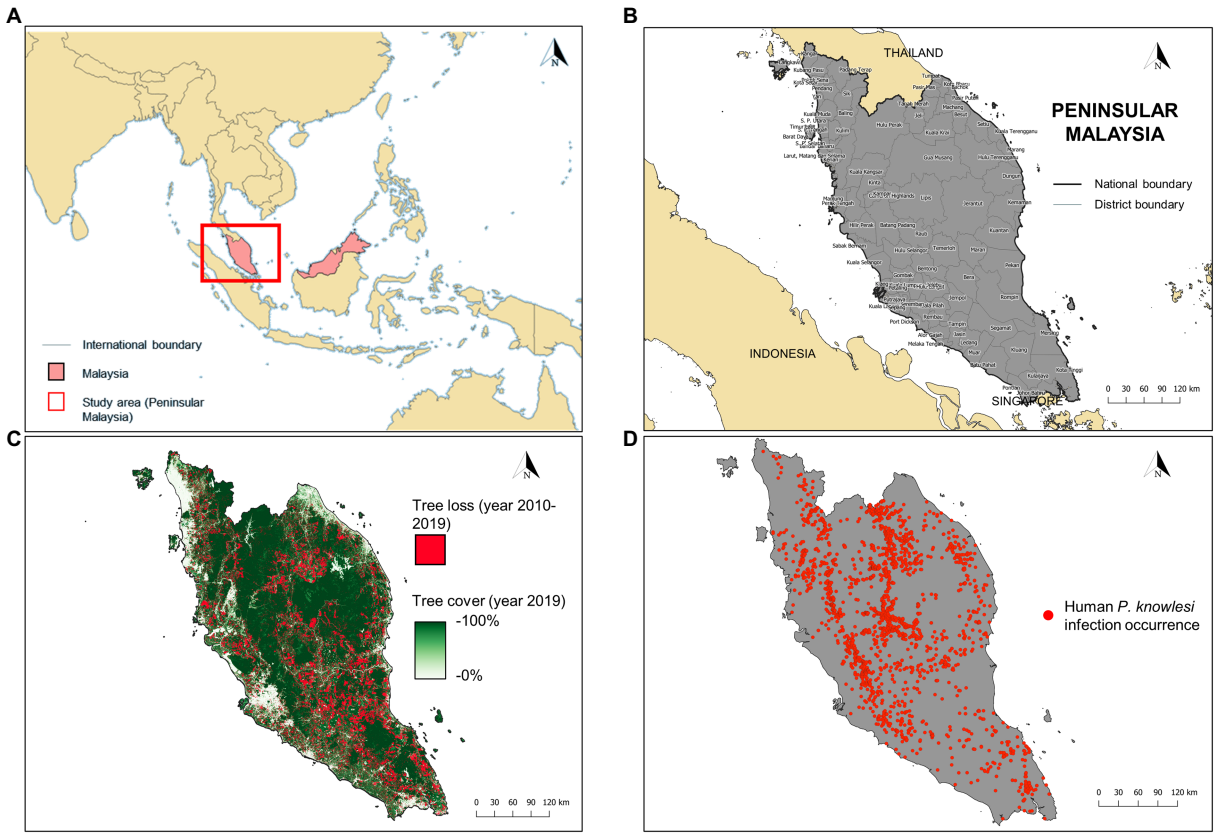


FIGURE 1
**(A)** Location of Peninsular Malaysia (red box). **(B)** The district-level administrative boundary of Peninsular Malaysia. **(C)** Extent of tree loss (recorded in years 2010–2019) and tree cover (recorded in year 2019) in Peninsular Malaysia. Tree loss data was acquired from Global Forest Change database (https://earthenginepartners.appspot.com/science-2013-global-forest/download_v1.7.html) whereas tree cover data was acquired from Copernicus Global Land Service (https://zenodo.org/record/3939050#.Yw-ZpXZBzIU). **(D)** Geolocated cases of human knowlesi malaria ($n$=2,873) throughout Peninsular Malaysia from years 2011–2019.

## 2.3. Human knowlesi malaria data

In Malaysia, all laboratory-diagnosed malaria cases are notified to the District Health Offices and State Health Departments, which will be subsequently compiled by the Ministry of Health Malaysia. Human knowlesi malaria cases are diagnosed *via* microscopic examination and/or nested PCR assay. In this study, retrospective data on knowlesi malaria cases from 1st January 2011 to 31st December 2019 were provided by the Ministry of Health Malaysia. Approximately 97.16% ($n$=2,873) of the reported indigenous knowlesi malaria cases (total=2,956) were able to be geolocated (Figure 1D). The source of infection reported for each case was manually geolocated as the occurrence point with reference to Google Maps (Google, 2022), Mapcarta (Mapcarta, 2022), Waze (Waze Mobile, 2022), as well as state and federal territory gazetteers (Ministry of Energy and Natural Resources Malaysia, 2022). For cases with no information on the source of infection addresses, household or working addresses were used as the replacement for occurrence point (9.89%, $n$=284, of the geolocated cases were georeferenced this way). Before running MaxEnt and XGBoost modeling, reports of cases within the same grid in a covariate layer were considered as a single unique record. This approach was used to reduce spatial clumping and avoid the inflation of model accuracy (Veloz, 2009). Overall, the case dataset consisted of 1,845 unique occurrence records. The overview of the modeling procedure is shown in Figure 2.

## 2.4. Spatial environmental covariate data collation and processing

ArcGIS Pro version 2.7.2 (Esri, Redlands, CA, United States) and QGIS version 3.6.3 (Open Source Geospatial Foundation, Beaverton, OR, United States) were used to visualize and process all spatial data. Original covariate data were acquired from multiple sources and processed as described in Supplementary Data and Supplementary Tables 1–3. The coordinate reference systems of all spatial data were projected to World Geodetic System (WGS) 84/Universal Transverse Mercator (UTM) zone 47 N. All covariates were resampled to produce raster layers with 1×1 km$^2$ pixel spatial resolution. A total of 36 constructed covariate spatial data consisted of landscape, climate, anthropogenic, and proximity characteristics were used for subsequent analysis (Supplementary Figures 1–3).

## 2.5. Multicollinearity test

A multicollinearity assessment was conducted to remove highly correlated covariates *via* two steps (Sillero et al., 2021). Firstly, a pairwise correlation matrix was constructed and Pearson's correlation coefficient $r \leq -0.8$ or $\geq 0.8$ were set as a threshold to selectively remove highly correlated covariates. Then, an assessment based on

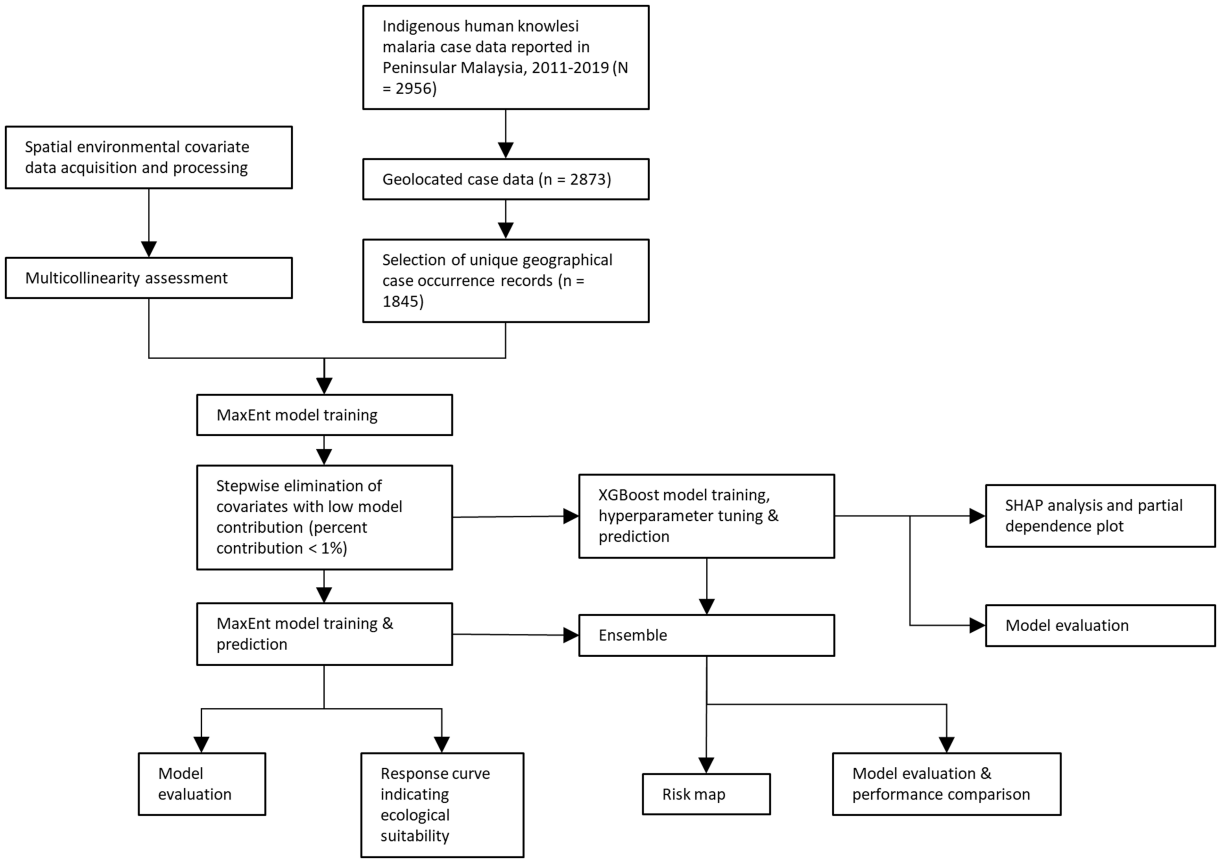


FIGURE 2
Schematic summarizing the modeling procedure in this study.

the variance inflation factor (VIF) was conducted to remove covariates with VIF ≥ 10.

## 2.6. Maximum entropy (MaxEnt) modeling procedure

MaxEnt is a machine learning approach which applies a maximum entropy algorithm to model potential distributions of an object based on presence-only datasets. MaxEnt version 3.4.4 (Phillips et al., 2006) was used in this study to construct the presence-background niche model for knowlesi malaria in Peninsular Malaysia. The unique case occurrence dataset was randomly partitioned into train dataset (70%) and test dataset (30%) through subsampling approach. Log-transformed value of human population density covariate was selected as the sampling bias layer. Sampling bias layer was included to account for the assumption of a greater likelihood of disease detection in populous places (Merow et al., 2013). The inclusion of sampling bias layer could also reduce the likelihood of false positives such as predicting highly populated areas as high-risk areas due to biased detection location. In this model, 10,000 background points were randomly sampled. The modeling software factors out bias by assigning weights to the background points based on the sampling bias layer value during modeling. The modeling parameters used include regularization multiplier of 1, 2000 iterations, and 0.00001 convergence threshold. The area under curve of receiver operating characteristic ($AUC_{ROC}$) was used to evaluate the performance of the model. The higher the $AUC_{ROC}$ value (ranging from 0 to 1), the higher its accuracy. The logistic output of the model was selected to present the predicted risk probability.

All environmental covariates (except human population density) that passed the multicollinearity assessment were included in the model training stage. Ten replicated models were fitted with each trained to a separate subsampled dataset. The relative importance of each covariate was ranked based on the percent contribution to the model. Backward stepwise elimination was applied to the to remove the covariates with the lowest percent contribution to the models until all remaining covariates have a percent contribution threshold of ≥1%.

To obtain a robust model, 30 replicated models were developed using the final covariate dataset (Convertino et al., 2012; Acharya et al., 2018). Mean output grids were calculated among the raster outputs of these 30 models and these grids were used to generate a 1×1 $km^2$ pixel spatial resolution predicted risk map of human knowlesi malaria. Ecological suitability ranges of the human knowlesi malaria transmission per covariate were demonstrated by response curves.

## 2.7. Extreme gradient boosting (XGBoost) modeling procedure

XGBoost is a machine learning algorithm based on gradient boosting, which can be utilized for both regression and classification problems. XGBoost is known for its ability to speed up data learning execution out of core computation (Chen and Guestrin, 2016). Similar to MaxEnt, we employed XGBoost as a presence-only model by using

the same dataset in the MaxEnt procedure, consisting of case occurrence and background points. This dataset was transformed into binary code of 1 and 0 to indicate case occurrence and background data, respectively. The covariates utilized for the final MaxEnt was similarly employed as predictors in XGBoost modeling. The partitioning of the case dataset into 70% train and 30% test datasets was the same as previously mentioned in the MaxEnt modeling procedure. We constructed the XGBoost model with a tree-based booster learning type and set the objective of binary logistic regression. It was noted that the background data make up a large proportion of the dataset by approximately five-fold as compared to the case occurrence data. This would lead to an imbalanced dataset, which can affect the model performance and cause biased prediction toward higher proportion class of background data. Therefore, we assigned a class weighted approach to reduce the impact of imbalanced data issue. The weight for each class (occurrence class weight, $w_1$, and background class weight, $w_0$) can be calculated as follows:

$$w_1 = \frac{N_{train}}{2N_{(train,1)}}$$

$$w_0 = \frac{N_{train}}{2N_{(train,0)}}$$

where $N_{train}$ is the total number of data points (both occurrence and background) in the train dataset, $N_{(train,1)}$ and $N_{(train,0)}$ are the numbers of occurrences and backgrounds, respectively, in train dataset. Weight assignment allows the handling of class imbalance by reducing model bias toward the majority class without manipulating the training data distribution (Johnson and Khoshgoftaar, 2019). Besides class weight, we included the bias layer of log-transformed human population density value as the instance weight for each corresponding occurrence and background points to adjust sampling bias. Class weight and instance weight were processed prior to input into the train dataset. $AUC_{ROC}$ was used to evaluate the performance of the model. During model training process, hyperparameter tuning was conducted to identify optimal parameters while maximizing the model training $AUC_{ROC}$. Five-fold cross-validation of the train dataset was performed during the tuning phase to avoid overfitting the model prediction. The final optimized parameters are described in Supplementary Table 4. Mean output grids were calculated among the raster outputs of 30 XGBoost replicates, and these grids were used to generate a $1\times1\,km^2$ pixel spatial resolution predicted risk map of human knowlesi malaria.

To provide better interpretations of environmental conditions and knowlesi malaria risk, we applied SHapley Additive exPlanations (SHAP) to disseminate and interpret the output of XGBoost model (Campbell et al., 2022). SHAP values were generated to evaluate the relative importance of covariates in the model. A high and positive SHAP value indicates that the covariate highly and positively affects the output of the prediction model and vice versa (Lundberg et al., 2020). Global SHAP summary plots and SHAP dependence plots were created to explain the relationship between covariates and the model prediction output. XGBoost modeling procedure was performed in R using maptools, raster, and usdm packages to manage digital mapping and data extraction, dplyr package for data manipulation, XGBoost package for running XGBoost algorithm, caret package for managing machine learning framework and hyperparameter tuning, pROC package for analyzing model $AUC_{ROC}$, and SHAPforxgboost package for generating SHAP value and plots.

## 2.8. Ensemble model procedure

Ensemble modeling involves the aggregation of outcome prediction from multiple model algorithms to generate a final prediction. Model ensemble approach is frequently applied to address machine learning issues such as incremental learning, imbalanced data, error correction, and confidence estimation, and it usually generates improved results (Polikar, 2012). An ensemble model was developed by averaging the outputs of MaxEnt and XGBoost models using the same subsampled datasets as used for constructing both MaxEnt and XGBoost. The averaged ensemble output was used to generate human knowlesi malaria risk map. The predictive performances of MaxEnt, XGBoost, and ensemble models were evaluated using $AUC_{ROC}$, sensitivity, specificity, and F1-score. To compare the prediction patterns produced by different models, 20,000 points were randomly sampled from the risk map outputs of the three models and converted by kernel density. District-level annual incidence rate in 1 million people was calculated by dividing the annual number of reported cases by estimated mid-year population size and multiplying by 1,000,000. Spearman's correlation test was conducted to determine the correlation between variables with *value of* $p$ $<0.05$ indicates statistical significance. The procedure of model development and validation was carried out in R software. The R script used to conduct XGBoost and ensemble modeling is available at https://github.com/WKPhang/XGBoost_EcologicalNicheModel/.

## 2.9. Identification of priority areas for intervention and surveillance

Priority zone maps were developed to identify priority areas for intervention targeting agricultural and logging workers, entomological surveillance, and macaque surveillance. Before the development of a priority zone map for intervention targeting agricultural and forest workers, the land cover of the workplace of agricultural and logging workers was estimated by overlaying the covariate layers of cropland, oil palm, and historical tree loss. For each pixel grid, the highest value of either of the overlaid value was selected to represent the value of the output map. A priority zone map highlighting important areas for intervention targeting agricultural and logging workers is important as this group of populations is considered at-risk and regularly exposed to potentially infective mosquitoes (Grigg et al., 2017; Chin et al., 2021). It was noted that 92% of tree cover loss in the year 2010–2019 was driven by deforestation (Global Forest Watch, 2021). Hence, it is important to consider the high likelihood of logging workers presence in areas where tree loss occurred. The relative occurrence probability maps of the *Anopheles* Leucophyrus group mosquito, *M. fascicularis*, and *M. nemestrina* were included in the development of priority zone maps for entomological and macaque surveillance. Threshold values indicating relative priority scores were set based on the quantile-based classification of each covariate and predicted risk map. We assigned the values in the first and second quarters a score of 0, values in the third quarter a score of 1, and values in the fourth quarter a score of 2. The score assignment of each covariate and risk map was described in Supplementary Table 5. For each objective, the

relative priority score of covariates and predicted risk map were summed to produce scores ranging between 1 (lowest priority) to 5 (highest priority).

# 3. Results

## 3.1. Model development and evaluation

Multicollinearity assessment *via* a pairwise correlation matrix revealed strong correlations between several covariates (Figure 3). Seven covariates with strong correlation relationships were removed while retaining relevant covariates in the modeling dataset. For instance, elevation has strong a negative correlation with three spatial climate covariates (historical minimum temperature with $r = -0.93$, historical maximum temperature with $r = -0.88$, and historical water vapor with $r = -0.97$). Thus, elevation is deemed more suitable to be maintained to represent these climate covariates. Besides, dense forest and secondary forest covariates were removed to ensure that the dataset achieved an overall VIF <10. Twenty-seven covariates were maintained for subsequent analysis after multicollinearity assessment. Before modeling, the human population density was excluded for inclusion as a sampling bias layer, leaving a balance of 26 covariates as predictors in starting model.

Backward stepwise elimination was conducted by initial MaxEnt modeling using 26 spatial covariates. Subsequently, we identified a reduced dataset of 14 covariates which fulfilled the criteria of having a percent contribution of ≥1 (Table 1). MaxEnt modeling using the final covariate dataset depicted high model performance with mean $AUC_{ROC}$ values of $0.835 \pm 0.003$ and $0.824 \pm 0.007$ for train and test datasets, respectively, (Table 2). The most important covariates were distance to coastline, forest cover, cropland, *M. fascicularis* occurrence probability, historical tree loss, and historical annual precipitation (Table 1).

XGBoost modeling using the final 14 covariates showed high predictive performance with $AUC_{ROC}$ values of $0.933 \pm 0.002$ and $0.854 \pm 0.007$ for the train and test datasets, respectively, (Table 2). The key covariates in the model fitting of XGBoost were distance to coastline, elevation, tree cover, historical annual precipitation, historical tree loss, and distance to forest (Figure 4). The output of ensemble model built showed higher $AUC_{ROC}$ than MaxEnt but lower than XGBoost ($AUC_{ROC} = 0.904 \pm 0.002$ for train dataset and $AUC_{ROC} = 0.845 \pm 0.008$ for test dataset). Despite XGBoost having a superior performance as compared to the other models, kernel density estimation showed a relatively similar distribution of predicted risk across models. There were statistically significant high positive correlations for all pairwise comparisons of the models: MaxEnt-XGBoost ($\rho = 0.899$, value of $p < 0.001$), MaxEnt-ensemble ($\rho = 0.969$, value of $p < 0.001$), and XGBoost-ensemble ($\rho = 0.977$, value of $p < 0.001$) (Figure 5).

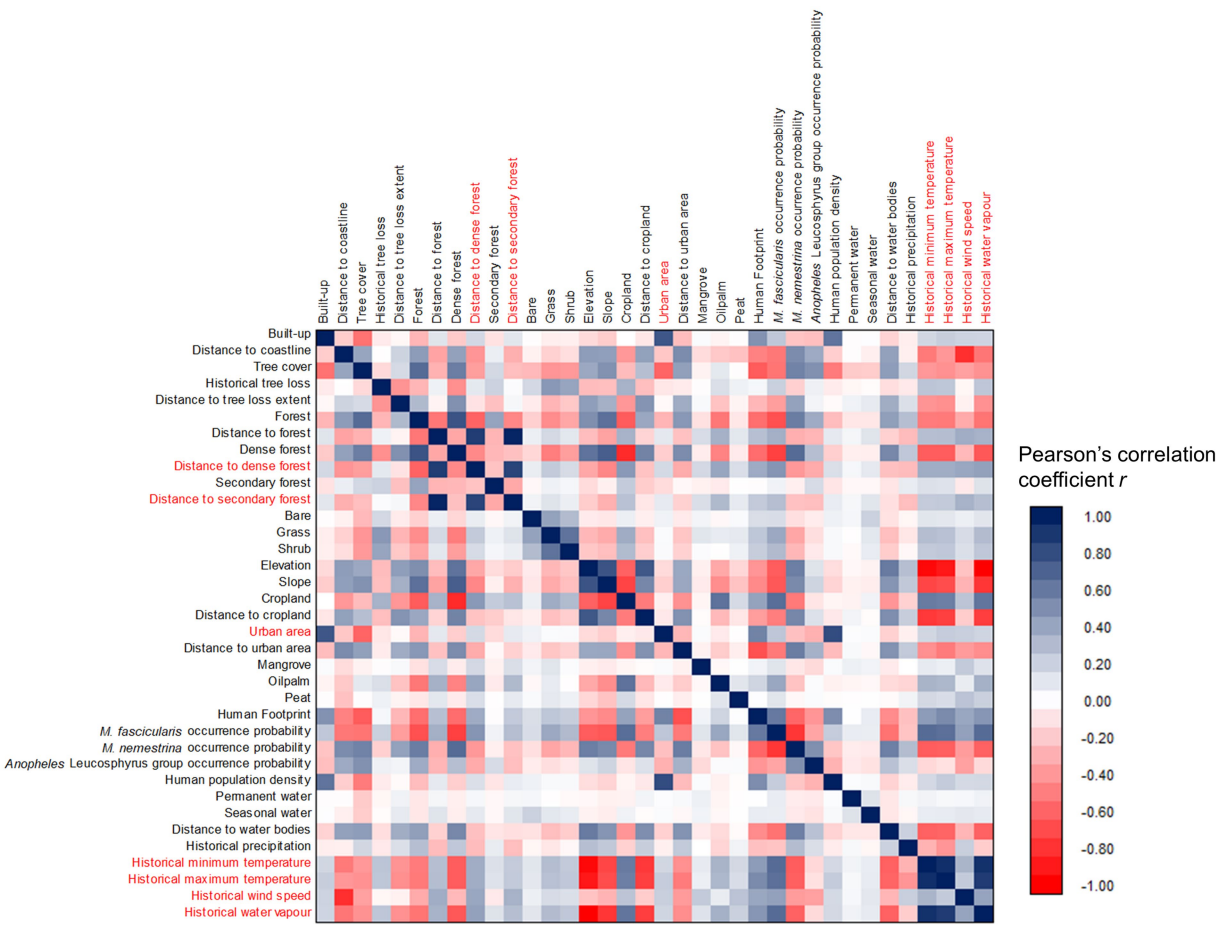


FIGURE 3
Correlation matrix of all spatial covariates. Covariates highlighted in red indicated high collinearity ($r \leq -0.8$ or $\geq 0.8$) with at least one of other covariates were removed from the modeling dataset.

## 3.2. Environmental suitability for the occurrence of human knowlesi malaria

Suitable range of each important environmental factor for the occurrence of human knowlesi malaria was identified based on the response curve of MaxEnt model and the partial dependence plot of XGBoost model (Figures 6, 7). Both models indicated that there was a higher risk of human knowlesi infection at inland areas distant from the coastline (>50 km distance in XGBoost or > 70 km distance in MaxEnt), experienced low intensity of tree loss (3–20% in XGBoost or 3–40% in MaxEnt), and with high annual precipitation (>2,500 mm in MaxEnt or > 2,640 mm in XGBoost). XGBoost demonstrated that there was a higher risk of human knowlesi malaria infection at lower elevation regions of 75–345 m above mean sea level, a wide range of tree cover (<82%), and near to forest landscape (<200 m). In association with various forest-related covariates, MaxEnt showed that the risk of knowlesi malaria increased at >32% forest cover.

As various forest-related covariates (forest cover, tree cover, historical tree loss, and distance to forest) were found to have significant influences on either of the two models, it was of interest to identify the type of forest where knowlesi malaria transmission is high. Thus, an alternative dataset was prepared by replacing the tree cover and forest cover with dense forest cover and secondary forest cover. An XGBoost analysis involving this dataset showed that knowlesi malaria cases have a higher probability to occur in areas with high secondary forest cover (>13%) and with low dense forest cover (<18%) (Supplementary Figure 4).

Besides, the knowlesi malaria environmental suitability range was found to be influenced by other spatial attributes such as *M. fascicularis* occurrence probability, and cropland in MaxEnt (Figure 6). This signifies that the occurrence of human knowlesi malaria has a specific ecological niche with multi-dimensional environmental factors playing roles in the disease transmission cycle.

TABLE 1 Relative importance of each covariate toward modeling of human knowlesi malaria risk based on MaxEnt model percent contribution.

| Covariates | Percent contribution |
|---|---|
| Distance to coastline | 22.643 ± 1.667 |
| Forest cover | 17.687 ± 2.555 |
| Cropland | 11.120 ± 2.600 |
| *M. fascicularis* occurrence probability | 9.634 ± 0.818 |
| Historical tree loss | 6.732 ± 1.219 |
| Historical annual precipitation | 5.681 ± 0.712 |
| Oil palm | 5.594 ± 1.876 |
| Tree cover | 4.493 ± 0.876 |
| Elevation | 3.980 ± 1.534 |
| Human footprint | 3.319 ± 1.219 |
| Built-up | 2.910 ± 0.298 |
| Distance to cropland | 2.506 ± 0.899 |
| Distance to forest | 2.337 ± 0.891 |
| *M. nemestrina* occurrence probability | 1.366 ± 0.306 |

## 3.3. Distribution of human knowlesi malaria in Peninsular Malaysia

The mean model outputs were used to generate predicted human *P. knowlesi* infection risk maps of 1×1 km$^2$ pixel spatial resolution (Figure 8). All models generated similar predicted spatial patterns across Peninsular Malaysia. Risk map generated by XGBoost was used as the final map output due to its higher performance compared to other models (Table 2). Based on the risk map, the models predicted that the ecological factors in the central-northern region of Peninsular Malaysia and the lower elevation areas along Titiwangsa mountain range are highly suitable for knowlesi malaria transmission. The mean predicted risk value was extracted for each district in Peninsular Malaysia. The district-level mean predicted risk is presented alongside the average annual human knowlesi malaria incidence rate in year 2011–2019 (Figures 9A,B). There is a significant positive correlation between mean predicted risk and disease incidence rate (in 1 million people) (Spearman's correlation coefficient $\rho = 0.76$, value of $p < 0.001$; Figure 9C).

## 3.4. Intervention and surveillance priority zone maps

The predicted risk map produced using XGBoost was subsequently selected for developing the intervention and surveillance priority zone maps (Figure 10). In coherence with the predicted risk map, most of the high-priority areas are situated in the central northern region of Peninsular Malaysia. For surveillance targeting agricultural and logging workers, the high-priority zones are mostly located in suburban areas in the central-northern Peninsular Malaysia region as well as near hills in the southern state of Johor (Figure 10A). *Anopheles* Leucosphyrus group mosquito priority zone maps indicated that key areas for enhanced surveillance are mostly located in the interior (Figure 10B). *M. fascicularis* surveillance priority zones are mainly situated in the peri-domestic areas as compared to *M. nemestrina* surveillance priority zones, which are mainly found in the interior part of Peninsular Malaysia.

TABLE 2 Performance comparison across MaxEnt, XGBoost, and ensemble models.

| Model | MaxEnt | | XGBoost | | Ensemble | |
|---|---|---|---|---|---|---|
| Dataset | Train | Test | Train | Test | Train | Test |
| $AUC_{ROC}$ | 0.833 ± 0.003 | 0.821 ± 0.009 | **0.933 ± 0.002** | **0.854 ± 0.007** | 0.904 ± 0.002 | 0.845 ± 0.008 |
| Sensitivity | 0.622 ± 0.006 | 0.606 ± 0.026 | **0.916 ± 0.004** | **0.742 ± 0.18** | 0.781 ± 0.005 | 0.684 ± 0.020 |
| Specificity | **0.874 ± 0.003** | **0.874 ± 0.003** | 0.816 ± 0.003 | 0.816 ± 0.003 | 0.848 ± 0.003 | 0.848 ± 0.003 |
| F1-score | 0.479 ± 0.007 | **0.312 ± 0.008** | **0.548 ± 0.005** | 0.293 ± 0.005 | 0.527 ± 0.005 | 0.308 ± 0.005 |

Bolded value indicates the best performance per evaluation metric ($AUC_{ROC}$, Sensitivity, Specificity, and F1-score) per train or test dataset across the three modeling methods.

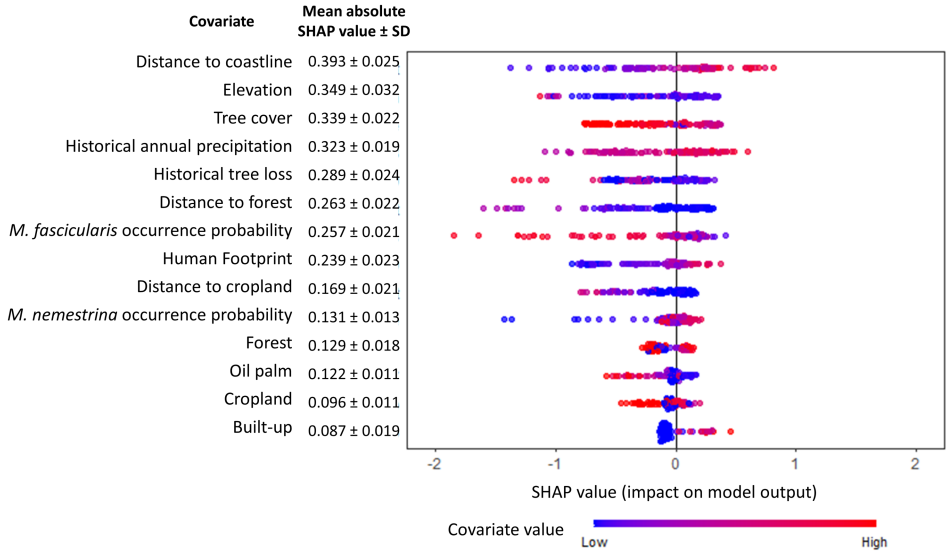


FIGURE 4
Global SHAP summary plot. The relative importance of each covariate toward human knowlesi malaria risk is indicated and ordered (most important covariate at the top) by the mean absolute SHAP value summarized over 30 model replicates. Warmer dot color indicates higher value of corresponding covariate.

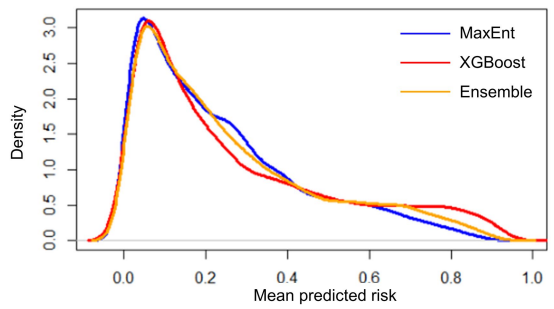


FIGURE 5
Distribution of mean predicted risk of each model output based on kernel density estimation. Spearman's correlation test was conducted for MaxEnt-XGBoost ($\rho$=0.899, value of $p$<0.001), MaxEnt-ensemble ($\rho$=0.969, value of $p$<0.001), and XGBoost-ensemble ($\rho$=0.977, value of $p$<0.001).

## 4. Discussion

This study incorporated diverse environmental data sources as well as the national knowlesi malaria case data to predict spatial knowlesi malaria transmission risk using machine learning approaches. Higher performance was observed in XGBoost as compared to other modeling approaches. XGBoost can generate high-resolution maps showing the risk of knowlesi malaria transmission to humans from known reservoirs, specifically *M. nemestrina* and *M. fascicularis*. One of the primary benefits of this map is that it allows for the identification of high-risk areas down to the village level. These high-risk areas can be prioritized for intervention or strengthening of existing surveillance systems.

In understanding the spatial heterogeneities of human knowlesi malaria occurrence, it is important to identify diverse environmental factors with optimal ranges that drive the transmission. For instance, forest cover was recognized as a key predictor in the MaxEnt model training, which reflects the role of forest environments as the habitats of macaque reservoirs and *Anopheles* mosquito vectors. Likewise, the XGBoost model showed that knowlesi malaria risk is higher in and near to the forest, which has also been observed in previous studies (Tan et al., 2008). A study in Sarawak found that the *P. knowlesi* vector *An. latens* had the highest sporozoite and oocyst rates in the forest as compared to farms (Tan et al., 2008). The association of increased knowlesi malaria occurrence with both forest and forest loss provides further support for the hypothesis that transmission occurs in forested areas undergoing substantial change (Fornace et al., 2016). Deforestation has been considered the main driver in the transmission of knowlesi malaria. As shown in this study, further classification of forest into dense forest and secondary forest revealed that the risk of knowlesi malaria is higher in areas mainly covered with secondary forest. An entomological study in Sabah found that the abundance of the local primary vector of knowlesi malaria, *An. balabacensis* is higher in the logged forest as compared to the primary forest (Brant et al., 2016). Another study revealed that higher percentage of infectious bites were likely to occur at households at forest edges (Fornace et al., 2019). This is related to the anthropogenic-induced conversion of forests into other land use such as cropland and settlements, which would affect macaque movements (Stark et al., 2019). For instance, the movement of macaques from forests to plantations and human settlements for food foraging would increase the contact between humans and macaques as well as the probability of zoonotic transmission of *P. knowlesi* can occur in the presence of efficient vectors (Imai et al., 2014).

In general, the predicted high-risk areas of knowlesi malaria are concentrated in lower elevation areas along the Titiwangsa mountain range and the central-northern region of Peninsular Malaysia. Other studies also indicated that geographical elevation was negatively associated with knowlesi malaria exposure (Fornace et al., 2016, 2019). This is because both the macaque hosts and vectors are more frequently found at lower elevation (Fooden, 1995). The risk of knowlesi malaria occurrence increased relative to distance from the coastline. This is apparent as forested areas where high transmission occurs are mainly

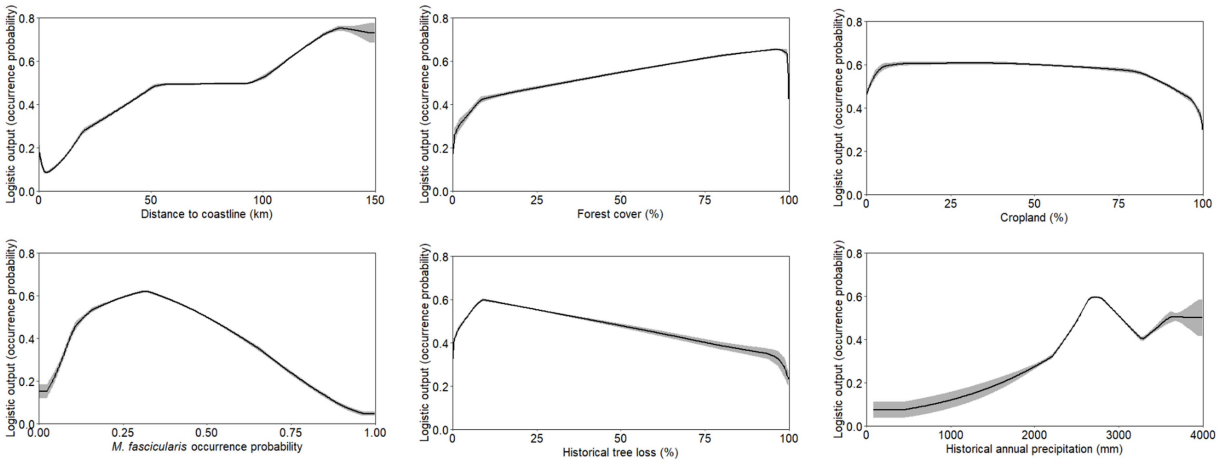


FIGURE 6
Response curve outputs from MaxEnt model demonstrating the range of suitability for human knowlesi malaria occurrence based on only key covariates with highest model percent contribution as described in Table 1. Grey band indicates standard deviation of the model output.

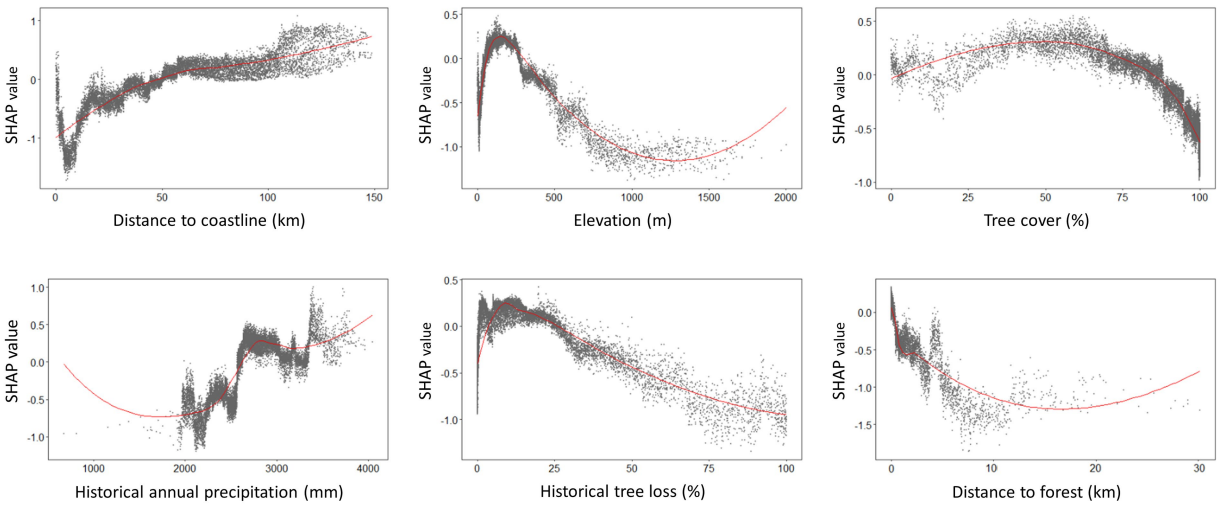


FIGURE 7
SHAP dependence plots generated from XGBoost model demonstrating the environmental suitability range for human knowlesi malaria occurrence based on only key covariates with highest mean absolute SHAP value as reported in Figure 4. Positive SHAP value indicates higher risk of knowlesi malaria infection whereas negative SHAP value indicates lower risk of knowlesi malaria infection. The plots were smoothed using LOESS (locally estimated scatterplot smoothing) curve in red.

situated inland. Greater urbanization nearer to the coastline has disrupted *Anopheles* mosquitoes' habitat and abundance, thus, transmission intensity in these areas is likely low (Ferraguti et al., 2016).

Both MaxEnt and XGBoost models explain that knowlesi malaria tends to occur in areas with high historical annual precipitation. Consistent rainfall with partial contribution from land-use changes would create favorable breeding sites for *Anopheles* mosquitoes and support larval development (Oo et al., 2002; Ahmad et al., 2018). In Sabah, an increase in knowlesi malaria cases was observed after 2 to 4 months of increased rainfall (William et al., 2014). Also, an increase in knowlesi malaria incidence 3 months after higher rainfall and higher humidity was found *via* univariate analyses in another study, but these associations were not statistically significant in multivariate analysis (Cooper et al., 2020). In Thailand, climate factors such as rainfall, temperature, and relative humidity were found to be associated with malaria incidence (Kotepui and Kotepui, 2018). Extreme rainfall may be unfavorable to malaria transmission as it would lead to a wash-out effect that disrupts vector breeding sites and causes larvae mortality (Thomson et al., 2005; Tompkins and Ermert, 2013). The utilization of time-series modeling would be able to help in explaining the non-linear relationship between rainfall and malaria transmission in detail. Also, there was a transient drop of number of knowlesi malaria cases throughout Malaysia in year 2015 and 2016, which was thought to be impacted by changing weather pattern and El Niño phenomenon (Cooper et al., 2020; Phang et al., 2020; Ooi et al., 2021). Nevertheless, other factors such as landscape factors such land-use change and deforestation play important roles in transmission patterns, which makes it difficult to fully understand the impact of climate change on knowlesi malaria transmission. More research is needed to fully understand the complex relationship between climate change and *P. knowlesi* transmission.

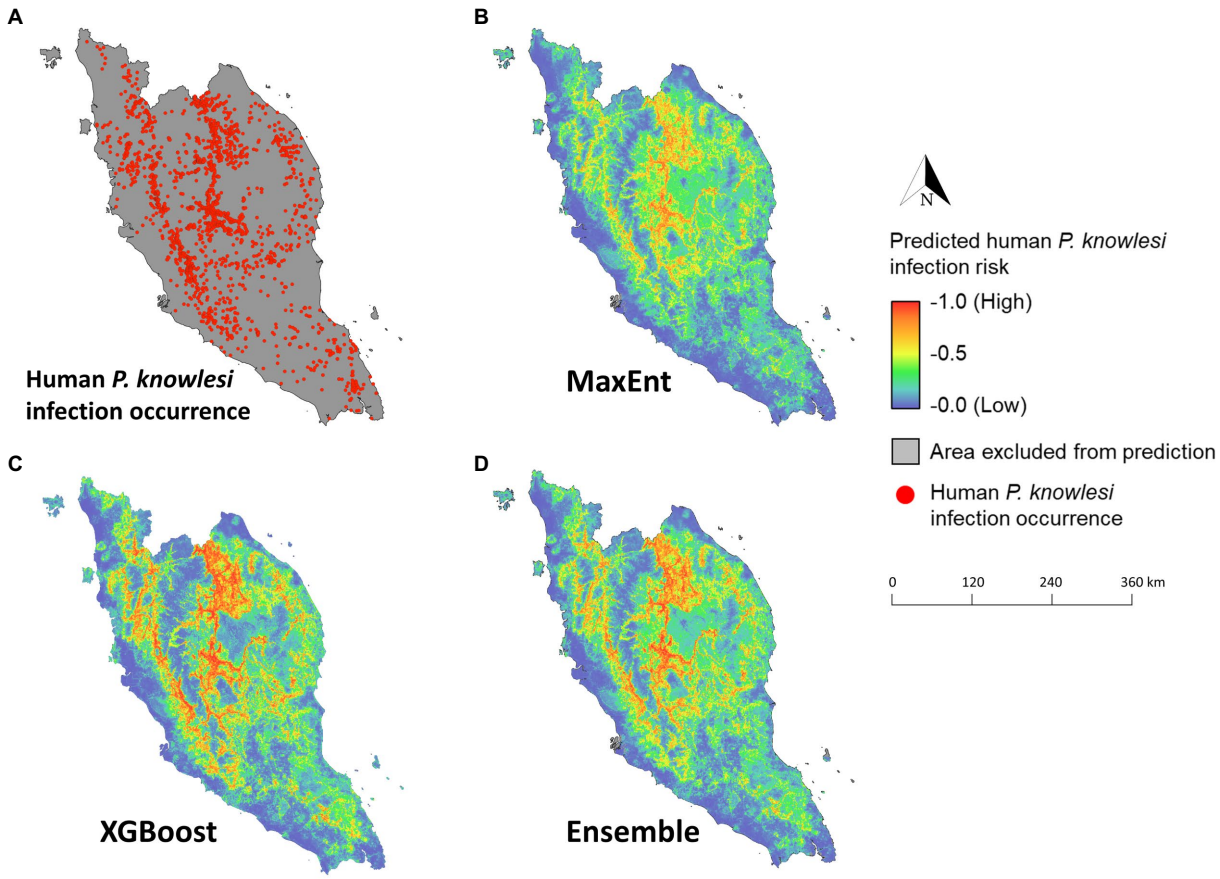


FIGURE 8
Maps of predicted human knowlesi malaria risk in Peninsular Malaysia. **(A)** Map of geolocated human knowlesi malaria occurrence throughout Peninsular Malaysia from years 2011–2019. Risk maps generated by MaxEnt **(B)**, XGBoost **(C)**, and ensemble models **(D)**. Warmer color indicates higher predicted risk of knowlesi malaria.

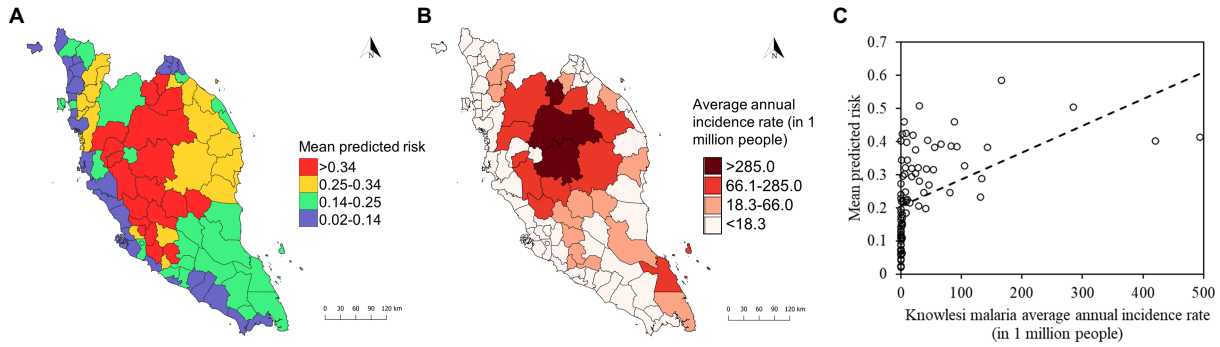


FIGURE 9
**(A)** District-level mean predicted risk of human knowlesi malaria based on XGBoost output. **(B)** Average annual incidence rate of knowlesi malaria from 2011 to 2019 by district. Color gradations for maps in **(A,B)** were determined using Jenks natural breaks. **(C)** Correlation plot shows statistically significant positive correlation (Spearman's correlation coefficient $\rho$=0.76, value of $p$ <0.001) between mean predicted risk and knowlesi malaria average annual incidence rate (in 1 million people).

The influence of *Anopheles* Leucosphyrus group mosquito occurrence was found to be less important in our models. This covariate was initially modeled using the scattered data collected before 2013 which may not present the reliable spatial distributions in the study region and resulted in its weak association with disease occurrence (Moyes et al., 2016). Breeding behavior, abundance, and distribution of certain mosquito species may change drastically over time due to landscape shifts, deforestation, and human encroachment (Burkett-Cadena and Vittor, 2018). At present, only *Anopheles* Leucosphyrus group mosquitoes are recognized as the vector of *P. knowlesi* in Peninsular Malaysia, but recent studies conducted in Sarawak have added *An. donaldi* from the Barbirostris group as well as *An. collessi* and

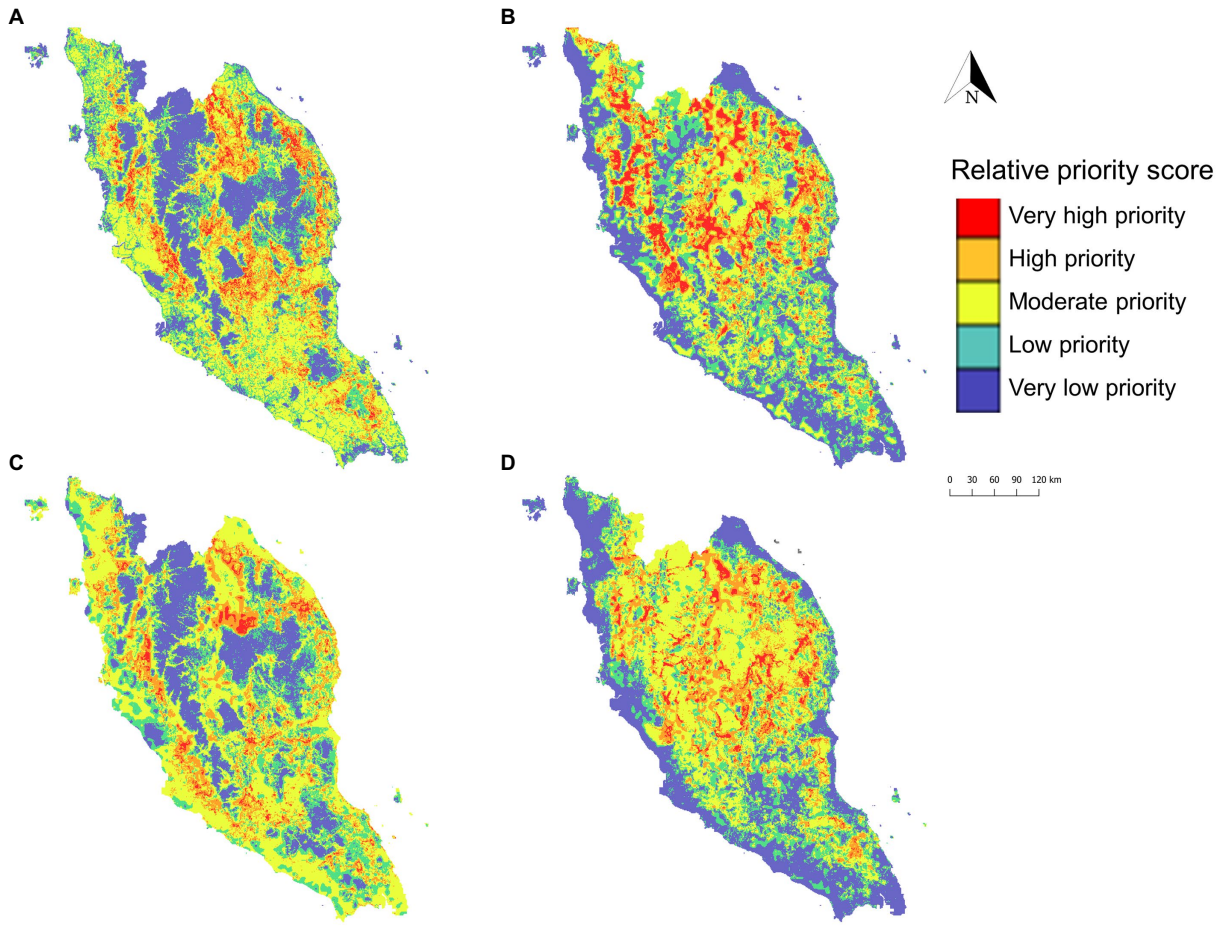


FIGURE 10
Priority zone maps developed to identify priority areas for intervention targeting agricultural and logging workers **(A)**, *Anopheles* Leucosphyrus group vector surveillance **(B)**, *M. fascicularis* surveillance **(C)**, and *M. nemestrina* surveillance **(D)**. The priority zone was determined using the sum of relative priority score of respective covariate spatial layer and XGBoost predicted risk of human knowlesi malaria.

*An. roperi* from the Umbrossus group into the list of potential vectors (Ang et al., 2020, 2021). It may be possible that there are efficient vectors other than the Leucosphyrus group mosquitoes in Peninsular Malaysia. It is necessary to implement continuous entomological surveillance for updating entomological data to monitor changes in *Anopheles* mosquito biology, to identify potentially new vectors, as well as to investigate the possible influence on receptivity across multiple localities in Malaysia. In addition, new tools are essential to enable efficient and cost-effective entomological fieldwork. For instance, the predictive risk map developed in this study has the potential to guide entomologists in identifying suitable surveillance locations. To complement the efficiency of vector sampling in the field, the use of commercialized mosquito traps as a safer alternative to human landing catch and the application of multiplex polymerase chain reaction assay for the accurate identification of certain *Anopheles* mosquito species should be considered (Jeyaprakasam et al., 2021b; Pramasivan et al., 2022).

The utility of MaxEnt has been well documented in various epidemiology-related ecological studies for its high performance in species distribution range prediction. However, this showed that XGBoost performed better than MaxEnt. Nevertheless, this may not indicate that XGBoost always offers superior performance compared to MaxEnt. This is because each model has different strengths and weaknesses with different outcomes. Therefore, an ensemble of multiple models is recommended to integrate the attributes of each involved model in a complementary manner. This approach is generally applied to address issues such as incremental learning, imbalanced data, error correction, and confidence estimation, and it usually generates improved results (Polikar, 2012). Some studies highlighted that combining relatively high-performing base models with low correlation or high diversity can generate ensemble models with higher performance (Pan et al., 2019; Yu et al., 2022). Nonetheless, our study demonstrated that the use of a single best-performing base model of XGBoost was adequate because the outputs from both base models, MaxEnt and XGBoost, were highly correlated with a lack of novel information to improve ensemble model performance.

The approach applied in this study demonstrated the importance of integrating empirical data from multiple agencies and developed a guide for future collaborative-based programs. From the zoonotic malaria control perspective, it is important to address the interdependence between humans, animals, and their environmental variations. The involvement of macaques as the natural hosts of *P. knowlesi* complicates the elimination and subsequent eradication of malaria and requires intervention strategies designed to specifically address zoonotic pathways, which is different from the strategy for tackling human malaria (Vythilingam et al., 2018; Mohammad et al., 2022). Thus, a unifying approach converging transdisciplinary and multisectoral

efforts is essential to combat the transmission of *P. knowlesi*, as advocated in the "One Health" concept. These efforts include sharing and co-assessment of intervention and data from epidemiologists, clinicians, zoologists, and entomologists, development of novel tools and platforms that can be adapted in different settings, as well as converging diagnostics for human, vector, and macaque reservoirs.

The development of intervention and surveillance priority zone map highlighted how the risk map can be further utilized to identify priority areas for concentrated efforts. For instance, the localities of the population at risk can be identified and effective interventions can be adapted to target populations. In this case, personal-level protective equipment such as insecticide-treated outdoor clothing, topical repellent, chemoprophylaxis, and spatial repellent shall be distributed more frequently to agricultural and logging workers, military personnel, as well as people living in high-risk areas (Vythilingam et al., 2021; Mohammad et al., 2022). Regular screening as well as awareness programs shall be conducted for communities in these areas. Specifically, in high-risk areas with a lack of accessible routes, the development and distribution of highly sensitive, mobile, and affordable tools such as novel rapid diagnostic test kits will enhance public health outreach (Tan et al., 2022).

Several potential strategies have been highlighted in relation to vector and wildlife controls. At present, indoor residual spraying and insecticide-treated net have been practiced as the core vector interventions in Malaysia (Ministry of Health Malaysia, 2022). However, the effectiveness of certain indoor-based interventions may be limited by the outdoor biting behaviors of the *P. knowlesi* vectors (Grigg et al., 2017; Vythilingam et al., 2021). Recent studies showed that outdoor-based applications such as outdoor residual sprays are effective against primary *P. knowlesi* vectors in Malaysian Borneo (Rohani et al., 2020, 2021). The distribution of vaccines or drug-treated oral baits for macaques has been proposed in wildlife-based intervention, and it is less invasive than macaque population culling, which is being debated for ethical reasons and uncertain implications (Cuenca et al., 2021). This similar method has been found promising in controlling other zoonoses such as Lyme disease (Dolan et al., 2017) and rabies (Rosatte et al., 2009; Maki et al., 2017). Nonetheless, there are currently no suitable vaccine or drug candidates that could be adapted for similar use in knowlesi malaria wildlife control programs. The use of oral baits will necessitate further research, and as suitable oral baits are developed in the future, they can be distributed to macaque populations in knowlesi malaria high-risk areas.

Surveillance, monitoring, and intervention are important aspects of zoonotic disease management and control because they serve as a guideline for detecting high-risk areas early in an outbreak and deciding how to allocate resources and manpower during disease outbreaks. The generated risk map had a high level of agreement with the actual data. Therefore, zoonotic disease management and control efforts should be targeted at the areas showing high probability of human knowlesi malaria occurrence. Furthermore, we propose that covariates with a high contribution be considered in field monitoring. We can identify the relative impact of environmental factors on knowlesi malaria occurrence by analyzing the partial dependence plots of each model. This data is required for epidemiologists, public health officials, and policymakers to effectively monitor and control knowlesi malaria.

There are several limitations to address concerning this study. Firstly, the ecological niche modeling approach in this study did not specifically consider the spatial variability of *P. knowlesi* infections in macaques and mosquitoes. To develop a surveillance system of macaques and vectors at priority zones will provide such information to enhance the accuracy of risk maps. Secondly, moderate F1-scores, which is caused by imbalanced data and random selection of background data near to reported cases, produced more false positive predictions. Elevated false positive rates may place additional demands on resources for monitoring and managing disease, however, this can be systematically reduced by alternative methods of identifying priority zones for targeted interventions. In addition, advanced deep learning algorithms can be considered to enhance model performance in the future.

## 5. Conclusion

Machine learning-based ecological niche modeling approaches such as MaxEnt and XGBoost are extremely useful in capturing diverse ecological signals relevant to spatial distributions of vector-borne diseases. The predictive risk maps produced in the present study can be used to identify high-risk areas of knowlesi malaria transmission and provide more precise information for decision-making of vector or reservoir surveillance and disease control, particularly when prevention resources are limited.

## Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The data of this study are available from the Ministry of Health Malaysia. Restrictions apply to the availability of these data. Data are available with the permission of the Ministry of Health Malaysia. The data generated in this study is available from the corresponding author on reasonable request. Requests to access these datasets should be directed to chtingwu@tmu.edu.tw.

## Ethics statement

The studies involving human participants were reviewed and approved by registered with the National Medical Research Register (NMRR-16-2109-32928), and ethical approval was obtained from the Malaysian Research Ethical Committee (MREC) [reference no. KKM/NIHSEC/P16-1782 (11)]. For all case data, information that identifies the patient was anonymized. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

## Author contributions

WP, T-WC, YL, and MF conceptualized and designed the study. MH, JJ, and RM were involved in data collection and provided the dataset for analysis. WP and T-WC conducted the data analysis. WP wrote the manuscript. All authors critically reviewed, revised, and approved the final manuscript.

## Funding

This study was supported by the Ministry of Higher Education, Malaysia Long Term Research Grant Scheme (LRGS/1/2018/

UM/01/1/1) and the Ministry of Science and Technology, Taiwan (MOST110-2621-M-038-001-MY2).

## Acknowledgments

The authors would like to thank the Ministry of Health Malaysia for providing data for this study.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1126418/full#supplementary-material

## References

Acharya, B. K., Cao, C., Xu, M., Khanal, L., Naeem, S., and Pandit, S. (2018). Present and future of dengue fever in Nepal: mapping climatic suitability by ecological niche model. *Int. J. Environ. Res. Public Health* 15:187. doi: 10.3390/ijerph15020187

Acharya, B. K., Chen, W., Ruan, Z., Pant, G. P., Yang, Y., Shah, L. P., et al. (2019). Mapping environmental suitability of scrub typhus in Nepal using MaxEnt and random forest models. *Int. J. Environ. Res. Public Health* 16:4845. doi: 10.3390/ijerph16234845

Ahmad, R., Ali, W., Ali, W., Omar, M., Azahary, A., Rahman, A., et al. (2018). Characterization of the larval breeding sites of *Anopheles balabacensis* (baisas), in Kudat, Sabah, Malaysia. *Southeast Asian J. Trop. Med.* 49, 566–579.

Akpan, G. E., Adepoju, K. A., Oladosu, O. R., and Adelabu, S. A. (2018). Dominant malaria vector species in Nigeria: modelling potential distribution of *Anopheles gambiae* sensu lato and its siblings with MaxEnt. *PLoS One* 13:e0204233. doi: 10.1371/journal.pone.0204233

Ang, J. X. D., Kadir, K. A., Mohamad, D. S. A., Matusop, A., Divis, P. C. S., Yaman, K., et al. (2020). New vectors in northern Sarawak, Malaysian Borneo, for the zoonotic malaria parasite, *Plasmodium knowlesi*. *Parasit. Vectors.* 13:472. doi: 10.1186/s13071-020-04345-2

Ang, J. X. D., Yaman, K., Kadir, K. A., Matusop, A., and Singh, B. (2021). New vectors that are early feeders for *Plasmodium knowlesi* and other simian malaria parasites in Sarawak, Malaysian Borneo. *Sci. Rep.* 11:7739. doi: 10.1038/s41598-021-86107-3

Bhatt, S., Cameron, E., Flaxman, S. R., Weiss, D. J., Smith, D. L., and Gething, P. W. (2017). Improved prediction accuracy for disease risk mapping using Gaussian process stacked generalization. *J. R. Soc. Interface* 14:20170520. doi: 10.1098/rsif.2017.0520

Brant, H. L., Ewers, R. M., Vythilingam, I., Drakeley, C., Benedick, S., and Mumford, J. D. (2016). Vertical stratification of adult mosquitoes (Diptera: Culicidae) within a tropical rainforest in Sabah, Malaysia. *Malar. J.* 15:370. doi: 10.1186/s12936-016-1416-1

Brock, P. M., Fornace, K. M., Grigg, M. J., Anstey, N. M., William, T., Cox, J., et al. (2019). Predictive analysis across spatial scales links zoonotic malaria to deforestation. *Proc. Biol. Sci.* 286:20182351. doi: 10.1098/rspb.2018.2351

Burkett-Cadena, N. D., and Vittor, A. Y. (2018). Deforestation and vector-borne disease: Forest conversion favors important mosquito vectors of human pathogens. *Basic Appl. Ecol.* 26, 101–110. doi: 10.1016/j.baae.2017.09.012

Burrows, H., Slatculescu, A. M., Feng, C. X., Clow, K. M., Guillot, C., Jardine, C. M., et al. (2022). The utility of a maximum entropy species distribution model for *Ixodes scapularis* in predicting the public health risk of Lyme disease in Ontario, Canada. *Ticks Tick Borne Dis.* 13:101969. doi: 10.1016/j.ttbdis.2022.101969

Campbell, T. W., Roder, H., Georgantas Iii, R. W., and Roder, J. (2022). Exact Shapley values for local and model-true explanations of decision tree ensembles. *Mach. Learn. Appl.* 9:100345. doi: 10.1016/j.mlwa.2022.100345

Chemison, A., Ramstein, G., Tompkins, A., Defrance, D., Camus, G., Charra, M., et al. (2021). Impact of an accelerated melting of Greenland on malaria distribution over Africa. *Nat. Commun.* 12:3971. doi: 10.1038/s41467-021-24134-4

Chen, T., and Guestrin, C. (2016). XGBoost: a scalable tree boosting system. Proceedings of the 22nd International Conference on Knowledge Discovery and Data Mining. New York, NY: ACM.

Chin, A. Z., Avoi, R., Atil, A., Awang Lukman, K., Syed Abdul Rahim, S. S., Ibrahim, M. Y., et al. (2021). Risk factor of *Plasmodium knowlesi* infection in Sabah Borneo Malaysia, 2020: a population-based case-control study. *PLoS One* 16:e0257104. doi: 10.1371/journal.pone.0257104

Chin, A. Z., Maluda, M. C. M., Jelip, J., Jeffree, M. S. B., Culleton, R., and Ahmed, K. (2020). Malaria elimination in Malaysia and the rising threat of *Plasmodium knowlesi*. *J. Physiol. Anthropol.* 39:36. doi: 10.1186/s40101-020-00247-5

Convertino, M., Welle, P., Muñoz-Carpena, R., Kiker, G. A., Chu-Agor, M. L., Fischer, R. A., et al. (2012). Epistemic uncertainty in predicting shorebird biogeography affected by sea-level rise. *Ecol. Model.* 240, 1–15. doi: 10.1016/j.ecolmodel.2012.04.012

Cooper, D. J., Rajahram, G. S., William, T., Jelip, J., Mohammad, R., Benedict, J., et al. (2020). *Plasmodium knowlesi* malaria in Sabah, Malaysia, 2015-2017: ongoing increase in incidence despite near-elimination of the human-only Plasmodium species. *Clin. Infect. Dis.* 70, 361–367. doi: 10.1093/cid/ciz237

Cuenca, P. R., Key, S., Jumail, A., Surendra, H., Ferguson, H. M., Drakeley, C. J., et al. (2021). "Chapter six – epidemiology of the zoonotic malaria *Plasmodium knowlesi* in changing landscapes" in *Advances in Parasitology*. ed. C. Drakeley (113: Academic Press), 225–286.

Cunze, S., Kochmann, J., Koch, L. K., Hasselmann, K. J. Q., and Klimpel, S. (2019). Leishmaniasis in Eurasia and Africa: geographical distribution of vector species and pathogens. *R. Soc. Open Sci.* 6:190334. doi: 10.1098/rsos.190334

Divis, P. C., Lin, L. C., Rovie-Ryan, J. J., Kadir, K. A., Anderios, F., Hisam, S., et al. (2017). Three divergent subpopulations of the malaria parasite *Plasmodium knowlesi*. *Emerg. Infect. Dis.* 23, 616–624. doi: 10.3201/eid2304.161738

Dolan, M. C., Schulze, T. L., Jordan, R. A., Schulze, C. J., Ullmann, A. J., Hojgaard, A., et al. (2017). Evaluation of doxycycline-laden oral bait and topical fipronil delivered in a single bait box to control *Ixodes scapularis* (acari: Ixodidae) and reduce *Borrelia burgdorferi* and *Anaplasma phagocytophilum* infection in small mammal reservoirs and host-seeking ticks. *J. Med. Entomol.* 54, 403–410. doi: 10.1093/jme/tjw194

Ferraguti, M., Martínez-de la Puente, J., Roiz, D., Ruiz, S., Soriguer, R., and Figuerola, J. (2016). Effects of landscape anthropization on mosquito community composition and abundance. *Sci. Rep.* 6:29002. doi: 10.1038/srep29002

Fooden, J. (1995). Systematics review of Southeast Asian longtail macaques, *Macaca fascicularis* (Raffles, 1821). *Fieldiana Zool.* 81, 2–3. doi: 10.5962/bhl.title.3456

Fornace, K. M., Abidin, T. R., Alexander, N., Brock, P., Grigg, M. J., Murphy, A., et al. (2016). Association between landscape factors and spatial patterns of *Plasmodium knowlesi* infections in Sabah, Malaysia. *Emerg. Infect. Dis.* 22, 201–209. doi: 10.3201/eid2202.150656

Fornace, K. M., Brock, P. M., Abidin, T. R., Grignard, L., Herman, L. S., Chua, T. H., et al. (2019). Environmental risk factors and exposure to the zoonotic malaria parasite *Plasmodium knowlesi* across northern Sabah, Malaysia: a population-based cross-sectional survey. *Lancet Planet. Health.* 3, e179–e186. doi: 10.1016/S2542-5196(19)30045-2

Global Forest Watch (2021). Tree cover in Malaysia. Available at: https://www.globalforestwatch.org/dashboards/country/MYS/ (Accessed March 21, 2022)

Google (2022). Google Maps. Available at: https://www.google.com/maps

Grigg, M. J., Cox, J., William, T., Jelip, J., Fornace, K. M., Brock, P. M., et al. (2017). Individual-level factors associated with the risk of acquiring human *Plasmodium knowlesi* malaria in Malaysia: a case-control study. *Lancet Planet. Health.* 1, e97–e104. doi: 10.1016/S2542-5196(17)30031-1

Hod, R., Mokhtar, S. A., Muharam, F. M., Shamsudin, U. K., and Hisham Hashim, J. (2022). Developing a predictive model for *Plasmodium knowlesi*–susceptible areas in Malaysia using geospatial data and artificial neural networks. *Asia Pac J Public Health* 34, 182–190. doi: 10.1177/10105395211048620

Imai, N., White, M. T., Ghani, A. C., and Drakeley, C. J. (2014). Transmission and control of *Plasmodium knowlesi*: a mathematical modelling study. *PLoS Negl. Trop. Dis.* 8:e2978. doi: 10.1371/journal.pntd.0002978

Jeyaprakasam, N. K., Liew, J. W. K., Low, V. L., Wan-Sulaiman, W.-Y., and Vythilingam, I. (2021a). *Plasmodium knowlesi* infecting humans in Southeast Asia: what's next? *PLoS Negl. Trop. Dis.* 14:e0008900. doi: 10.1371/journal.pntd.0008900

Jeyaprakasam, N. K., Pramasivan, S., Liew, J. W. K., Van Low, L., Wan-Sulaiman, W.-Y., Ngui, R., et al. (2021b). Evaluation of Mosquito Magnet and other collection tools for Anopheles mosquito vectors of simian malaria. *Parasit. Vectors* 14:184. doi: 10.1186/s13071-021-04689-3

Jia, P., and Joyner, A. (2015). Human brucellosis occurrences in Inner Mongolia, China: a spatio-temporal distribution and ecological niche modeling approach. *BMC Infect. Dis.* 15:36. doi: 10.1186/s12879-015-0763-9

Johnson, J. M., and Khoshgoftaar, T. M. (2019). Survey on deep learning with class imbalance. *J. Big Data* 6:27. doi: 10.1186/s40537-019-0192-5

Kopczewska, K. (2022). Spatial machine learning: new opportunities for regional science. *Ann. Reg. Sci.* 68, 713–755. doi: 10.1007/s00168-021-01101-x

Kotepui, M., and Kotepui, K. U. (2018). Impact of weekly climatic variables on weekly malaria incidence throughout Thailand: a country-based six-year retrospective study. *J. Environ. Public Health* 2018:8397815. doi: 10.1155/2018/8397815

Kulkarni, M. A., Duguay, C., and Ost, K. (2022). Charting the evidence for climate change impacts on the global spread of malaria and dengue and adaptive responses: a scoping review of reviews. *Glob. Health* 18:1. doi: 10.1186/s12992-021-00793-2

Liu, X., Rajarethinam, J., Shi, Y., Liang, S., Yap, G., and Ng, L. C. (2016). Development of predictive dengue risk map using random forest. *Int. J. Infect. Dis.* 45:346. doi: 10.1016/j.ijid.2016.02.746

Lundberg, S. M., Erion, G., Chen, H., DeGrave, A., Prutkin, J. M., Nair, B., et al. (2020). From local explanations to global understanding with explainable AI for trees. *Nat. Mach. Intell.* 2, 56–67. doi: 10.1038/s42256-019-0138-9

Maki, J., Guiot, A.-L., Aubert, M., Brochier, B., Cliquet, F., Hanlon, C. A., et al. (2017). Oral vaccination of wildlife using a vaccinia–rabies-glycoprotein recombinant virus vaccine (RABORAL V-RG®): a global review. *Vet. Res.* 48:57. doi: 10.1186/s13567-017-0459-9

Mapcarta (2022). Mapcarta. Available at: https://mapcarta.com/

Medone, P., Ceccarelli, S., Parham, P. E., Figuera, A., and Rabinovich, J. E. (2015). The impact of climate change on the geographical distribution of two vectors of Chagas disease: implications for the force of infection. *Phil. Trans. R. Soc. B.* 370:20130560. doi: 10.1098/rstb.2013.0560

Merow, C., Smith, M. J., and Silander, J. A. Jr. (2013). A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. *Ecography* 36, 1058–1069. doi: 10.1111/j.1600-0587.2013.07872.x

Ministry of Energy and Natural Resources Malaysia (2022). Data from: Pangkalan data nama geografi dan gazetir kebangsaan. Ministry of Energy and Natural Resources Malaysia. Available at: https://mygeoname.mygeoportal.gov.my/index.jsp

Ministry of Health Malaysia (2022). Guidelines for malaria vector control in Malaysia. Available at: https://www2.moh.gov.my/moh/resources/Penerbitan/Garis%20Panduan/Pengurusan%20KEsihatan%20&%20kawalan%20pykit/Dari%20En.Zainudin%20BKP/3rd/GUIDELINES_FOR_MALARIA_VECTOR_CONTROL_IN_MALAYSIA_TAHUN_2022.pdf

Mischler, P., Kearney, M., McCarroll, J. C., Scholte, R. G., Vounatsou, P., and Malone, J. B. (2012). Environmental and socio-economic risk modelling for Chagas disease in Bolivia. *Geospat. Health* 6, 59–66. doi: 10.4081/gh.2012.123

Mohammad, A. H., Naserrudin, N. A., Syed Abdul Rahim, S. S., Jelip, J., Atil, A., Sazali, M. F., et al. (2022). Narrative review of the control and prevention of knowlesi malaria. *Tropical Med. Int. Health* 7:178. doi: 10.3390/tropicalmed7080178

Morand, S., and Lajaunie, C. (2021). Outbreaks of vector-borne and zoonotic diseases are associated with changes in forest cover and oil palm expansion at global scale. *Front. Vet. Sci.* 8:661063. doi: 10.3389/fvets.2021.661063

Moyes, C. L., Shearer, F. M., Huang, Z., Wiebe, A., Gibson, H. S., Nijman, V., et al. (2016). Predicting the geographical distributions of the macaque hosts and mosquito vectors *of Plasmodium knowlesi* malaria in forested and non-forested areas. *Parasit. Vectors* 9:242. doi: 10.1186/s13071-016-1527-0

Oo, T. T., Storch, V., and Becker, N. (2002). Studies on the bionomics of Anopheles dirus (Culicidae: Diptera) in Mudon, Mon State, Myanmar. *J. Vector. Ecol.* 27, 44–54. PMID: 12125872

Ooi, C. H., Phang, W. K., Liew, J. W. K., and Lau, Y. L. (2021). Spatial and temporal patterns of *Plasmodium knowlesi* malaria in Sarawak from 2008 to 2017. *Am J Trop Med Hyg.* 104, 1814–1819. doi: 10.4269/ajtmh.20-1304

Pan, I., Thodberg, H. H., Halabi, S. S., Kalpathy-Cramer, J., and Larson, D. B. (2019). Improving automated pediatric bone age estimation using ensembles of models from the 2017 RSNA machine learning challenge. *Radiol. Artif. Intell.* 1:e190053. doi: 10.1148/ryai.2019190053

Phang, W. K., Hamid, M. H. A., Jelip, J., Mudin, R. N., Chuang, T. W., Lau, Y. L., et al. (2020). Spatial and temporal analysis of *Plasmodium knowlesi* infection in peninsular Malaysia, 2011 to 2018. *Int. J. Environ. Res. Public Health* 17:9271. doi: 10.3390/ijerph17249271

Phillips, S. J., Anderson, R. P., and Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecol. Model.* 190, 231–259. doi: 10.1016/j.ecolmodel.2005.03.026

Polikar, R. (2012). "Ensemble learning" in *Ensemble Machine Learning: Methods and Applications*. eds. C. Zhang and Y. Ma (Boston, MA: Springer US), 1–34.

Pramasivan, S., Liew, J. W., Jeyaprakasam, N. K., Low, V. L., Ngui, R., and Vythilingam, I. (2022). Multiplex PCR assay for the identification of four species of the *Anopheles* Leucosphyrus sub-group in Malaysia. *Insects* 13:195. doi: 10.3390/insects13020195

Richman, R., Diallo, D., Diallo, M., Sall, A. A., Faye, O., Diagne, C. T., et al. (2018). Ecological niche modeling of Aedes mosquito vectors of chikungunya virus in southeastern Senegal. *Parasit. Vectors* 11:255. doi: 10.1186/s13071-018-2832-6

Rohani, A., Fakhriy, H. A., Suzilah, I., Zurainee, M. N., Najdah, W. M. A. W., Ariffin, M. M., et al. (2020). Indoor and outdoor residual spraying of a novel formulation of deltamethrin K-Othrine® (Polyzone) for the control of simian malaria in Sabah, Malaysia. *PLoS One* 15:e0230860. doi: 10.1371/journal.pone.0230860

Rohani, A., Zurainee, M. N., Wan Najdah, W. M. A., Ahmad Fakhriy, H., David, L., Mohd Ariffin, M., et al. (2021). Outdoor residual spray for the control of monkey malaria vectors in Sarawak, Malaysia. *Int. J. Mosq. Res.* 8, 54–62. doi: 10.22271/23487941.2021.v8.i2a.519

Rosatte, R. C., Donovan, D., Davies, J. C., Allan, M., Bachmann, P., Stevenson, B., et al. (2009). Aerial distribution of Onrab® baits as a tactic to control rabies in raccoons and striped skunks in Ontario, Canada. *J. Wildl. Dis.* 45, 363–374. doi: 10.7589/0090-3558-45.2.363

Sato, S., Tojo, B., Hoshi, T., Minsong, L. I. F., Kugan, O. K., Giloi, N., et al. (2019). Recent incidence of human malaria caused by *Plasmodium knowlesi* in the villages in Kudat peninsula, Sabah, Malaysia: mapping of the infection risk using remote sensing data. *Int. J. Environ. Res. Public Health* 16:2954. doi: 10.3390/ijerph16162954

Shartova, N., Mironova, V., Zelikhina, S., Korennoy, F., and Grishchenko, M. (2022). Spatial patterns of West Nile virus distribution in the Volgograd region of Russia, a territory with long-existing foci. *PLoS Negl. Trop. Dis.* 16:e0010145. doi: 10.1371/journal.pntd.0010145

Shearer, F. M., Huang, Z., Weiss, D. J., Wiebe, A., Gibson, H. S., Battle, K. E., et al. (2016). Estimating geographical variation in the risk of zoonotic *Plasmodium knowlesi* infection in countries eliminating malaria. *PLoS Negl. Trop. Dis.* 10:e0004915. doi: 10.1371/journal.pntd.0004915

Sillero, N., Arenas-Castro, S., Enriquez-Urzelai, U., Vale, C. G., Sousa-Guedes, D., Martínez-Freiría, F., et al. (2021). Want to model a species niche? A step-by-step guideline on correlative ecological niche modelling. *Ecol. Model.* 456:109671. doi: 10.1016/j.ecolmodel.2021.109671

Singh, B., Lee, K. S., Matusop, A., Radhakrishnan, A., Shamsul, S. S., Cox-Singh, J., et al. (2004). A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. *Lancet* 363, 1017–1024. doi: 10.1016/S0140-6736(04)15836-4

Stark, D. J., Fornace, K. M., Brock, P. M., Abidin, T. R., Gilhooly, L., Jalius, C., et al. (2019). Long-tailed macaque response to deforestation in a *Plasmodium knowlesi*-endemic area. *EcoHealth* 16, 638–646. doi: 10.1007/s10393-019-01403-9

Tan, A. F., Sakam, S. S. B., Rajahram, G. S., William, T., Abd Rachman Isnadi, M. F., Daim, S., et al. (2022). Diagnostic accuracy and limit of detection of ten malaria parasite lactate dehydrogenase-based rapid tests for plasmodium knowlesi and *P. falciparum*. *Front. Cell. Infect. Microbiol.* 12:1023219. doi: 10.3389/fcimb.2022.1023219

Tan, C. H., Vythilingam, I., Matusop, A., Chan, S. T., and Singh, B. (2008). Bionomics of Anopheles latens in Kapit, Sarawak, Malaysian Borneo in relation to the transmission of zoonotic simian malaria parasite *Plasmodium knowlesi*. *Malar. J.* 7:52. doi: 10.1186/1475-2875-7-52

Temenos, A., Tzortzis, I. N., Kaselimi, M., Rallis, I., Doulamis, A., and Doulamis, N. (2022). Novel insights in spatial epidemiology utilizing explainable AI (XAI) and remote sensing. *Remote Sens.* 14:3074. doi: 10.3390/rs14133074

Thomson, M. C., Mason, S. J., Phindela, T., and Connor, S. J. (2005). Use of rainfall and sea surface temperature monitoring for malaria early warning in Botswana. *Am. J. Trop. Med. Hyg.* 73, 214–221. doi: 10.4269/ajtmh.2005.73.214

Tompkins, A. M., and Ermert, V. (2013). A regional-scale, high resolution dynamical malaria model that accounts for population density, climate and surface hydrology. *Malar. J.* 12:65. doi: 10.1186/1475-2875-12-65

Veloz, S. D. (2009). Spatially autocorrelated sampling falsely inflates measures of accuracy for presence-only niche models. *J. Biogeogr.* 36, 2290–2299. doi: 10.1111/j.1365-2699.2009.02174.x

Vythilingam, I., Chua, T. H., Liew, J. W. K., Manin, B. O., Ferguson, H. M., and Four, C. (2021). "The vectors of *Plasmodium knowlesi* and other simian malarias Southeast Asia: challenges in malaria elimination" in *Advances in Parasitology*. ed. C. Drakeley (113: Academic Press), 131–189.

Vythilingam, I., Wong, M. L., and Wan-Yussof, W. S. (2018). Current status of *Plasmodium knowlesi* vectors: a public health concern? *Parasitology* 145, 32–40. doi: 10.1017/s0031182016000901

Waze Mobile (2022). Waze. Available at: https://www.waze.com

William, T., Jelip, J., Menon, J., Anderios, F., Mohammad, R., Awang Mohammad, T. A., et al. (2014). Changing epidemiology of malaria in Sabah, Malaysia: increasing incidence of *Plasmodium knowlesi*. *Malar. J.* 13:390. doi: 10.1186/1475-2875-13-390

Wong, M. L., Chua, T. H., Leong, C. S., Khaw, L. T., Fornace, K., Wan-Sulaiman, W. Y., et al. (2015). Seasonal and spatial dynamics of the primary vector of *Plasmodium knowlesi* within a major transmission focus in Sabah, Malaysia. *PLoS Negl. Trop. Dis.* 9:e0004135. doi: 10.1371/journal.pntd.0004135

Yu, J., Pan, R., and Zhao, Y. (2022). High-dimensional, small-sample product quality prediction method based on MIC-stacking ensemble learning. *Appl. Sci.* 12:23. doi: 10.3390/app12010023

Zhao, X., Xia, N., Xu, Y., Huang, X., and Li, M. (2021). Mapping population distribution based on XGBoost using multisource data. *IEEE J. Sel. Top. Appl. Earth Obs. Remote Sens.* 14, 11567–11580. doi: 10.1109/JSTARS.2021.3125197

frontiers | Frontiers in Microbiology

TYPE Correction
PUBLISHED 16 March 2023
DOI 10.3389/fmicb.2023.1178864



OPEN ACCESS

APPROVED BY
Frontiers Editorial Office,
Frontiers Media SA, Switzerland

*CORRESPONDENCE
Ting-Wu Chuang
✉ chtingwu@tmu.edu.tw

SPECIALTY SECTION
This article was submitted to Infectious Agents and Disease, a section of the journal Frontiers in Microbiology

RECEIVED 03 March 2023
ACCEPTED 06 March 2023
PUBLISHED 16 March 2023

CITATION
Phang WK, Hamid MHbA, Jelip J, Mudin RNb, Chuang T-W, Lau YL and Fong MY (2023) Corrigendum: Predicting *Plasmodium knowlesi* transmission risk across Peninsular Malaysia using machine learning-based ecological niche modeling approaches.
*Front. Microbiol.* 14:1178864.
doi: 10.3389/fmicb.2023.1178864



# Corrigendum: Predicting *Plasmodium knowlesi* transmission risk across Peninsular Malaysia using machine learning-based ecological niche modeling approaches

Wei Kit Phang[1], Mohd Hafizi bin Abdul Hamid[2], Jenarun Jelip[2], Rose Nani binti Mudin[3], Ting-Wu Chuang[4]*, Yee Ling Lau[1] and Mun Yik Fong[1]

[1]Department of Parasitology, Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia, [2]Disease Control Division, Ministry of Health Malaysia, Putrajaya, Malaysia, [3]Sabah State Health Department, Ministry of Health Malaysia, Kota Kinabalu, Sabah, Malaysia, [4]Department of Molecular Parasitology and Tropical Diseases, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

KEYWORDS

***Plasmodium knowlesi*, Peninsular Malaysia, ecological niche modeling, XGBoost, ensemble modeling, maximum entropy**

A corrigendum on
Predicting *Plasmodium knowlesi* transmission risk across Peninsular Malaysia using machine learning-based ecological niche modeling approaches

by Phang, W. K., Hamid, M. H. b. A., Jelip, J., Mudin, R. N. b., Chuang, T. –W., Lau, Y. L. and Fong, M. Y. (2023). *Front. Microbiol.* 14:1126418. doi: 10.3389/fmicb.2023.1126418

In the published article, there was an error in the **Funding** statement. "This study was supported by the Ministry of Higher Education, Malaysia Long Term Research Grant Scheme (LRGS/1/2018/UM/01/1/1) and the Ministry of Science and Technology, Taiwan (MOST108-2638-H-002-002-MY2)." The correct Funding statement appears below.

## Funding

This study was supported by the Ministry of Higher Education, Malaysia Long Term Research Grant Scheme (LRGS/1/2018/UM/01/1/1) and the Ministry of Science and Technology, Taiwan (MOST110-2621-M-038-001-MY2).

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

## Publisher's note

frontiers | Frontiers in Microbiology

TYPE Review
PUBLISHED 21 March 2023
DOI 10.3389/fmicb.2023.1135977



OPEN ACCESS

EDITED BY
Jose G. Estrada-Franco,
National Polytechnic Institute,
Mexico

REVIEWED BY
Sheng-Qun Deng,
Anhui Medical University,
China
Devojit Kumar Sarma,
ICMR-National Institute for Research in Environmental Health,
India

*CORRESPONDENCE
Wenn-Chyau Lee
✉ leewc_88@hotmail.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Infectious Agents and Disease, a section of the journal Frontiers in Microbiology

RECEIVED 02 January 2023
ACCEPTED 28 February 2023
PUBLISHED 21 March 2023

CITATION
Wong ML, Zulzahrin Z, Vythilingam I, Lau YL, Sam I-C, Fong MY and Lee W-C (2023) Perspectives of vector management in the control and elimination of vector-borne zoonoses.
*Front. Microbiol.* 14:1135977.
doi: 10.3389/fmicb.2023.1135977



# Perspectives of vector management in the control and elimination of vector-borne zoonoses

Meng Li Wong[1†], Zulhisham Zulzahrin[1†], Indra Vythilingam[1], Yee Ling Lau[1], I-Ching Sam[2,3], Mun Yik Fong[1] and Wenn-Chyau Lee[1,4*]

[1]Department of Parasitology, Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia, [2]Department of Medical Microbiology, Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia, [3]Department of Medical Microbiology, University Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia, [4]A*STAR Infectious Diseases Labs (A*STAR ID Labs), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore

The complex transmission profiles of vector-borne zoonoses (VZB) and vector-borne infections with animal reservoirs (VBIAR) complicate efforts to break the transmission circuit of these infections. To control and eliminate VZB and VBIAR, insecticide application may not be conducted easily in all circumstances, particularly for infections with sylvatic transmission cycle. As a result, alternative approaches have been considered in the vector management against these infections. In this review, we highlighted differences among the environmental, chemical, and biological control approaches in vector management, from the perspectives of VZB and VBIAR. Concerns and knowledge gaps pertaining to the available control approaches were discussed to better understand the prospects of integrating these vector control approaches to synergistically break the transmission of VZB and VBIAR in humans, in line with the integrated vector management (IVM) developed by the World Health Organization (WHO) since 2004.

KEYWORDS

**zoonoses, vector-borne, vector management, control, prevention**

## Introduction

Zoonoses are infections transmitted from animals to humans (Murphy, 1998). In fact, "zoonosis" is a relatively new word coined by German scientist Rudolf Virchow in the late 19th century, which combines Greek words "zoon" (animal) and "noson" (disease) (Chomel, 2009). Due to increased overlap of habitats by humans and wildlife, climate change, certain economic, cultural and dietary practices, as well as invasion of alien species and convenient international travels, the healthcare and economic burden exerted by zoonoses has increased significantly (Woolhouse and Gowtage-Sequeria, 2005; Jones et al., 2008; Grace et al., 2012; Karesh et al., 2012; Kulkarni et al., 2015). Zoonoses are caused by a variety of pathogens encompassing viruses, bacteria, parasites, fungi, and prions (Taylor et al., 2001; Woolhouse and Gowtage-Sequeria, 2005; Jones et al., 2008). Theoretically, the transmission of a zoonosis can be prevented by segregating humans and the animals that serve as natural hosts of the pathogen (Chomel, 2009; Karesh et al., 2012). However, control and prevention strategies may face additional

challenges when the zoonosis is vector-borne, as reflected by knowlesi malaria, a potentially fatal zoonosis transmitted by simio-anthropophilic anopheline mosquitoes (Tan et al., 2008; Jiram et al., 2012; Vythilingam et al., 2014; Wong et al., 2015; Lau et al., 2016). Similar obstacles happen with vector-borne infections possessing animal reservoirs, such as the tsetse fly-transmitted *Trypanosoma brucei*, the sand fly-transmitted leishmaniasis, and the mosquito-borne Sindbis virus (SINV), Zika virus (ZIKV), and yellow fever virus (Balfour, 1914; Njiokou et al., 2006; Singh et al., 2013; Vorou, 2016; Steyn et al., 2020; Kushwaha et al., 2022). Since the involving animals cannot be culled just to break the transmission circuit to humans (Lee et al., 2022), vector control is a critical component of breaking the transmission of vector-borne zoonoses (VBZ) and vector-borne infections with animal reservoirs (VBIAR). Vector control programs aim at either reducing the population of the vectors, or avoiding, if not reducing the exposure of the targeted vectors to humans (Wilson et al., 2020). Notably, a wide variety of arthropods and arachnids with different biological behaviors have been verified as medically important disease vectors (Table 1). The diverse array of vectors, animal reservoirs, and activities engaged by humans in the vicinity contribute different challenges to the control and elimination of these diseases. Here, we discussed the main vector control strategies in the current scenario, highlighted the strengths, limitations, and concerns arising from these approaches, knowledge gaps that deserve to be filled, and possibility of integrating multiple approaches of vector management into the control and elimination of VBZ and VBIAR.

## The trilogy of vector control strategies

In general, vector control strategies can be classified into chemical, biological and environmental management approaches (Bos, 1991). Each of these approaches gained research and public attention at different time points and comes with its own advantages and disadvantages. These approaches are inter-related, where simultaneous application of multiple approaches can produce either synergistic effect against the propagation of vectors, or antagonistic effect that disputes the vector control program. Therefore, a thorough understanding on each vector control approach is crucial for a successful vector control that can lead to the eradication of respective vector-borne diseases (WHO, 2012). This is particularly crucial for the management of VBZ and VBIAR, as the transmission profiles of these infections are usually more complex, involving more organisms. In fact, some of these diseases have multiple transmission cycles. For example, *Trypanosoma cruzi* has an urban transmission cycle involving humans, and sylvatic cycle involving wildlife (Orozco et al., 2013), whereas yellow fever virus has sylvatic, intermediate/savannah and urban transmission cycles (Valentine et al., 2019; Cunha et al., 2020; Gabiane et al., 2022). Of note, each transmission cycle may involve different vectors with distinct biological properties and behaviors that further complicate transmission blocking *via* vector control program. Worse still, many of these infections have incompletely deciphered transmission risk factors (Swei et al., 2020). Due to such complexity, a well-designed multi-pronged strategy that integrates multiple approaches may be more suitable to control the transmission of VBZ and VBIAR.

## Environment management approach

The environment management approach was the predominant vector control method prior to World War II (WWII). During this period, comprehensive understanding on local vector behavior and ecology dynamics, along with specifically tailored environmental management plans were the prerequisites toward a successful vector control (Quiroz-Martinez and Rodriguez-Castro, 2007; Wilson et al., 2020). The environment approach revolves around behavioral manipulation and landscape modification (Figure 1). Behavioral manipulation can be directed at humans, animals or the vectors involved (Ault, 1994). For example, community members can be trained to practice good sanitary measures around their housing compound, set up barrier proofing against mosquitoes (such as usage of bed net and mosquito screens), and employs personal protection when exploring places with high vector density (Demers et al., 2018; Wilson et al., 2020). Zooprophylaxis can be employed to distract vectors from biting humans (or animals that serve as natural reservoirs of the targeted pathogen), by introducing another animal with similar or better feeding attractiveness to the targeted vectors (Charlwood et al., 1985; Sousa et al., 2001). In this context, the mosquito behavior is manipulated. On the other hand, landscape modification revolves around temporary and permanent strategies of water management, with the goal of removing suitable breeding grounds for the vectors (Watsons, 1921). Vector control *via* environment management has been employed against the transmission of malaria (Le Prince and Orenstein, 1916; Watsons, 1921; Utzinger et al., 2001; Lindsay et al., 2002; Ferroni et al., 2012), lymphatic filariasis (van den Berg et al., 2013; Davis et al., 2021), yellow fever (Le Prince and Orenstein, 1916; Soper and Wilson, 1943), African trypanosomiasis (Jackson, 1941; Jackson, 1943; Jackson, 1948; Scott, 1966; Hargrove, 2003; Headrick, 2014), and leishmaniasis (Busvine, 1993; Steverding, 2017) in different parts of the world. However, this approach does not work in a "one size fits all" manner. For example, the zooprophylaxis approach reported promising results in Papua New Guinea and São Tomé (Charlwood et al., 1985; Sousa et al., 2001). However, this approach experienced failure in places such as Ethiopia, the Gambia, and Pakistan (Bouma and Rowland, 1995; Ghebreyesus et al., 2000; Bøgh et al., 2001). Such contradicting outcomes were due to various factors, including the types of vectors targeted in these studies. Indeed, the success of zooprophylaxis relies on the prerequisites that the involving vectors must be zoophilic and exophilic (outdoor feeders), in addition to the adequate segregation between the human and animal living spaces (Asale et al., 2017).

A thorough evaluation and understanding on the stakeholders and targeted areas, along with long-term engagement (commitment) by the government and community members are needed to ensure a higher success rate of vector control *via* environment management. However, these can only be achieved with adequate time, financial support, and sustainable manpower. In addition, the benefits brought by this approach may be shadowed by unpredictable and potentially irreversible negative impact cast upon the environment, as exemplified by the bush clearing effort in parts of Africa during the 1950s and 1960s to control the population of tsetse flies (Scott, 1966; Hargrove, 2003; Pilossof, 2016). Hence, this vector control approach may not be an ideal solution for all diseases. Nevertheless, this approach is still a valuable tool for a sustained elimination of the targeted vector-borne diseases, provided that the approach is designed carefully by taking all

TABLE 1 Vector-borne diseases (VBDs) and the respective vectors and animal reservoirs.

| VBDs | Causative agent | Vector | Animal reservoir | Refs |
|---|---|---|---|---|
| Malaria | Human: *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale* spp. | Mosquito: *Anopheles* spp. | N/A | Warren and Wharton (1963), Vythilingam et al. (2001, 2005, 2006), da Rocha et al. (2008), Tan et al. (2008), Sinka et al. (2010), Sinka et al. (2011), Jiram et al. (2012), Sinka et al. (2012), Vythilingam (2012), Vythilingam and Hii (2013), Vythilingam et al. (2013), Vythilingam et al. (2014), Wong et al. (2015), Lau et al. (2016), Liew et al. (2021), and Vythilingam et al. (2021) |
| | Zoonotic: *Plasmodium knowlesi, Plasmodium cynomolgi, Plasmodium inui, Plasmodium simium, Plasmodium brasilianum* | Mosquito: *Leucosphyrus* group of mosquitoes | Simian primates | |
| Babesiosis | *Babesia microti, Babesia divergens, Babesia duncani, Babesia venatorum* | Tick: *Ixodes* spp. | Cattle, roe deer and rodents | Donnelly and Peirce (1975), Spielman (1976), Lewis and Young (1980), Walter and Weber (1981), Mylonakis (2001), Gray et al. (2002), and Bonnet et al. (2009) |
| Dengue | Dengue virus (DENV) | Mosquito: *Aedes aegypti, Ae. albopictus, Ae. polynesiensis, Ae. scutellaris* group | Monkey (sylvatic dengue strains) | Siler et al. (1926), Chan et al. (1971), Rosen and Gubler (1974), Gubler (1988), Moore and Mitchell (1997), Rigau-Perez and Gubler (1997), Scott et al. (1997), Hotta (1998), Tsuda et al. (2002), Gubler et al. (2007), Lambrechts et al. (2010), and Higa (2011) |
| Yellow fever | Yellow Fever virus (YFV) | Mosquito: *Aedes* spp., *Haemagogus* spp. | Monkeys | Haddow (1969), Barrett and Higgs (2007), and Young et al. (2014) |
| Chikungunya | Chikungunya virus (CHIKV) | Mosquito: *Ae. aegypti*, *Ae. albopictus* | Primates | Niyas et al. (2010) and Kumar et al. (2012) |
| O'nyong'nyong fever | O'nyong'nyong virus (ONNV) | Mosquito: *Anopheles* spp. | N/A | Shore (1961), Johnson (1988), and Young et al. (2014) |
| Sindbis fever | Sindbis virus (SINV) | Mosquito: *Culex* spp., *Culiseta* spp. | Birds | Laine et al. (2004), Young et al. (2014), and Sang et al. (2017) |
| Zika | Zika virus (ZIKV) | Mosquito: *Aedes* spp. | Primates | Dick (1952), Marchette et al. (1969), and Vorou (2016) |
| Rift Valley fever | Rift Valley fever virus (RVFV) | Mosquito: *Aedes* spp., *Culex* spp. | Ruminants | Davies (1975), Davies and Highton (1980), Chevalier et al. (2004), and Sang et al. (2017) |
| West Nile fever | West Nile virus (WNV) | Mosquito: *Culex* spp. | Birds | Taylor and Hurlbut (1953) and Shirato et al. (2005) |
| Japanese encephalitis | Japanese encephalitis virus (JEV) | Mosquito: *Culex* spp., *Ae. togoi, Ae. japonicus, Ae. vexans nipponii, An. annularis, An. vagus* | Birds, pigs (amplifier host) | Sucharit et al. (1989), Vythilingam et al. (1994), Vythilingam et al. (1995), Vythilingam et al. (1997), Weng et al. (1999), Das et al. (2005), Nitatpattana et al. (2005), van den Hurk et al. (2006), Seo et al. (2013), and de Wispelaere et al. (2017) |
| Murray Valley encephalitis | Murray Valley encephalitis virus (MVEV) | Mosquito: *Culex annulirostris* | Birds | Marshall (1988), Mackenzie et al. (1994), Kurucz et al. (2005), and Floridis et al. (2018) |
| Tick-borne encephalitis | Tick-borne encephalitis virus (TBEV) | Tick: *Ixodes* spp., *Dermacentor* spp. | Small mammals | Kozuch and Nosek (1971), Labuda and Randolph (1999), and Biernat et al. (2014) |
| Kunjin encephalitis | Kunjin virus (KUNV) | Mosquito: *Culex annulirostris* | Birds | Doherty et al. (1963), Kay et al. (1984), Marshall (1988), Hall et al. (2002), and Hall et al. (2006) |
| Colorado tick fever | Colorado tick fever virus (CTFV) | Tick: *Dermacentor andersoni* | Squirrels, chipmunks, mice | Florio et al. (1944), Florio and Miller (1948), Florio et al. (1950), and Emmons (1988) |
| Lymphatic filariasis | Human: *Wuchereria bancrofti, Brugia malayi, Brugia timori* | Mosquito: *Anopheles* spp., *Culex* spp., *Aedes* spp., *Mansonia* spp. | Cats, dogs, monkeys, pangolins (*B. malayi*) | Edeson and Wilson (1964), Cheong et al. (1981), Chiang et al. (1984), Hii et al. (1984), Zahedi and White (1994), Kanjanopas et al. (2001), Vythilingam (2012), Muslim et al. (2013), Vythilingam et al. (2013), WHO (2013), Aagaard et al. (2015), Mulyaningsih et al. (2019), and Nunthanid et al. (2020) |
| | Zoonotic: *Brugia pahangi* | Mosquito: *Armigeres subalbatus* | Cats and dogs | |
| Serous cavity filariasis | *Mansonella perstans, Mansonella ozzardi* | Midge: *Culicoides* spp. | N/A | Manson (1891) |

*(Continued)*





10.3389/fmicb.2023.1135977

TABLE 1 (Continued)

| VBDs | Causative agent | Vector | Animal reservoir | Refs |
|---|---|---|---|---|
| Subcutaneous filariasis | Loiasis: *Loa loa* | Deer fly: *Chrysops* spp. | N/A | Kleine (1915), Connal (1921), Macfie and Corson (1922), Fischer et al. (1997), Lawrence (2004), Boussinesq (2006), Kelly-Hope et al. (2017), and Hendy et al. (2018) |
| | *Mansonella streptocerca* | Midge: *Culicoides* spp. | N/A | |
| | Onchocerciasis / river blindness: *Onchocerca volvulus* | Black fly: *Simulium* spp. | N/A | |
| Sleeping sickness (African trypanosomiasis) | *Trypanosoma brucei rhodesiense, Trypanosoma brucei gambiense* | Tsetse fly: *Glossina* spp. | Cattle (*T. brucei rhodesiense*) Primates & ungulates (*T. brucei gambiense*)[#] | Bruce (1895, 1915) and Njiokou et al. (2006) |
| Chagas disease (American trypanosomiasis) | *Trypanosoma cruzi* | True bug/kissing bug/triatomine/ reduviid bug: *Rhodnius prolixus, Triatoma infestans* | Small rodents | Chagas (1909), Jurberg and Galvão (2006), Rassi et al. (2010), and WHO (2015) |
| Leishmaniasis | *Leishmania* | Phlebotomine sandfly: *Phlebotomus* spp., *Lutzomyia* spp. | Dogs | Swaminath et al. (1942), Mukhopadhyay et al. (2000), and Guerbouj et al. (2007) |
| Epidemic typhus (louse-borne typhus) | *Rickettsia prowazekii* | Human body louse: *Pediculus humanus humanus* | Flying squirrels (sylvatic typhus) | McDade et al. (1980), McDade and Newhouse (1986), and Durden (2019) |
| Rocky Mountain spotted fever (RMSF) | *Rickettsia rickettsii* | Tick: *Dermacentor variabilis, Dermacentor andersoni, Rhipicephalus sanguine* | Small mammals | Kohls (1947) and Ahantarig et al. (2013) |
| Queensland tick typhus (QTT) | *Rickettsia australis* | Tick: *Ixodes holocyclus*, *I. tasmania* | Bandicoots, rodents | Fenner (1946), Domrow and Derrick (1964), Sexton et al. (1991), and Barker and Walker (2014) |
| Scrub typhus | *Orientia tsutsugamushi* | Mite: *Leptotrombidium* spp. | Rodents | Shirai et al. (1981), Pham et al. (2001), Lerdthusnee et al. (2003), and Weitzel et al. (2022) |
| Tularemia (rabbit fever) | *Francisella tularensis* | Tick: *Amblyomma* spp., *Dermacentor* spp., *Haemaphysalis* spp., *Ixodes* spp. | Rabbits, hares, other small rodents | Parker et al. (1924), Gurycová (1998), Sjöstedt (2007), Kugeler et al. (2009), Männikkö (2011), Maurin et al. (2011), Yeni et al. (2021), and Troha et al. (2022) |
| | | Deer fly: *Chrysops discalis* | Deers | |
| Lyme disease | *Borrelia burgdorferi, Borrelia mayonii* | Tick: *Ixodes* spp. | Avians, mammals | Wilson et al. (1985), Steere (2001), Lo Re et al. (2004), and Couper et al. (2020) |
| Bubonic plague | *Yersinia pestis* | Oriental rat flea: *Xenopsylla cheopis* | Rodents | Bacot and Martin (1914), Bacot (1915), Burroughs (1947), and Pollitzer (1954) |
| Anaplasmosis | *Anaplasma phagocytophilum* | Tick: *Ixodes* spp. | Mammals, birds | Chen et al. (1994), Ohashi et al. (2005), Katargina et al. (2012), and Bakken and Dumler (2015) |
| Ehrlichiosis | *Ehrlichia chaffeensis, Ehrlichia ewingii, Erhlichia muris eauclairensis* | Ticks: *Amblyomma* spp., *Ixodes* spp. | Mammals | Anderson et al. (1993), Lockhart et al. (1997), and Ganguly and Mukhopadhayay (2008) |

[#]Humans are the main reservoir for *T. brucei gambiense* but this parasite has been isolated from primates and ungulates.





10.3389/fmicb.2023.1135977

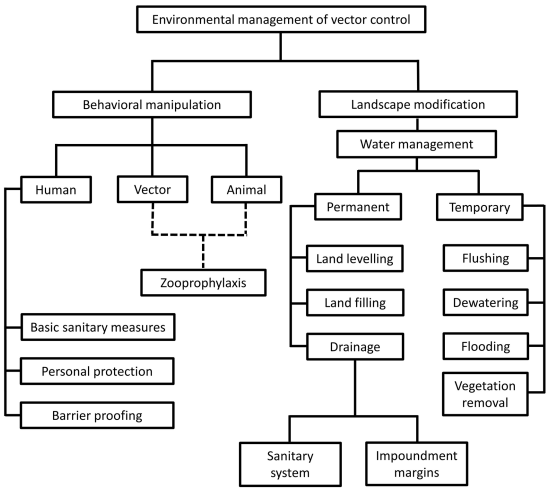


FIGURE 1
Different strategies under the environmental management of vector control. This approach revolves around behavioral alteration of humans, animals and vectors, as well as landscape modification, to create barriers between humans and vectors. Of note, the "zooprophylaxis" under "behavioral manipulation" involves the introduction of animals that are not pathogen reservoirs, to distract the blood-seeking vectors from humans and animals that serve as natural reservoirs of pathogens. This method involves behavioral alteration of animals and vectors, as indicated by the dotted lines in the diagram. On the other hand, landscape modification consists of permanent and temporary water management strategies to change the breeding environment of vectors.

biological, environmental, legal and socio-economic factors into consideration.

## Chemical vector control

Chemical vector control strategies have gained popularity, especially after the WWII, due to the rapid and potent effect of these methods. The development, marketing and application of various insecticides has been the mainstream of chemical vector control strategy. Attempts to employ chemicals for pest control were recorded as early as the 1840s (Table 2). However, the discovery of dichloro-diphenyl-trichloroethane (DDT) revolutionized the approach to control vector population. The insecticidal properties of DDT were discovered in 1939 (Mellanby, 1992; Davies et al., 2007). Following the halted supply of chrysanthemum-derived pyrethrum from Japan due to WWII, DDT became the mainstream chemical player in vector control (Wilson et al., 2020), especially after its involvement in the successful control of typhus outbreak in Europe (Wheeler, 1946). Following this much publicized success against lice, DDT was proven to be potent against many other vectors such as the mosquito, tsetse fly, sandfly and blackfly (Ismail et al., 1975; Loyola et al., 1990, 1991; Roberts and Alecrim, 1991; Casas et al., 1998; Hargrove, 2003; Dias, 2007; Rijal et al., 2019). Nevertheless, the negative impacts brought by DDT to non-targeted organisms and environment were discovered after years of mass application. As a result, the application of this powerful chemical was discontinued abruptly in the 1970s (Davies et al., 2007). Subsequently, other insecticide groups such as organophosphates, carbamates and synthetic pyrethroid gained

TABLE 2 Brief overview of insecticides in vector control.

| Year | Description | Methods | Refs |
|---|---|---|---|
| 1840 | Discovery of insecticidal properties of a *Tanacetum* (*Chrysanthemum*) *cinerariifolium* (Compositae)-derived compound (pyrethrum), subsequently its successful extraction and commercial production | N/A | Ujváry (2010) |
| 1930s | Discovery of insecticidal properties of organophosphates (OP) and carbamate. | N/A | Hill (1995) and Glaser (1999) |
| 1939 | Discovery of insecticidal properties of dichlorodiphenyltrichloroethane (DDT) against flies, mosquitoes and beetles by Paul Muller | N/A | Mellanby (1992) and Davies et al. (2007) |
| 1943 | First application of DDT in Italy to control typhus epidemic | Dusting 10% DDT powder onto clothing of infested individuals to kill body lice | Wheeler (1946) |
| 1946–1991 | Widespread application of DDT and other organochlorines (OC) in various locations to control vector-borne diseases | Aerial spraying and indoor residual spraying (IRS) | Ismail et al. (1975), Loyola et al. (1990), Loyola et al. (1991), Roberts and Alecrim (1991), Casas et al. (1998), and Hargrove (2003) |
| 1949 | Development of the first synthethic pyrethroids | N/A | Davies et al. (2007) and Matsuo (2019) |
| 1955–1969 | Introduction and implementation of Global Malaria Eradication Program by WHO | Control program varied across different locations | Najera et al. (2011) |
| 1972 | DDT usage was banned by US Environment Agency | N/A | Mellanby (1992) and Davies et al. (2007) |
| 1970s – present | Development of pyrethroid-treated net (ITN) for malaria control. Organophosphates and carbamates are more widely used as replacements for OC due to hazardous effect imposed by DDT | Organophosphates: residual spraying, space spraying and larviciding. Carbamates: residual spraying | Bonsall and Goose (1986), Dorta et al. (1993), van den Berg et al. (2012), Tangena et al. (2020), and van den Berg et al. (2021a,b) |

popularity in many vector control programs. This has stimulated various chemical-oriented vector combating strategies, such as the long-lasting insecticidal net (LLIN), indoor residual spraying (IRS), as well as outdoor residual spraying (ORS; Bonsall and Goose, 1986; Bhatt et al., 2015; Rohani et al., 2020; Tangena et al., 2020; Chaumeau et al., 2022).

Various chemicals have been developed and marketed as readily available larvicides and adulticides. The high availability and instantaneous killing effect of these products have created a dogma that the chemicals are the best way forward in vector management (Casida and Quistad, 1998; Thomas, 2018). Nevertheless, the biology of arthropods plays a critical role in determining the success rate of insecticide-mediated vector control programs. For instance, IRS and LLIN are not suitable for exophagic and exophilic mosquitoes with peak biting time in the early evening (Dolan et al., 1993; Rohani et al., 1999; Smithuis et al., 2013; Wong et al., 2015). Besides, behavioral adaptation of endophilic mosquitoes toward avoidance of insecticide-treated houses or rapid exit from the insecticide-treated buildings will minimize the exposure of these vectors to the insecticides, compromising the efficacy of the applied insecticide (Killeen, 2014). Importantly, the rampant usage of these chemicals has fuelled insecticide resistance in arthropods (Kleinschmidt et al., 2018; Tangena et al., 2020). Moreover, these chemicals may cast negative impacts to the ecosystem, although of lower toxicity than DDT. For example, synthetic pyrethroids are harmful to aquatic environment (Thatheyus and Selvam, 2013; Prusty et al., 2015), whereas organophosphates poisoning remains prevalent among communities involved in agricultural industry, despite being classified as non-persistent pesticides (Jaipieam et al., 2009; Kaushal et al., 2021). Due to these disadvantages, the chemical approach must be considered carefully in vector control programs against VBZ and VBIAR, particularly those with sylvatic transmission cycle.

Despite the non-specific harm to the environment due to their toxicity, the rapid and potent effect of insecticides against different vectors grants them the high popularity in pest and vector control. Many researchers have investigated ways of accelerating the degradation of these chemicals to minimize their adverse effects to the environment, while retaining their potency against the pests (Zhang and Qiao, 2002; Kaushal et al., 2021; Zhao et al., 2022). Meanwhile, the discovery of pyrethrum from chrysanthemum plant continues to inspire scientists to find novel compounds that can serve as bio-insecticides. For example, bioactive metabolites of *Streptomyces* have been reported to demonstrate good potential of becoming bio-insecticide candidates (Amelia-Yap et al., 2022). Such discovery has been driven by the need of novel, environment-friendly insecticide compounds, following rapid development of insecticide resistance and concerns over environment harm cast by chemical-based insecticides.

## Vector biocontrol approach

Among the vector control strategies, biocontrol approaches have received increasing attention and popularity over the past two decades. Therefore, various organisms and strategies have been put forward as potential vector biocontrol candidates. In general, biocontrol approach explores the potential of using organisms and microorganisms to control the vector population (van den Bosch et al., 1982; Kamareddine, 2012; Okamoto and Amarasekare, 2012; Benelli et al., 2016; Huang et al., 2017; Kwenti, 2017; Thomas, 2018), based on the natural predation, pathobiological or parasitism relationship between the candidates and the targeted vectors (Table 3). Biological manipulation targeting certain vital functions of the vectors have been explored as a new approach in vector biocontrol (Gillette, 1988; Iturbe-Ormaetxe et al., 2011; Benelli et al., 2016). Theoretically, the biocontrol approach is more target-specific, thus of lower risk of imparting off-target effects to the environment. Prior to the new millennium, biocontrol approach was not as widely applied as its chemical and environmental counterparts, due to the relative ease of implementing the other two approaches (Quiroz-Martinez and Rodriguez-Castro, 2007; Shaalan and Canyon, 2009; Vershini and Kanagappan, 2014; Vinogradov et al., 2022). Nevertheless, biocontrol approach has received increasing attention following encouraging results obtained from the mass-application of *Wolbachia*-infected *Aedes aegypti*, genetically modified mosquitoes, sterile male triatomine bugs and tsetse flies. In fact, with the increased prevalence of VBZ and VBIAR, vector biocontrol approach may offer novel and sustainable strategies to control the transmission of these infections. Biological control approach can be categorized based on the natural relationship between the biocontrol agents and the respective vectors (Figure 2), as elaborated in the next few paragraphs of this review.

## Biocontrol *via* predators

The potential of prey–predator relationship in vector control was explored before the era of mass insecticide application. For example, attempts to reduce the larval population of *Stegomyia calopus* (vector of yellow fever) in Ecuador with freshwater fish were initiated as early as the 1910s (Connor, 1922). Various aquatic and amphibian animals were put forward as potential candidates to control mosquito population, based on their predatory nature to the targeted pests. In this review, emphasis is given to medically relevant examples. Of note, most of these predator-driven strategies target the aquatic stages of mosquitoes because the mosquito larvae share a relatively confined living space with the predators. Thus, the aquatic prey–predator encounter does not rely as much on the overlapping active hours of the prey and predator, as compared to the flying adults. In addition, efficient and persistent predation on the vector offspring will inevitably control the vector population, and hence disease transmission (Kumar and Hwang, 2006; Walker and Lynch, 2007; Louca et al., 2009; Griffin and Knight, 2012).

Among the predators, larvivorous fishes have a prolific history as a biocontrol agent against pests, particularly mosquitoes. Larvivorous fishes were introduced into over 60 countries in 20th century to control vector populations (Gerberich and Laird, 1985). Their popularity was attributed mainly to their adaptability to a wide variety of natural and man-made water bodies that serve as mosquito breeding grounds, as well as their rapid reproduction rates (Hadjinicolaou and Betzios, 1973; Motabar, 1978; Chandra et al., 2008a). Numerous field trials with these predators demonstrated between 70 and 97% reduction of mosquito larvae (Connor, 1922; Menon and Rajagopalan, 1978; Fletcher et al., 1992; Kumar et al., 1998; Chandra et al., 2008a; Louca et al., 2009; Griffin and Knight, 2012). For instance, *Aphanius dispar* (Arabian toothcarp) managed to suppress the population of *Anopheles arabiensis* and *Anopheles gambiae* in wells, cisterns and barrels in Djibouti (Louis and Albert,

TABLE 3 List of available vector biocontrol agents.

| Biocontrol agent type | Biocontrol agent | Commonly used strains/ species | Remark | Limitation | Refs |
|---|---|---|---|---|---|
| Predator | Larvivorous fish | *Aphanius dispar Aplocheilus* spp. *Chanda nama Colisa* spp. *Carassius auratus Catla catla Cirrhinus mrigala Ctenopharyngodon idella Cyprinodontidae Cyprinus carpio Danio rerio Gambusia affinis Labeo rohita Macropodus cupanus Nothobranchiius guentheri Oreochromis* spp. *Oryzias melastigma Poecilia reticulata Sarotherodon niloticus Tilapia* spp. | Natural predator of larvae: reduces number of mosquito larvae | A threat to native aquatic fauna. Inconsistency in terms of efficacy | Connor (1922), Menon and Rajagopalan (1978), Rupp (1996), Walton (2007), Chandra et al. (2008a), Louca et al. (2009), Griffin and Knight (2012), and Subramaniam et al. (2015) |
| | Dragonfly | Nymph and adult *Anax immaculifrons Brachydiplax sobrina Neurothemis fluctuans Orthetrum chrysis Orthethrum sabina* | Reduces the number of the vector population through feeding on immature and adult | Critically affected by water quality, thus field application can be limited | Sebastian et al. (1990), Singh et al. (2003), Chatterjee et al. (2007), Quiroz-Martinez and Rodriguez-Castro (2007), Shaalan and Canyon (2009), Vershini and Kanagappan (2014), Vatandoost (2021), and Ramlee et al. (2022) |
| | Larvivorous mosquito larva | *Psorophora* subgenus *Psorophora Sabethes cyaneus Toxorhynchites* spp. *Lutzia* spp. *Sabethes* spp. *Trichoprosopon* spp. *Runchyomyia* spp. *Culex fuscanus Anopheles barberi Tripteroides* spp. *Topomyia* spp. *Wyeomyia* subgenus *Dendromyia Eretmapodites* spp. *Aedes* subgenus *Alanstomea Aedes* subgenus *Mucidus* | Decreases number of mosquito larvae | Spatial limitations for application, especially for some sylvatic species. Risk of cannibalism among larvivorous mosquito larva | Chapman (1974), Lounibos (1980), Focks et al. (1985), Annis et al. (1989), Annis et al. (1990), Rawlins et al. (1991), Brown (1996), Mogi and Chan (1996), Amalraj and Das (1998), Collins and Blackwell (2000), Aditya et al. (2006), Benelli et al. (2016), Huang et al. (2017), Donald et al. (2020), and Hancock et al. (2022) |
| | Larvivorous copepod | *Megacyclops* spp. *Mesocyclops* spp. *Macrocylops* spp. | Reduces mosquito larvae density | Copepods are affected by water temperature, low oxygen content and accumulation of toxins in water. Some copepods are intermediate host for guinea-worm and fish tape worm | Marten et al. (1989), Lardeux et al. (1992), Manrique-Saide et al. (1998), Schaper (1999), Vu et al. (2005), Marten and Reid (2007), Soumare and Cilek (2011), Mahesh Kumar et al. (2012), and Vinogradov et al. (2022) |
| | Beetle | Diving beetle (Dystiscidae) Water scavenger beetle (Hydrophilidae) | Reduces number of vector immatures | Incomplete habitats overlap. Alternative prey preference. Emigration. Limited research | Juliano and Lawton (1990), Lundkvist et al. (2003), Chandra et al. (2008b), Shaalan and Canyon (2009), and Vinogradov et al. (2022) |
| | Water bug | Backswimmer (Notonectidae) Giant water bugs (Belostomatidae) Waterboatmen (Corixidae) | Reduces number of vectors: feeds by holding its prey with pincers and injecting a strong liquefying enzyme into it | Greatly affected by water quality, limiting its spatial reach to the vectors. Difficulty in mass production | Bay (1974), Murdock et al. (1984), Venkatesan and Jeyachandra (1985), Sankaralingam and Venkatesan (1989), Aditya et al. (2004), Aditya et al. (2005), Shaalan et al. (2007), Shaalan and Canyon (2009), Selvarajan and Kakkassery (2019), and Vinogradov et al. (2022) |

*(Continued)*





10.3389/fmicb.2023.1135977

TABLE 3 (Continued)

| Biocontrol agent type | Biocontrol agent | Commonly used strains/ species | Remark | Limitation | Refs |
|---|---|---|---|---|---|
| | Mite | *Acari* spp. *Eustigmaeus johnstoni* (affects sand fly) *Pimeliaphilus plumifer* (affects true bugs) | Feeds on vector immature. Affects the physiological aspects of vector: reduces nymph molting rate, reduces adult longevity, increases mortality in 3rd–5th instar nymph, reduces number of viable eggs laid by infected female | Difficulty is mass-rearing | Martinez-Sanchez et al. (2007), Badakhshan et al. (2013), and Dinesh et al. (2014) |
| | Spider | Web-building spider. Hunting spiders (Active and passive hunter) | Feeds on vector immatures and adults | Consideration on different biological factors to ensure successful establishment of control | Ximena et al. (2005), Hadole and Vankhede (2013), Fischhoff et al. (2018), and Ndava et al. (2018) |
| | Lizard | *Gehydra dubia Hemidactylus frenatus Tarentola mautitanica* (prey: true bug) | Feeds on adults | Possible threat to native fauna | Castello and Gil Rivas (1980) and Canyon and Hii (1997) |
| | Frog and toad | *Bufo* spp. *Euphlycytis* spp. *Hoplobatrachus* spp. *Polypedates cruciger Ramanella* spp. | Predates on eggs of mosquito | Can be invasive toward native fauna | Raghavendra et al. (2008) and Bowatte et al. (2013) |
| | Bird | Scrub jay Chicken Yellow-billed oxpecker (*Buphagus africanus*) Red-billed oxpecker (*Buphagus erythrorhycus*) | Predates on ticks (scrub jay: ticks on deer; chicken: ticks on cattle; yellow-billed oxpecker: ticks on buffaloes; red-billed oxpecker: ticks on ungulate) | Oxpecker could induce wound enlargement on the mammalian host given that it prefers host with most ticks. Assessment of tick population needs to be performed before introduction programme (scrub jay and oxpeckers) | Moreau (1933), van Someren (1951), Mundy and Cook (1975), Bezuidenhout and Stutterheim (1980), Isenhart and DeSante (1985), Hassan et al. (1991), Mooring and Mundy (1996), Weeks (1999), and Plantan et al. (2012) |
| | Rodent | *Sorex araneus* | Predates on ticks | Not advisable as rodent transmits several diseases | Short and Norval (1982) |
| Parasitism | Parasitoid arthropods | Tachinid fly (parasitizes true bug) Chalcid wasp (parasitizes tick) *Ixodiphagus hookeri* (Encyrtid wasp-parasitizes tick) | Immatures of vector is attacked when the eggs of the parasitoid arthropods hatch and feed on it | Highly sensitive to insecticides. Mass-rearing in laboratory can be difficult, especially the diet preparation | Mather et al. (1987), Tijsse-Klasen et al. (2011), Wang et al. (2014), Kwenti (2017), Wang et al. (2019), and Buczek et al. (2021) |
| Pathogens | Nematode | Mermithid nematode (*Perutilimermis culicis*, *Romanomermis* spp., *Reeseimermis nielseni*, *Diximermis peterseni*, *Hydromermis churchillensis*). Rhabditoid nematode (*Neoaplectana carpocapsae*) Stenernematid nematode (ticks) | Parasitic relationship: Reduces number of mosquitoes. Causes biological castrations through interference in mosquito reproduction | Limited resources on the parasitic effects of nematodes against the adult mosquitoes. Environmental parameters limitations such as temperature, pH, salinity, and oxygen level | Petersen et al. (1972), Petersen and Willis (1972), Reynolds (1972), Chapman (1974), Mitchell et al. (1974), Levy and Miller (1977), Molloy and Jamnback (1977), Zhioua et al. (1995), Peng et al. (1998), Samish and Glazer (2001), Secundio et al. (2002), and Poinar (2018) |

*(Continued)*





10.3389/fmicb.2023.1135977

TABLE 3 (Continued)

| Biocontrol agent type | Biocontrol agent | Commonly used strains/ species | Remark | Limitation | Refs |
|---|---|---|---|---|---|
| | Entomopathogenic fungus | *Beauveria* spp. *Coelomomyces* spp. *Culicinomyces* spp. *Entomophthora* spp. *Lagenidium* spp. *Metarhizium* spp. *Phytium* spp. *Smittium* spp. *Fusarium oxysporum* | Upon contact to external cuticle, toxins are released by the infective spores. Modifies physiology of insect: reduces likelihood for blood-feeding, survival, and fecundity | Slow killing. Production of zoospore is difficult and affected by UV irradiation. Some strains can affect non-target arthropods. *Beauveria bassiana* are inactive against adults in laboratory (*Anopheles, Aedes, Culex*). *Entomophthora coronata* has been reported to cause phycomycosis in man and horses. *Smittium* spp. has reduced pathogenicity against mosquitoes | Clark et al. (1966), Clark et al. (1968), Anderson and Ringo (1969), Ginsberg et al. (2002), Scholte et al. (2004), Scholte et al. (2007), Paula et al. (2011a,b), and Fischhoff et al. (2018) |
| | Non-spore-forming unicellular eukaryotes | Ciliate: *Tetrahymena* spp. Flagellate: (*Crithidia* spp. *Blastocrithidia* spp. *Eugregarine Ascogregarina culicis Psychodiella* spp. (found only in sand flies) Schizogregarine: *Caulleryella* spp. Helicosporida) | Stunts growth of larvae and increased mortality. Effects on host's biological aspects especially on females are more profound in nutrient-deficient conditions | Pathogenicity highly depends on internal and external conditions. Host-specific | Corliss (1954, 1960), Chapman et al. (1967), Anderson (1968), Barrett (1968), McCray et al. (1970), Reynolds (1972), Wu and Tesh (1989), Sulaiman (1992), Mourya et al. (2003), Albicócco and Vezzani (2009), Lantova et al. (2011), and Lantova and Volf (2012, 2014) |
| | *Microsporida* | *Thelohania* spp. *Nosema* spp. *Pleistophora* spp. *Stempellia* spp. | Swollen thorax and abdomen/ benign subcutaneous pale spots on mosquito larvae. Reduces life span of infected female mosquito | Most of them cannot be transmitted perorally. Spores from different species are difficult to identify morphologically | |
| | Bacteria | *Bacillus sphaericus Bacillus thuriengiensis Bacillus thuringiensin* var. *thuringiensin Cedecca lapegei Proteus mirabilis* Different Wolbachia strains | Pathobiological effect against vectors: target is killed by an enterotoxin from crystal protein of spore. Suppresses late instars and pupae. Affects reproductive system. Shortens vectors' life | Inconsistent efficacy | Lacey and Inman (1985), Novak et al. (1986), Arredondo-Jimenez et al. (1990), Hassanain et al. (1997), Robert et al. (1997), Stouthamer et al. (1999), Armengol et al. (2006), Lacey (2007), Panteleev et al. (2007), Hedges et al. (2008), Werren et al. (2008), Brelsfoard and Dobson (2009), Kambris et al. (2009), Moreira et al. (2009a), Wiwatanaratanabutr and Kittayapong (2009), Bian et al. (2010), Ritchie et al. (2010), Ahantarig and Kittayapong (2011), Hoffmann et al. (2011), Iturbe-Ormaetxe et al. (2011), Walker et al. (2011), Mousson et al. (2012), van den Hurk et al. (2012), Bian et al. (2013), Aliota et al. (2016a,b), Dutra et al. (2016), Jeffries and Walker (2016), Ahmad et al. (2017), Chouin-Carneiro et al. (2019) and Nazni et al. (2019) |

*(Continued)*





10.3389/fmicb.2023.1135977

TABLE 3 (Continued)

| Biocontrol agent type | Biocontrol agent | Commonly used strains/ species | Remark | Limitation | Refs |
|---|---|---|---|---|---|
| | Virus | Mosquito-specific densovirus (MDV) Cytoplasmic polyhedrosis virus (CPV or near reovirus) Nuclear polyhedrosis virus (NPV or baculovirus) Deltabaculovirus (dipteran-specific NPVs) *Mosquito Iridescent Virus* (MIV or iridovirus) Entomopoxvirus (EPV) | Intranuclear protein inclusions in *Anopheles subpictus.* Infections in nuclei in midgut and gastric caeca of *An. sollicitans.* Kills fourth instar mosquito larvae | Host-specific. Slow killing, hence, studies are being performed by genetic modification of the virus to have quicker effect on the vectors | Anderson (1970), Warburg and Pimenta (1995), Jehle et al. (2006), Szewczyk et al. (2009), Szewczyk et al. (2011) |
| Vital function modification | Sterile Insect Technique (SIT) | Tsetse fly Mosquito | Genetic suppression strategy by creating sterile male vector | Sex segregation of sterile insects in mass production. Inconsistent lifespan affecting release to the wildlife | Alphey and Andreasen (2002), Phuc et al. (2007), and Vreysen et al. (2014) |
| | Release of Insects carrying a Dominant Lethal (RIDL) | Mosquito | Release of male vector carrying dominant lethal transgene to mate with wild female vector will results in the death of progeny due to the lethal effect from the transgene | Reduced biological fitness of modified insect, affecting release to the wild | Fu et al. (2010), Harris et al. (2012), Carvalho et al. (2015), Dong et al. (2018), and O'Leary and Adelman (2020) |
| | Genetic Sexing Strain (GSS) | Mosquito New World screwworm fly (Agriculture pest) | Genetically engineered male with insecticide resistance phenotype. Accidentally "leaked" females will be killed by the respective insecticide prior to release | Production difficulty | Fu et al. (2010) and Dong et al. (2018) |
| | CRISPR/Cas 9 system | Mosquito Sand fly | Manipulation of gene expression to alter vectorial capacity, survival and fertility of vector | Production difficulty, stability issues | Dong et al. (2018) |





10.3389/fmicb.2023.1135977

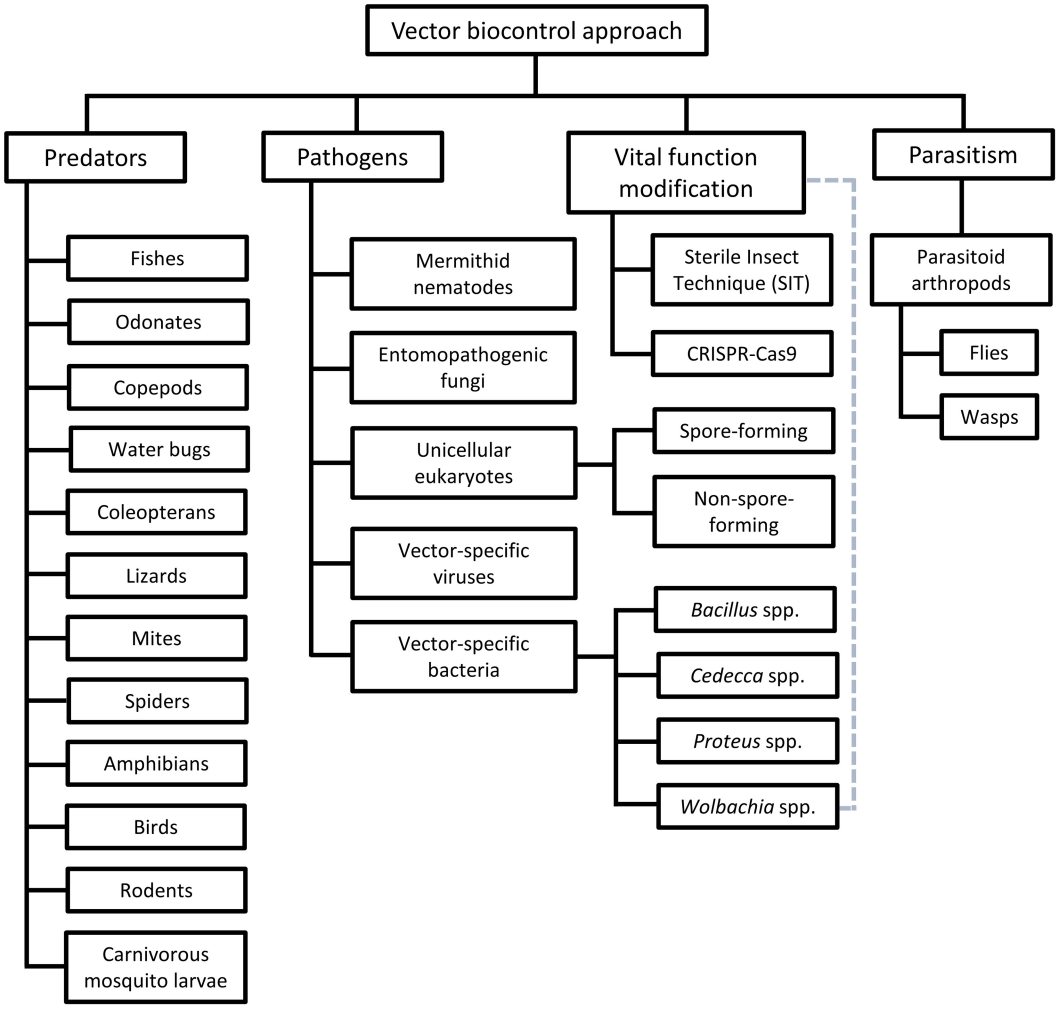


FIGURE 2
Different groups of vector biocontrol approach. Gray dotted lines reflect the characteristics of the *Wolbachia* method that combines the features of biocontrol approaches mediated by pathogens and vital function modification.

1988). However, the application of larvivorous fish has raised several concerns. The effect of an alien species to the native fauna and flora needs to be considered and monitored carefully. For example, the continuous introduction of *Gambusia affinis* (Western mosquitofish) into Greece from 1927 to 1937 resulted in the decline of an endemic species *Valencia letourneuxi* (Corfu toothcarp), due to living resource competition between the two species (Economidis, 1995; Economidis et al., 2000). Similar adverse effects associated with *G. affinis* have been reported from Australia and United States (Motabar, 1978; Arthington, 1991; Walton, 2007).

Similarly, odonates (particularly the larvae) are ferocious and imperative predators of many insects. Members of the order Odonata include various dragonflies and damselflies (Shaalan and Canyon, 2009; Vatandoost, 2021). Given their high predation capacity, relatively long aquatic life cycle (usually 1–2 years), and shared aquatic larval habitat with mosquito juveniles, odonates are potential vector biocontrol candidates. Indeed, field trials demonstrated significant reduction of mosquito larvae in water reservoirs by dragonfly nymphs (Sebastian et al., 1980, 1990; Chatterjee et al., 2007; Mandal et al., 2008). For example, a trial release of dragonfly nymphs in Myanmar reported a significant decrease of *Ae. aegypti* population in 2–3 weeks, and the effect persisted till the end of the 4-month-long trial (Sebastian et al., 1990). Similar findings were reported from India (Mandal et al., 2008). The odonate adults are agile aerial predators that prey on many insects (Vatandoost, 2021). Nevertheless, diet analyses of wild-caught dragonfly adults inferred that mosquitoes are rarely taken in large numbers by odonate adults (Pritchard, 1964; Sukhacheva, 1996; Pfitzner et al., 2015). In addition, the active hours (feeding time) of odonate adults (most species are diurnal) do not overlap with the active hours of many medically important vectors (Pfitzner et al., 2015; Vatandoost, 2021). Furthermore, the lifespan of odonate adults is relatively short (1–8 weeks). Hence, the potential of odonate adults as vector biocontrol agents is not as attractive as their juveniles.

The population of many mosquito vectors can be controlled by another mosquito *via* predation. Larvae of mosquitoes from 13 genera prey upon larvae of other arthropods (Harbach, 2007). All members of genera *Toxorhynchites, Lutzia* and *Psorophora* (subgenus *Psorophora*) are obligate predators of other arthropod larvae (Steffan and Evenhuis, 1981; Annis et al., 1990; Rawlins et al., 1991; Collins and Blackwell, 2000; Aditya et al., 2006; Wilkerson et al., 2021;

Hancock et al., 2022), whereas larvae of *Sabethes*, several species of *Culex* and *Anopheles* are facultative predators (Lounibos, 1980; Mogi and Chan, 1996; Shaalan and Canyon, 2009; Hancock et al., 2022). Of these, *Toxorhynchites* has received relatively high research attention, mainly because the adult is non-hematophagous (blood feeding), hence not imposing risk as pest or disease vector (Shaalan and Canyon, 2009). Previously, the release of *Toxorhynchites amboinensis* larvae led to a 45% reduction of *Ae. aegypti* population in urban areas of New Orleans (Focks et al., 1985). Similar success was reported with *T. splendens* (Annis et al., 1989; Aditya et al., 2006) and *T. moctezuma* (Rawlins et al., 1991). Apart from their direct effect *via* ferocious predation, the presence of *Toxorhynchites* larvae can delay the prey's developmental time and increase the prey's mortality. This is probably due to the stress experienced by the prey in the presence of the predator, or the predator-derived kairomones (Andrade, 2015; Zuharah et al., 2015). Nevertheless, the mechanism behind this effect has yet to be completely deciphered. Despite the earlier reported success, the application of *Toxorhynchites* as biocontrol agent has been hindered by several factors. Firstly, sylvatic species such as *T. rutilus* are not well adapted to urban environment, which restricts its application despite the good predation capacity (Focks et al., 1983). Nevertheless, a more recent surveillance demonstrated the presence of *T. rutilus* in urban areas, albeit of low numbers (Wilke et al., 2019). Indeed, this discovery has reignited the hope of applying *Toxorhynchites* as a vector biocontrol agent in urban areas (Schiller et al., 2019). Besides, the slow population expansion of *Toxorhynchites* is another challenge that needs to be overcome. Under the natural settings, *Toxorhynchites* produces few offspring, which limits their efficacy and capacity in vector biocontrol. This is further aggravated by the cannibalistic nature of *Toxorhynchites* immatures, especially under food-restricted conditions (Donald et al., 2020).

Copepods of genera *Megacyclops, Mesocyclops*, and *Macrocyclops* are crustaceans that feed primarily on the first instar of mosquito larvae. Copepods can adapt to a large variety of water bodies and micro aquatic habitats such as phytotelmata (structures of terrestrial plants that allow formation of water pockets). Such high adaptability allows copepods to be explored as vector biocontrol agents in different settings (Vinogradov et al., 2022). In fact, the discovery of copepod's potential in vector biocontrol was rather accidental, following an observed reduction of *Ae. aegypti* and *Ae. polynesiensis* larvae from ovitraps set in a study site at Tahiti, after unintentional introduction of copepods to the ovitraps (Riviere and Thirel, 1981). Subsequently, field trials from different regions confirmed the effectiveness of copepods in various water bodies (including drains and land crab burrows) against larvae of medically important mosquitoes, particularly of genera *Aedes* and *Ochlerotatus* (Lardeux et al., 1992; Kay et al., 2002). Importantly, the introduced copepods can adapt and colonize nearby water bodies, allowing sustained effort of mosquito larval control (Kay et al., 2002). Although being used mainly against *Aedes* spp., copepods have been used against other vectors such as *Anopheles* spp. and *Culex* spp. (Riviere and Thirel, 1981; Marten et al., 1989; Lardeux, 1992; Lardeux et al., 1992; Vu et al., 1998; Schaper, 1999; Marten et al., 2000; Kay et al., 2002; Zoppi de Roa et al., 2002; Soumare and Cilek, 2011). Despite their ability to adapt to different sizes of water bodies, copepods are particularly sensitive to temperature changes, chlorine content, low oxygen levels, and presence of toxin within the water (Brown, 1996; Vinogradov et al., 2022). Moreover, it is important to highlight that several species of copepods serve as the intermediate hosts of medically important parasites such as *Drancunculus medinensis* (guinea-worm) and *Dibothriocephalus latus*/ *Diphyllobothrium latum* (fish tape worm; Marten and Reid, 2007; Vinogradov et al., 2022). Therefore, careful consideration and planning must be done prior to application of this method. For example, non-vector copepod species can still be considered as biocontrol agents in certain parts of Africa that are endemic for dracunculiasis (Marten and Reid, 2007).

Water bugs, such as the backswimmers (family: Notonectidae), giant water bugs (family: Belostomatidae) and waterboatmen (family: Corixidae) are important predaceous insects under the order Hemiptera (Shaalan and Canyon, 2009). The potential of *Anisops assimilis* (common backswimmer) to control mosquito population was reported officially for the first time in 1939, following the observation that the backswimmer-harboring water containers were void of mosquito larvae, in contrast to the surrounding backswimmer-free water bodies that were infested with active mosquito larvae (Graham, 1939). Although field and laboratory trials using water bugs to control mosquito larvae exhibited promising results, they are hardly utilized as biocontrol agents due to the high cost and difficulty of mass rearing, as well as logistical challenges (Bay, 1974; Murdock et al., 1984; Venkatesan and Jeyachandra, 1985; Sankaralingam and Venkatesan, 1989; Aditya et al., 2004, 2005, 2006; Selvarajan and Kakkassery, 2019).

Predatory coleopterans from the families Dytiscidae (diving beetle) and Hydrophilidae (water scavenger beetle) are commonly found in ground pools, permanent and temporary ponds (Shaalan and Canyon, 2009). Despite the lower research interest, several studies on the predatory effect of beetles on mosquito reported promising results (Nilsson and Soderstrom, 1988; Juliano and Lawton, 1990; Nilsson and Savensson, 1994; Aditya et al., 2006; Chandra et al., 2008b). However, the efficacy of coleopterans as vector biocontrol agents may be compromised by their diet preference (when mosquitoes are not the only insects presented), species emigration and cannibalism (Juliano and Lawton, 1990; Lundkvist et al., 2003).

Currently, the potential of predators discussed above has not been thoroughly explored, and most of the reported studies focused on mosquitoes (Kim and Merritt, 1987; Werner and Pont, 2003). Notably, several natural predator-based biocontrol strategies have been attempted against non-mosquito vectors, notably the parasitic VBIAR. For example, *Tarentola mautitanica*, an insectivorous lizard, has been proposed as a candidate to control the population of *Triatoma infestans* (kissing bug) that spreads Chagas disease (Castello and Gil Rivas, 1980). Mites and spiders have been suggested as biocontrol agents of *Phlebotomus* spp. (sand fly) that transmits leishmaniasis (Dinesh et al., 2014).

## Pathogenesis-mediated vector biocontrol

Besides predatory animals, pathogens have been proposed as biocontrol agents against vectors. In fact, a number of these pathogens have been applied in the field. These candidates vary in sizes and behavior, encompassing both prokaryotic and eukaryotic organisms. The nematodes are probably the largest candidates on the list. The mermithids are members of an endoparasitic nematode family. These nematodes are highlighted as potential vector biocontrol candidates,

due to their parasitic relationship with various arthropods and several arachnids (Stabler, 1952; Chapman, 1974). The hatched pre-parasitic juveniles of mermithid nematodes aggressively infect mosquito larvae (usually the early instars) by paralyzing the targeted hosts, followed by penetration of cuticular wound to establish the infection (Sanad et al., 2017). Once infected, the mermithid parasites take over the cellular function regulatory authority of their hosts. If infection occurs during the early larvae instar, the parasitized mosquito larvae are halted from pupating as the infecting parasites develop within (Stabler, 1952; Allahverdipour et al., 2019). When the nutrient resource supplied by the infected host is exhausted, the nematode, now at its third-stage juvenile post-parasite stage, emerges out of the host, which results in the death of the host (Stabler, 1952). The emerged post-parasite stage then molts into the free-living adult to reproduce and lay eggs. Multiple mermithids may repeatedly infect an already infected larva, giving rise to a phenomenon called superparasitism (Sanad et al., 2017). Different research groups have demonstrated the mosquito larvicidal effect of several mermithids such as *Romanonermis iyengari* (against *Ae. aegypti*, *Ae. albopictus*, *An. gambiae*, *Anopheles culicifacies*, *Anopheles stephensi*, *Anopheles subpictus*, *Armigeres subalbatus*, *Culex pipiens, Culex quinquefasciatus, Culex sitiens, Culex tritaeniorhynchus, and Mansonia annulifera*), *Diximermis peterseni* (against *Anopheles crucians*, *Anopheles quadrimaculatus*, and *Anopheles punctipennis*) and *Strelkovimermis spiculatus* (against *Aedes albifasciatus* and *Cx. pipiens*; Petersen and Willis, 1974; Levy and Miller, 1977; Poinar and Camino, 1986; Santamarina Mijares and Perez Pacheco, 1997; Paily and Balaraman, 2000; Sanad et al., 2013, 2017; Abagli and Alavo, 2019; Abagli et al., 2019). However, the lack of culturable mermithids hinders mass application of this nematode as a biocontrol agent (Kendie, 2020).

Entomopathogenic fungi are another group of insect pathogens that have been explored as a potential vector biocontrol agent (Chapman, 1974). Fungi of genera *Beauveria* and *Metarhizium* have been shown to exert high mortality to medically important mosquitoes of genera *Anopheles*, *Culex* and *Aedes* (Blanford et al., 2011; Accoti et al., 2021). The fungal infection exhausts the mosquitoes due to increased metabolic rate and reduces their frequency of taking blood meals. As a result, the lifespan, oviposition rate, as well as the chance of infected mosquitoes to acquire and transmit medically important pathogens reduces greatly (Blanford et al., 2011). Interestingly, the fungi have been reported to affect both the larval and adult stages of mosquitoes (Blanford, 2005; Blanford et al., 2011). However, the virulence of fungi is influenced by various factors (Scholte et al., 2007; Paula et al., 2011b; Alkhaibari et al., 2017). For example, most fungi may lose their potency after a few months (Scholte et al., 2007). Besides, the lethality of entomopathogenic fungi is influenced by the nutritional state of the targeted vector (Paula et al., 2011b). Furthermore, different forms of fungi may demonstrate different potency against the mosquitoes. For instance, *Ae. aegypti* is more susceptible to the blastospores of *Metarhizium*, whereas *Cx. quinquefasciatus* is more susceptible to the conidia forms. On the other hand, *An. stephensi* is susceptible to both forms of *Metarhizium* (Alkhaibari et al., 2017). Notably, it is difficult to culture and mass produce fungi (Accoti et al., 2021). More importantly, these entomopathogenic fungi have been reported to cause symptomatic infections in immuno-compromised humans, raising safety concerns regarding this vector biocontrol agent (Henke et al., 2002; Tucker et al., 2004; Lara Oya et al., 2016; Goodman et al., 2018). These drawbacks render fungi a less attractive vector biocontrol option.

*Bacillus thuringiensis* var. *israelis* (*Bti*) is a bacterium commonly used as a household larvicide. This bacterium produces delta endotoxins (known as the "Cry" or "Cyt" toxins) during its sporulation, which are potent insecticide proteins (Tabashnik, 1992; Wu et al., 1994; Ben-Dov et al., 1995). The toxin has been demonstrated to kill larvae of *Ae. aegypti* and *Ae. albopictus* effectively, by disrupting the osmotic balance of the midgut epithelial cells upon ingestion (Chapman, 1974; Promdonkoy and Ellar, 2003; Lacey, 2007). Importantly, *Bti* does not pose direct ecological or health threats as it does not affect any off-target organisms including fishes, birds, mammals, and many other insects (Fayolle et al., 2015; Poulin and Lefebvre, 2018; Poulin et al., 2022). Nevertheless, research is underway to evaluate the indirect impact of *Bti* application, particularly its impacts on local ecological systems (Novak et al., 1986; Arredondo-Jimenez et al., 1990; Kumar et al., 1998; Ritchie et al., 2010; Fayolle et al., 2015; Poulin and Lefebvre, 2018; Poulin et al., 2022). Resistance against Cry toxin has yet to be reported. Nevertheless, development of tolerance toward some of the Cry toxins (Cry4Aa and Cry11Aa) was reported in a population of *Ae. sticticus* (Tetreau et al., 2013).

Viruses, such as the mosquito-specific densoviruses (MDV) may be used against the vectors too (Chapman, 1974). MDVs are highly infectious to its targets due to its capability of establishing vertical and horizontal transmission (Johnson and Rasgon, 2018). Upon infection, MDV causes a plethora of pathogeneses on their targets, which lead to apoptosis of infected larvae (Roekring and Smith, 2010), and shortening of adult lifespan (Suchman et al., 2006). Interestingly, MDV has been shown to reduce the viral load of type II DENV in *Ae. albopictus* (Wei et al., 2006). Besides, MDV can be genetically modified to cater for different conditions of vector control. For instance, a recombinant *Ae. aegypti* densovirus (AeDNV) expressing BmK IT1(an insect-specific toxin) was demonstrated to exert higher pathogenicity to *Ae. albopictus* (Gu et al., 2010). Despite these advantages, large-scale implementation of MDV-mediated vector biocontrol strategy may not be easy due to the relatively low stability of viral particles outside the hosts (Johnson and Rasgon, 2018). Nevertheless, advancement of technology may make this method more feasible for mass application in the future.

The potential vector biocontrol candidates above share a drawback that need to be overcome for mass application. It remains uncertain how sustainable these biocontrol agents can exist in the environment for a long-lasting controlling effect against the vector population. This is especially crucial for VBZ and VBIAR with complex and sporadic transmission profiles. Besides, candidates with healthcare risk concerns should not be employed until all doubts are scientifically cleared. Nevertheless, biocontrol candidates such as *Bti* and entomopathogenic fungi have been commercialized recently (Akutse et al., 2020).

## Manipulation of vital biological functions

Alternative approaches that revolve around the manipulation of vector's biology have been explored to develop a strategy that preserves the relatively target-specific nature of most pathogenesis-mediated biocontrol approaches while overcoming the drawbacks faced by these strategies. Hence, genetic manipulation of vector's vital functions has gained increasing research attention. Sterile Insect Technique (SIT) is

one of the successful examples of such approach (Baumhover et al., 1955; Lofgren et al., 1974; Patterson et al., 1977). In the SIT approach, the male vector is made infertile *via* radiation exposure or chemosterilization (Serebrovsky, 1940; Baxter, 2016). Subsequently, when these sterile males are released into the wild and mate with females, non-viable offspring are produced. As a result, the targeted vector population is reduced. This technique was successfully employed to control the infestation of the New World screwworm fly (*Cochliomyia hominivorax*) in the United States, whose maggots are capable of causing myiasis with severe tissue damages (Baumhover et al., 1955). SIT worked well against *C. hominivorax* because each female fly mates only once. As SIT-modified insects do not produce any offspring, the success of this technique depends on the persistent release of sterile male specimens to compete with the fertile wild type (WT) males for mating. Subsequently, this technique was attempted against mosquitoes in the 1970s, which yielded encouraging results. The population of *Anopheles albimanus* in El Salvador was reduced by 99% after implementing this technique for 5 months (Lofgren et al., 1974). Several mosquito-targeting field trials were performed in Burkina Faso, France, India, Myanmar, and United States. The experimented mosquitoes were *Ae. aegypti*, *An. gambiae, An. quadrimaculatus*, *Cx pipiens*, and *Cx quinquefasciatus* (Weidhaas et al., 1962; Dame et al., 1964; Laven, 1967; Davidson et al., 1970; Patterson et al., 1970; Curtis, 1976; Grover et al., 1976; Patterson et al., 1977; Curtis et al., 1982). These field trials yielded mixed results. For example, in India, the population of targeted mosquitoes was not effectively controlled with this approach, due to the immigration of mated WT females from the locations adjacent to the trial sites. In addition, political turmoil significantly affected the execution of this approach, which confounded the success of this strategy (Curtis, 1976; Curtis et al., 1982).

Despite the reported success, SIT is accompanied with several drawbacks. Firstly, there are concerns among the public members regarding the off-target effect of chemosterilizing agents to the environment (Bartumeus et al., 2019). Laboratory bioassays on non-target predators such as the common house spider (*Achaeranea tepidariorum*) revealed the significant reduction in fertility among the spiders that consumed the chemosterilized mosquitoes (Bracken and Dondale, 1972). Nevertheless, this issue can be overcome *via* simple bulk detoxification using acid and alkaline, which eliminates residues of chemosterilizing agents without compromising the efficacy of this method (Sharma, 1976). Secondly, the difficulty to precisely segregate male and female specimens in the insect colony implies the possibility of sterilizing female specimens by mistake (Sharma et al., 1976; McInnis et al., 1994; Parker, 2005). Accidental release of these mistakenly treated females will result in mating competition with the fertile WT females. As a result, the dispersal of sterile males will be compromised. In addition, radiation used in sterilization will significantly shorten the lifespan of these irradiated insects, which compromises the success of this technique in the field (Alphey and Andreasen, 2002). To overcome this issue, the concept of homozygous female-specific lethal genes has been applied, giving rise to techniques such as Genetic Sexing Strain (GSS) and Release of Insects carrying a Dominant Lethal Gene (RIDL; Franz, 2005; Fu et al., 2010; Harris et al., 2011; Carvalho et al., 2015; O'Leary and Adelman, 2020). RIDL enables selection of the developmental stage corresponding to the manifestation of engineered lethal traits. The insertion of a repressible dominant lethal transgene into the mosquito genome confers conditional fatality (such as tetracycline-dependent survival) to its late juvenile stage. In this approach, the engineered male mosquitoes are released to mate with the WT females. Instead of completing metamorphosis, the produced juveniles that carry a copy of the engineered gene will die in the absence of tetracycline (Phuc et al., 2007). Indeed, field trials of *Ae. aegypti* OX513A in Cayman Islands and Brazil demonstrated strong suppression of the targeted mosquito population (Harris et al., 2012; Carvalho et al., 2015). In addition, female-specific flightless phenotype and DENV-susceptible phenotype that are genetically engineered in *Ae. aegypti* have improved the gender segregation and impeded vector competence to DENV, respectively (de Valdez et al., 2011; Buchman et al., 2020). These techniques minimize the "leakage" of "accidentally treated females" into the wild (Franz, 2002; Calkins and Parker, 2005; Franz, 2005; Koskinioti et al., 2021). In general, the attempts to overcome the shortcomings of SIT revolve around gene editing, which was highly challenging decades ago. However, the discovery and establishment of CRISPR/Cas9 system allows gene editing to be performed much more easily (Gupta et al., 2019). This molecular advancement facilitates the application of SIT against different vectors.

Besides facilitating SIT in vector biocontrol approach, CRISPR/Cas9 can be applied to genetically design arthropod vectors that are not receptive to pathogens transmitted by them under normal circumstances. For example, the knock-out of *FREP1* gene has been shown to reduce the susceptibility of *An. gambiae* to *Plasmodium* spp. (Dong et al., 2018). Gene drive is another genetic engineering concept that has enjoyed a great push in vector control research following the establishment of CRISPR/Cas9 technology. The CRISPR/Cas9-integrated gene drive method allows the targeted genes to be propagated and inherited much more rapidly than the Mendelian rates, resulting in fast replacement or displacement of the targeted traits in a population (Leung et al., 2022). Recently, this technology has been applied on *An. gambiae*, resulting in a successful halting of *Plasmodium* development within the genetically modified mosquitoes, as well as compromising the survival of the homozygous transgenic females (Hoermann et al., 2022). In addition, other gene editing methods, such as the application of homing endonuclease genes (HEG) have been explored to control the malaria vectors (Windbichler et al., 2007; Deredec et al., 2011). Nevertheless, such genetically engineered mosquitoes suffered compromised fitness that hindered their sustainable establishment in the wild. This drawback is in fact a major concern, as modification of one gene may lead to unexpected outcomes on the experimented organism (Resnik, 2014, 2017). If the mutants with unexpected and undesirable traits (following gene editing) thrive in the wild, the ecosystem may be threatened in an unprecedented manner. Nevertheless, the successful application of vital function modification to control a myiasis causative agent with wild and domestic animals as reservoirs reflects the great potential of this approach to control VBZ and VBIAR. Importantly, techniques stemming from this approach should be tested, evaluated, and validated thoroughly before mass application.

## *Wolbachia* as a novel vector biocontrol approach

As elaborated earlier, the pathogenesis-mediated biocontrol agents are arthropod pathogens that shorten the lifespan of vectors,

whereas genetic manipulation of arthropod vital functions works by halting the vectors' population expansion. The application of *Wolbachia* in vector biocontrol is a unique approach that combines the characteristics of both approaches. *Wolbachia* are maternally inherited, gram-negative, obligate intracellular endosymbiotic bacteria found in many arthropods such as mites, spiders, scorpions and isopods (Werren et al., 2008). *Wolbachia* are found in various organs and tissues within the infected arthropod (Werren, 1997; Werren et al., 2008). Besides, medically important filarial nematodes carry *Wolbachia* as well (Lau et al., 2015).

Approximately 60% of the insects are positive for *Wolbachia*. Interestingly, *Ae. aegypti* is *Wolbachia*-free under normal condition (Kittayapong et al., 2000; Rasgon and Scott, 2004). In the early 2000s, Xi et al. (2005) successfully performed an experimental infection on *Ae. aegypti* with *Wolbachia w*AlbB strain (henceforth *w*AlbB) derived from *Ae. albopictus*. Subsequently, this finding was explored further, with trial release of *w*AlbB-infected *Ae. aegypti* in several locations reported increased resistance of the vector to DENV, ZIKV, and CHIKV (Bian et al., 2010; Aliota et al., 2016a,b; Chouin-Carneiro et al., 2019; Nazni et al., 2019). Meanwhile, the infection of *Ae. aegypti* by another more virulent strain of *Wolbachia* (*w*MelPop strain) has been shown to reduce the number of *Ae. aegypti* significantly (Rasgon et al., 2003; Ritchie et al., 2015). These findings highlight the potential of *Wolbachia* as a tool in vector control program. Therefore, the mechanisms behind the effects cast by *Wolbachia* on the infected mosquitoes have received increasing research attention over the past two decades.

*Wolbachia* have evolved and developed various mechanisms to manipulate the host's cellular biology toward their survival advantage, namely cytoplasmic incompatibility (CI), parthenogenesis, feminization, and male killing (O'Neill et al., 1997; Werren, 1997; Werren et al., 2008). CI happens when a *Wolbachia*-infected male mates with either a *Wolbachia*-negative female or a female infected with a different strain of *Wolbachia*, resulting in non-viable progeny (Werren, 1997). This principle forms the basis of "Incompatible Insect Technique (IIT)" that drives many *Wolbachia*-mediated biocontrol programs against *Ae. aegypti* in China, the United States, and Singapore (Ritchie et al., 2015; Mains et al., 2019; Zheng et al., 2019; Soh et al., 2021). Parthenogenesis refers to the development of eggs into progenies without fertilization, whereas feminization involves development of genetic male into female. *Wolbachia* has been shown to induce feminization in several crustaceans and insects (Werren, 1997; Cordaux et al., 2001; Kageyama et al., 2002; Negri et al., 2006; Werren et al., 2008; Asgharian et al., 2014; Scola et al., 2015). Meanwhile, male killing happens when the affected males experience a significantly shorter lifespan than the affected females. CI, parthenogenesis, feminization, and male killing trigger disruption of gender ratio in the affected population toward female dominance. Using these strategies, *Wolbachia* manipulates the population structure of the infected arthropods, which facilitates the spread and establishment of *Wolbachia* in the wild (Hoffman and Turelli, 1997; Werren, 1997; Werren et al., 2008). Coupled with the reported resistance to virus infection by the *Wolbachia*-infected mosquitoes, the establishment of *Wolbachia* in the vector population may suppress the transmission of these pathogens to humans. In fact, countries such as Malaysia, Indonesia, Laos, Vietnam, Sri Lanka, Australia, Fiji, Vanuatu, Brazil, Colombia, and Mexico have released *Wolbachia* -infected female *Ae. aegypti* to establish a stable *Wolbachia*-infected mosquito colony in the wild (Nazni et al., 2019; World Mosquito Program, 2022). Besides mosquitoes, *Wolbachia* has been explored for the control of black flies and sand flies. However, difficulties in colony maintenance of black flies and sand flies, coupled with the relatively low *Wolbachia* load post-infection in these insects giving rise to the undetectable CI among these insects. This implied the unsuitability of *Wolbachia* for the control of these non-mosquito vectors. Therefore, the versatility of the *Wolbachia* biocontrol approach remains to be validated (Crainey et al., 2010; Bordbar et al., 2014).

Despite the promising advantages of *Wolbachia*-mediated vector biocontrol approach, this method has several shortcomings and concerns. Similar to SIT, the *Wolbachia* method faces the issue of accidental "female leakage" that may compromise the efficacy of IIT-driven vector control strategy. For instance, IIT that incorporates *Wolbachia* can be dampened by mass production. Accidental release of *Wolbachia*-infected females during field trial could affect the population suppression goal. Nevertheless, this may not be considered as an absolute disadvantage, as *Wolbachia*-infected mosquitoes have been claimed to be less susceptible to the medically important pathogens that they carry (Bian et al., 2010; Nazni et al., 2019). To date, the long-term impact of *Wolbachia* on the targeted mosquitoes has not been well studied, partly due to the relatively short discovery history of this vector biocontrol candidate. Furthermore, the interaction dynamics among the arthropod host, *Wolbachia*, and the medically important pathogens carried by the arthropod remains to be fully deciphered. Nevertheless, few studies on this topic revealed interesting findings. For example, a previous study demonstrated that the *Wolbachia*-infected *Culex tarsalis* became more susceptible to West Nile Virus, with much higher viral load post-infection, as compared to the *Wolbachia*-free specimens (Dodson et al., 2014). Since *Wolbachia* has been shown to interfere with interactions between the arthropod host and the medically important pathogens that it carries, it is of utmost importance to consistently assess the efficacy and impact of *Wolbachia* deployed in vector control programs. Besides, the *w*MelPop-related strains have been demonstrated to be temperature-sensitive, raising doubts about the sustainability of this approach in areas with higher temperature (Ulrich et al., 2016; Ross et al., 2017). Succinctly, these concerns deserve more research attention despite the higher research difficulty, where longitudinal study covering adequately long duration is needed.

Besides, concerns have been raised regarding the possibility of *Wolbachia* to cause pathology to humans. Although *Wolbachia* can be found in mosquito salivary glands, the bacteria are not available in saliva, as backed by polymerase chain reaction (PCR) screenings (Wu et al., 2004; Moreira et al., 2009b). In addition, *Wolbachia* are larger than the mosquito salivary duct (Moreira et al., 2009b). Hence, it is relatively unlikely for the bacteria to be transmitted to humans *via* mosquito bites. Moreover, human volunteers exposed to *Wolbachia*-infected mosquitoes over extended period of time revealed absence of antibody specific to *Wolbachia* in their blood (Popovici et al., 2010). Of note, responses to *Wolbachia* or *Wolbachia*-derived antigens by other key players in human immune system remained unclear. Based on currently available information, *Wolbachia* application has been considered as a relatively safe vector biocontrol approach. There are environmental concerns regarding this approach as well. In fact, the major environmental concern is extrapolated from the public health concern, where *Wolbachia* may spread to other organisms across the mosquito-related food chain in the ecosystem. This may disrupt the

ecosystem dynamics, hence threatening the biodiversity in the affected environment. Fortunately, *Wolbachia* has been proven to be unable to establish itself throughout the mosquito-associated food chain (Popovici et al., 2010). Notably, natural cross-species infestation of *Wolbachia* is extremely rare (Werren et al., 1995; Werren, 1997), let alone a sustained establishment that allows vertical transmission (Hoffman and Turelli, 1997; Turelli, 2010).

Importantly, *Wolbachia* vector control approach may work well against a disease transmission that involves only one species of arthropod as vector. The effects of *Wolbachia* infection varies with different species of vectors. Hence, infections with multiple vectors, or those with incomplete list of vectors cannot employ this method as vector control program. The clearance of one vector by the bacteria may allow other vectors to thrive, rendering the disease control futile. Knowlesi malaria is an example of VBZ with multiple vectors, and the list of knowlesi malaria vectors is expanding for the moment (Ang et al., 2020; Jeyaprakasam et al., 2020; Pramasivan et al., 2021; Vythilingam et al., 2021). Apart from this, the actual efficacy of *Wolbachia* approach to reduce disease transmission has been questioned. Over the past few years, an increasing number of countries have participated in the release of *Wolbachia*-infected *Ae. aegypti*. Nevertheless, many of these countries still experience increased burden of dengue transmission after persistent release of these mosquitoes (Kementerian Kesihatan Malaysia, 2022). The difficulty to establish stable *Wolbachia* colony within the environment, stability and sustainability of this method in the field, and relative attractiveness of *Wolbachia*-infected mosquitoes during mating may contribute to the challenges faced by this approach to secure a more obvious disease transmission chain breakage in these countries. Obviously, more investigations are needed to better understand this approach, and its practicality, as well as its sustainability in the field.

## Prospects and challenges of vector control against VBZ and VBIAR

The control and eradication of VBZ and VBIAR hardly rely on a single approach of vector management, due to the complexity of their transmission circuit. Hence, the application of integrated vector management (IVM) that incorporates multiple vector control approaches may increase the success rate of breaking the transmission circuit of these infections (WHO, 2012). To implement a successful and sustainable IVM, the components of IVM triads (biological, environmental, and chemical) should be covered during the designing of the vector control plan (Figure 3). Adaptation and customization of vector control strategies according to the targeted locations are required to ensure high success. For example, the landscape of a targeted location can be modified to facilitate the implementation of vector biocontrol strategies. At the same time, environment-friendly chemicals that can promote biocontrol strategy (such as predator attractants and pheromone-like substances) can be applied. IVM is a multi-prong approach against the vectors, where the selected strategies may complement each other to bring down the vector population. Moreover, IVM may minimize the risk of complete failure faced by a vector control program, as other components in the IVM may continue to work normally when one component is breaking down. For instance, ORS (chemical approach) may be completely stopped during the total lockdown of sudden onset (as exemplified by the COVID-19 pandemic-triggered lockdown in many countries). If the

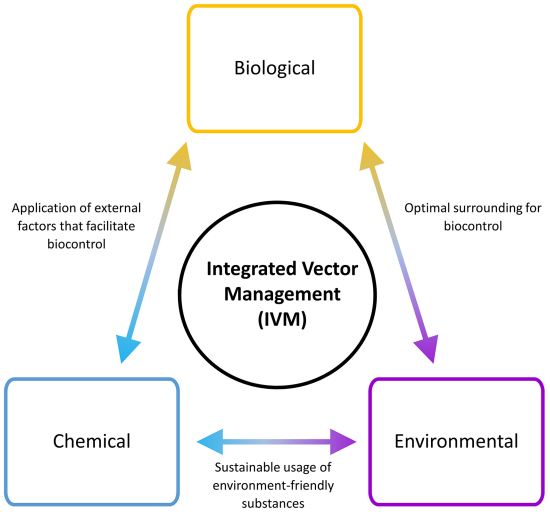


FIGURE 3
Integrated Vector Management (IVM) involving different components in planning and implementation. Multiple components are factored into an IVM strategy to optimize the output of vector control.

affected area has a well-constructed and maintained drainage system that hampers oviposition by the vectors (environmental management approach), the vector population in that area may not increase after ORS is brought to an abrupt halt. In short, multiple components should be explored to synergize the vector control effort.

While promoting IVM against VBZ and VBIAR, we should not overlook other co-existing factors in the surrounding that may confound the outcomes of the vector control strategy. For example, knowlesi malaria is one of the most prevalent VBZ in Southeast Asia, with Malaysia serving as the epicenter of transmission. Unlike *P. falciparum* that has developed resistance strategies against artemisinin (the current first line anti-malarial treatment; Fairhurst and Dondorp, 2016; Lee et al., 2021), *P. knowlesi* remains susceptible to artemisinin and other anti-malarials in the market (Fatih et al., 2013; van Schalkwyk et al., 2017). Nevertheless, this zoonotic parasite can cause hyperparasitaemia and life-threatening pathogenesis in humans (Lee et al., 2013; Singh and Daneshvar, 2013). Hence, various strategies have been considered to control and eliminate this infection, including the vector management. The potential of different chemical-based approaches has been investigated (Rohani et al., 2020), and IVM against knowlesi malaria transmission has been proposed (Lee et al., 2022). Currently, it is challenging to implement an all-rounded IVM against knowlesi malaria in many hyperendemic areas as the vector profile of this VBZ has yet to be completely deciphered. As mentioned earlier, environment management demands a thorough evaluation of vector profile, transmission dynamics and socio-economic activities in the targeted area. Landscape modification that aims against the incriminated vector may effectively clear the targeted vector's population. However, the altered landscape may become a conducive breeding ground for another species of anophelines capable of transmitting *P. knowlesi*. Besides, many places affected by knowlesi malaria are endemic to other vector-borne diseases such as dengue and filariasis (Murphy et al., 2020; Zakaria and Avoi, 2022). Therefore, strategies aimed at reducing the transmission of knowlesi malaria should not facilitate the expansion of vectors responsible for other vector-borne diseases. Given the complexity of disease transmission dynamics in many areas

endemic to knowlesi malaria, it is not surprising that chemical-based vector control approaches are preferred over other approaches as chemicals are effective against broader range of vectors, despite the potential harmful effects to the environment. Nevertheless, biocontrol strategies with lower target specificity (such as the predator–prey approaches) deserve more attention. Interestingly, edible fishes can be explored as biocontrol candidates, as demonstrated in western Kenya (Howard et al., 2007). The Nile tilapia used in this earlier study is a commonly farmed and eaten fish. In this study, the Nile tilapia significantly reduced the population of *An. gambiae* s.l., *An. funestus* and culicine mosquitoes (Howard et al., 2007). Such integration of vector biocontrol and socioeconomic activity can ensure better sustainability of the implemented vector control efforts. Chemical-based approaches, such as IRS and ORS may be considered and implemented with caution. In addition, environment management *via* human behavioral changes should be emphasized, particularly for VBZ like knowlesi malaria, in which the transmission is associated with socioeconomic activities near or within forested areas, such as tourism, logging, and subsistence cropping (Singh and Daneshvar, 2013; Müller and Schlagenhauf, 2014; Lee et al., 2022). The challenges faced in the control and prevention of knowlesi malaria in Malaysia are applicable to other VBZ and VBIAR. Obviously, there are numerous knowledge gaps that need to be filled with properly designed studies to put forward better vector control programs. Notably, the long-term safety, efficacy, and sustainability of all proposed methods should be investigated thoroughly prior to mass application. Such information is needed to convince the public members and secure their support and compliance to a proposed vector control program, which is crucial to many IVMs. In short, various factors must be taken into consideration when designing a control strategy against VBZ and VBIAR, particularly in areas endemic to multiple vector-borne diseases.

## Conclusion

Vector control has always been a crucial component of breaking the transmission circuit of vector borne diseases. The increased prevalence of vector-borne diseases, including several VBZ and VBIAR in different parts of the world implies a more important role of vector control in healthcare sector. Indeed, there is no "silver bullet" for outbreak management, even more so for the management of the more complex VBZ and VBIAR. Careful integration of multiple vector control approaches in the vector management program may increase the success of disease control and prevention. While battling these pathogens with large investment in the research and development for treatments and vaccines, continuous efforts of discovering novel vector control approaches should be made concurrently, to reduce the prevalence of these infections without compromising the wellbeing of the environment, humans, and the animals involved in the transmission circuit.

## Author contributions

W-CL, MLW, and ZZ conceptualized the review. W-CL, MLW, and ZZ performed literature review and information interpretation. W-CL, MLW, ZZ, IV, YL, MYF, and I-CS involved in manuscript preparation. All authors contributed to the article and approved the submitted version.

## Funding

We would like to thank the Ministry of Higher Education, Malaysia, for the Fundamental Research Grant Scheme (FRGS/1/2022/SKK12/UM/02/9) awarded to W-CL. MLW and MYF were supported by the Long-Term Research Grant Scheme (LRGS) by the Ministry of Higher Education, Malaysia (LRGS/1/2018/UM/01/1/1). IV was supported by another LRGS of the Ministry of Higher Education, Malaysia (LRGS/1/2018/UM/01/1/3). YL was supported by another LRGS of the Ministry of Higher Education, Malaysia (LRGS/1/2018/UM/01/1/4).

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

W-CL is an editorial board member of Frontiers in Microbiology, and one of the guest associate editors for the research topic "Zoonoses – a rising threat to healthcare system." The authors agree to abide by the rules, ethical standards and practices of Frontiers; and this does not alter the authors' adherence to all the Frontiers policies on publishing and sharing of information.

## Publisher's note



## References

Aagaard, K., Luna, R. A., and Versalovic, J. (2015). "The human microbiome of local body sites and their unique biology" in *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*. eds. J. E. Bennett, R. Dolin and M. J. Blaser, vol. *2. 8th* ed (Philadelphia, PA: Elsevier/Saunders), 12–21.

Abagli, A. Z., and Alavo, T. B. C. (2019). Biocontrol of *Culex quinquefasciatus* using the insect parasitic nematode, Romanomermis iyengari (Nematoda: Mermithidae). *Trop. Biomed.* 36, 1003–1013.

Abagli, A. Z., Alavo, T. B. C., Perez-Pacheco, R., and Platzer, E. G. (2019). Efficacy of the mermithid nematode, Romanomermis iyengari, for the biocontrol of Anopheles gambiae, the major malaria vector in sub-Saharan Africa. *Parasit. Vectors* 12:253. doi: 10.1186/s13071-019-3508-6

Accoti, A., Engdahl, C. S., and Dimopoulos, G. (2021). Discovery of novel entomopathogenic fungi for mosquito-borne disease control. *Front. Fungal Biol.* 2:637234. doi: 10.3389/ffunb.2021.637234

Aditya, G., Ash, A., and Saha, G. K. (2006). Predatory activity of Rhantus sikkimensis and larvae of Toxorhynchites splendens on mosquito larvae in Darjeeling, India. *J. Vector Borne Dis.* 43, 66–72. PMID: 16967818

Aditya, G., Bhattacharyya, S., Kundu, N., and Saha, G. K. (2005). Frequency-dependent prey-selection of predacious water bugs on *Armigeres subalbatus* immatures. *J. Vector Borne Dis.* 42, 9–14. PMID: 15999455

Aditya, G., Bhattacharyya, S., Kundu, N., Saha, G. K., and Raut, S. K. (2004). Predatory efficiency of the water bug Sphaerodema annulatum on mosquito larvae (Culex quinquefasciatus) and its effect of the adult emergence. *Bioresour. Technol.* 95, 169–172. doi: 10.1016/j.biortech.2004.02.007

Ahantarig, A., and Kittayapong, P. (2011). Endosymbiotic Wolbachia bacteria as biological control tools of disease vectors and pests. *J. Appl. Entomol.* 135, 470–486. doi: 10.1111/j.14390418.2011.0 1641.x

Ahantarig, A., Trinachartvanit, W., Baimai, V., and Grubhoffer, L. (2013). Hard ticks and their bacteria endosymbionts (or would be pathogens). *Folia Microbiol.* 58, 419–428. doi: 10.1007/sl2223-013-0222-1

Ahmad, N. A., Vythilingam, I., Lim, Y. A. L., Zabari, N. Z. A. M., and Lee, H. L. (2017). Detection of Wolbachia in Aedes albopictus and their effects on chikungunya virus. *Am. J. Trop. Med. Hyg.* 96, 148–156. doi: 10.4269/ajtmh.16-0516

Akutse, K. S., Subramaniam, S., Maniania, N., Dubois, T., and Ekesi, S. (2020). Biopesticide research and product development in Africa for sustainable agriculture and food security-experiences from the International Centre of Insect Physiology and Ecology (ICIPE). *Front. Sustain. Food Syst.* 4:563016. doi: 10.3389/fsufs.2020.563016

Albicócco, A. P., and Vezzani, D. (2009). Further study on Ascogregarina culicis in temperate Argentina: prevalence and intensity in Aedes aegypti larvae and pupae. *J. Invertebr. Pathol.* 101, 210–214. doi: 10.1016/j.jip.2009.05.003

Aliota, M. T., Peinado, S. A., Velez, I. D., and Osorio, J. E. (2016a). The wMel strain of Wolbachia reduces transmission of Zika virus by Aedes aegypti. *Sci. Rep.* 6:28792. doi: 10.1038/srep28792

Aliota, M. T., Walker, E. C., Uribe Yepes, A., Dario Velez, I., Christensen, B. M., and Osorio, J. E. (2016b). The wMel strain of Wolbachia reduces transmission of Chikungunya virus in Aedes aegypti. *PLoS Negl. Trop. Dis.* 10:e0004677. doi: 10.1371/journal.pntd.0004677

Alkhaibari, A. M., Carolino, A. T., Bull, J. C., Samuels, R. I., and Butt, T. M. (2017). Differential pathogenicity of Metarhizium blastospores and conidia against larvae of three mosquito species. *J. Med. Entomol.* 54, 696–704. doi: 10.1093/jme/tjw223

Allahverdipour, H. H., Talaei-Hassanloui, R., Karimi, J., Wang, Y., Rochlin, I., and Gaugler, R. (2019). Behavior manipulation of mosquitoes by a mermithid nematode. *J. Invertebr. Pathol.* 168:107273. doi: 10.1016/j.jip.2019.107273

Alphey, L., and Andreasen, M. (2002). Dominant lethality and insect population control. *Mol. Biochem. Parasitol.* 121, 173–178. doi: 10.1016/S0166-6851(02)00040-3

Amalraj, D. D., and Das, P. K. (1998). Estimation of predation by the larvae of Toxorhynchites splendens on the aquatic stages of Aedes aegypti. *Southeast Asian J. Trop. Med. Public Health* 29, 177–183.

Amelia-Yap, Z. H., Azman, A. S., AbuBakar, S., and Low, V. L. (2022). Streptomyces derivatives as an insecticide: current perspectives, challenges and future research needs for mosquito control. *Acta Trop.* 229:106381. doi: 10.1016/j.actatropica.2022.106381

Anderson, J. F. (1968). Microsporidia parasitizing mosquitoes collected in Connecticut. *J. Invertebr. Pathol.* 11, 440–455. doi: 10.1016/0022-2011(68)90192-4

Anderson, J. F. (1970). An iridescent virus infecting the mosquito *Aedes stimulans*. *J. Invertebr. Pathol.* 15, 219–224. doi: 10.1016/0022-2011(70)90238-7

Anderson, J. F., and Ringo, S. L. (1969). *Entomophthora aquatica* sp. n. Infecting larvae and pupae of floodwater mosquitoes. *J. Invertebr. Pathol.* 13, 386–393. doi: 10.1016/0022-2011(69)90191-8

Anderson, B. E., Sims, K. G., Olson, J. G., Childs, J. E., Piesman, J. F., Happ, C. M., et al. (1993). *Amblyomma americanum*: a potential vector of human ehrlichiosis. *Am. J. Trop. Med. Hyg.* 49, 239–244. doi: 10.4269/ajtmh.1993.49.239

Andrade, M. R. (2015). *Container-dwelling mosquitoes: habitat size, direct and indirect effects of predation*. Minas Gerais, Brazil: Universidade Federal de Viçosa.

Ang, J. X. D., Kadir, K. A., Mohamad, D. S. A., Matusop, A., Divis, P. C. S., Yaman, K., et al. (2020). New vectors in northern Sarawak, Malaysian Borneo, for the zoonotic malaria parasite, *Plasmodium knowlesi*. *Parasit. Vectors* 13:472. doi: 10.1186/s13071-020-04345-2

Annis, B., Krisnowardojo, S., Atmosoedjono, S., and Supardi, P. (1989). Suppression of larval Aedes aegypti populations in household water storage containers in Jakarta, Indonesia through releases of first-instar Toxorhynchites splendens larvae. *J. Am. Mosq. Control Assoc.* 5, 235–238.

Annis, B., Nalim, S., Hadisuwasono, W., and Boewono, D. T. (1990). Toxorhynchites amboinensis larvae released in domestic containers fail to control dengue vectos in a rural village in Central Java. *J. Am. Mosq. Control Assoc.* 6, 75–78.

Armengol, G., Hernandez, J., Velez, J. G., and Orduz, S. (2006). Long-lasting effects of a bacillus thuringiensis serovar israelensis experimental tablet formulation for Aedes aegypti (Diptera: Culicidae) control. *J. Econ. Entomol.* 99, 1590–1595. doi: 10.1603/0022-0493-99.5.1590

Arredondo-Jimenez, J. I., Lopez, T., Rodriguez, M. H., and Bown, D. N. (1990). Small scale field trials of Bacillus sphaericus (strain 2362) against anopheline and culicine mosquito larvae in southern Mexico. *J. Am. Mosq. Control Assoc.* 6, 300–305. PMID: 2370538

Arthington, A. H. (1991). Ecological and genetic impacts of introduced and translocated freshwater fishes in Australia. *Can. J. Fish. Aquat. Sci.* 48, 33–43. doi: 10.1139/f91-302

Asale, A., Duchateau, L., Devleesschauwer, B., Huisman, G., and Yewhalaw, D. (2017). Zooprophylaxis as a control strategy for malaria caused by the vector anopheles arabiensis (Diptera: Culicidae): a systematic review. *Infect. Dis. Poverty* 6:160. doi: 10.1186/s40249-017-0366-3

Asgharian, H., Chang, P. L., Mazzoglio, P. J., and Negri, I. (2014). Wolbachia is not all about sex: male-feminizing Wolbachia alters the leafhopper *Zyginidia pullula* transcriptome in a mainly sex-independent manner. *Front. Microbiol.* 5:430. doi: 10.3389/fmicb.2014.00430

Ault, S. K. (1994). Environmental management: a re-emerging vector control strategy. *Am. J. Trop. Med. Hyg.* 50, 35–49. doi: 10.4269/ajtmh.1994.50.35

Bacot, A. W. (1915). LXXXI. Further notes on the mechanism of the transmission of plague by fleas. *J. Hyg.* 14, 774–776.3.

Bacot, A. W., and Martin, C. J. (1914). LXVII. Observations on the mechanism of the transmission of plague by fleas. *J. Hyg.* 13, 423–439.

Badakhshan, M., Sadraei, J., and Moin-Vaziri, V. (2013). The first report of Eustigmaeus johnstoni (Acari: Stigmaeidae) parasitic mite of Phlebotominae sand flies from Iran. *J. Arthropod. Borne Dis.* 7, 94–98. PMID: 23785700

Bakken, J. S., and Dumler, J. S. (2015). Human granulocytic anaplasmosis. *Infect. Dis. Clin. N. Am.* 29, 341–355. doi: 10.1016/j.idc.2015.02.2007

Balfour, A. (1914). The wild monkey as a reservoir for the virus of yellow fever. *Lancet* 183, 1176–1178. doi: 10.1016/S0140-6736(01)56899-3

Barker, S. C., and Walker, A. R. (2014). Ticks of Australia: the species that infect domestic animals and humans. *Zootaxa* 3816, 1–144. doi: 10.11646/zootaxa.3816.1.1

Barrett, W. L. J. (1968). The damage caused by *Lankesteria culicis* (Ross) to *Aedes aegypti* (L.). *Mosq. News* 28, 441–444.

Barrett, A. D. T., and Higgs, S. (2007). Yellow fever: a disease that has yet to be conquered. *Annu. Rev. Entomol.* 52, 209–229. doi: 10.1146/annurev.ento.52.110405.091454

Bartumeus, F., Costa, G. B., Eritja, R., Kelly, A. H., Finda, M., Lezaun, J., et al. (2019). Sustainable innovation in vector control requires strong partnerships with communities. *PLoS Negl. Trop. Dis.* 13:e0007204. doi: 10.1371/journal.pntd.0007204

Baumhover, A. H., Graham, A. J., Bitter, B. A., Hopkins, D. E., New, W. D., Dudley, F. H., et al. (1955). Screw-worm control through release of sterilized flies. *J. Econ. Entomol.* 48, 462–466. doi: 10.1093/JEE/48.4.462

Baxter, R. H. G. (2016). Chemosterilants for control of insects and insect vectors of disease. *Chimia (Arau)* 70, 715–720. doi: 10.2533/chimia.2016.715

Bay, E. C. (1974). Predatory-prey relationships among aquatic insects. *Annu. Rev. Entomol.* 19, 441–453. doi: 10.1146/annurev.en.19.010174.002301

Ben-Dov, E., Boussiba, S., and Zaritsky, A. (1995). Mosquito larvicidal activity of *Escherichia coli* with combinations of genes from bacillus thuringiensis subsp. Israelensis. *J. Bacteriol.* 177, 2851–2857. doi: 10.1128/jb.177.10.2851-2857.1995

Benelli, G., Jeffries, C. L., and Walker, T. (2016). Biological control of mosquito vectors: past, present, and future. *Insects* 7:52. doi: 10.3390/insects7040052

Bezuidenhout, J. D., and Stutterheim, C. J. (1980). A critical evaluation of the role played by the red-billed oxpecker *Buphagus erythrorhynchus* in the biological control of ticks. *Onderstepoort J. Vet. Res.* 47, 51–75. PMID: 7413164

Bhatt, S., Weiss, D. J., Cameron, E., Bisanzio, D., Mappin, B., Darymple, U., et al. (2015). The effect of malaria control on plasmodium falciparum in Africa between 2000 and 2015. *Nature* 526, 207–211. doi: 10.1038/nature15535

Bian, G., Joshi, D., Dong, Y., Lu, P., Zhou, G., Pan, X., et al. (2013). Wolbachia invades *Anopheles stephensi* populations and induces refractoriness to plasmodium infection. *Science* 340, 748–751. doi: 10.1126/science.1236192

Bian, G., Xu, Y., Lu, P., Xie, Y., and Xi, Z. (2010). The endosymbiotic bacterium Wolbachia induces resistance to dengue virus in Aedes aegypti. *PLoS Pathog.* 6:e1000833. doi: 10.1371/journal.ppat.1000 833

Biernat, B., Karbowiak, G., Werszko, J., and Stańczak, J. (2014). Prevalence of tick-borne encephalitis virus (TBEV) RNA in Dermacentor reticulatus ticks from natura and urban environment, Poland. *Exp. Appl. Acarol.* 64, 543–551. doi: 10.1007/s10493-014-9836-5

Blanford, S. (2005). Fungal pathogen reduces potential for malaria transmission. *Science* 308, 1638–1641. doi: 10.1126/science.1108423

Blanford, S., Shi, W., Christian, R., Marden, J. H., Koekemoer, L. L., Brooke, B. D., et al. (2011). Lethal and pre-lethal effects of a fungal biopesticide contribute to substantial and rapid control of malaria vectors. *PLoS One* 6:e23591. doi: 10.1371/journal.pone.0023591

Bøgh, C., Clarke, S. E., Pinder, M., Sanyang, F., and Lindsay, S. W. (2001). Effect of passive zooprophylaxis on malaria transmission in the Gambia. *J. Med. Entomol.* 38, 822–828. doi: 10.1603/0022-2585-38.6.822

Bonnet, S., Brisseau, N., Hermouet, A., Jouglin, M., and Chauvin, A. (2009). Experimental in vitro transmission of *Babesia* sp. (EU1) by Ixodes ricinus. *Vet. Res.* 40:21. doi: 10.1051/vetres/2009004

Bonsall, J. L., and Goose, J. (1986). The safety evaluation of bendiocab, a residual insecticide for vector control. *Toxicol. Lett.* 33, 45–59. doi: 10.1016/0378-4274(86)90070-6

Bordbar, A., Soleimani, S., Fardid, F., Zolfaghari, M. R., and Parvizi, P. (2014). Three strains of *Wolbachia pipientis* and high rates of injection in Iranian sandfly species. *Bull. Entomol. Res.* 104, 195–202. doi: 10.1017/S0007485313000631

Bos, R. (1991). Cost-effectiveness of environmental management for vector control in resource development projects. *Ann. Soc. Belg. Med. Trop.* 71, 243–255.

Bouma, M., and Rowland, M. (1995). Failure of passive zooprophylaxis: cattle ownership in Pakistan is associated with a higher prevalence of malaria. *Trans. R. Soc. Trop. Med. Hyg.* 89, 351–353. doi: 10.1016/0035-9203(95)90004-7

Boussinesq, M. (2006). Loiasis. *Ann. Trop. Med. Parasitol.* 100, 715–731. doi: 10.1179/136485906X 112194

Bowatte, G., Perera, P., Senevirathne, G., Meegaskumbura, S., and Meegaskumbura, M. (2013). Tadpoles as dengue mosquito (*Aedes aegypti*) egg predators. *Biol. Control* 67, 469–474. doi: 10.1016/j.biocontrol.2013.10.005

Bracken, G. K., and Dondale, C. D. (1972). Fertility and survival of *Achaeranea tepidariorum* (Araneida: Theridiidae) on a diet of chemosterilized mosquitoes. *Can. Entomol.* 104, 1709–1712. doi: 10.4039/ent 104709-11

Brelsfoard, C. L., and Dobson, S. L. (2009). Wolbachia-based strategies to control insect pests and disease vectors. *Asia-Pac. J. Mol. Biol. Biotechnol.* 17, 55–63.

Brown, M. D. (1996). Evaluation of Mesocyclops aspericornis (Cyclopoida: Cyclopidae) and *Toxorhynchite speciosus* as integrated predators of mosquitoes in tire habitats in Queensland. *J. Am. Mosq. Control Assoc.* 12, 414–420. PMID: 8887220

Bruce, D. (1895). *Preliminary report on the tsetse fly disease or nagana in Zululand*. Durban: Bennett & Davis.

Bruce, D. (1915). The Croonian lectures on trypanosomes causing disease in man and domestic animals in Central Africa: delivered before the Royal College of Physicians of London. *BMJ* 2, 5–10. doi: 10.1136/bmj.2.284.5

Buchman, A., Gamez, S., Li, M., Antoshechkin, I., Li, H.-H., Wang, H.-W., et al. (2020). Broad dengue neutralization in mosquitoes expressing an engineered antibody. *PLoS Pathog.* 16:e1008103. doi: 10.1371/journal.ppat.1008103

Buczek, A., Buczek, W., Bartosik, K., Kulisz, J., and Stanko, M. (2021). Ixodiphagus hookeri wasps (hymenoptera: Encyrtidae) in two sympatric tick species Ixodes ricinus and Haemaphysalis concinna (Ixodida: Ixodidae) in the Sloavak karst (Slovakia): ecological and biological considerations. *Sci. Rep.* 11:11310. doi: 10.1038/s41598-021-90871-7

Burroughs, A. L. (1947). Sylvatic plague studies: the vector efficiency of nine species of fleas compared with *Xenopsylla cheopis*. *J. Hyg.* 45, 371–396. doi: 10.1017/s0022172400014042

Busvine, J. R. (1993). *Disease transmission by insects: Its discovery and 90 years of effort to prevent it*. New York/Berlin: Springer.

Calkins, C. O., and Parker, A. G. (2005). "Sterile insect quality" in *Sterile insect technique: Principles and practice in area-wide integrated pest management*. eds. V. A. Dyck, J. Hendrichs and A. S. Robinson (The Netherlands: Springer)

Canyon, D. V., and Hii, J. L. (1997). The gecko: an environmentally friendly biological agent for mosquito control. *Med. Vet. Entomol.* 11, 319–323. doi: 10.1111/j.1365-2915.1997.tb00416.x

Carvalho, D. O., McKemey, A. R., Garziera, L., Lacroix, R., Donnelly, C. A., Alphey, L., et al. (2015). Suppression of a field population of Aedes aegypti in Brazil by sustained release of transgenic male mosquitoes. *PLoS Negl. Trop. Dis.* 9:e0003864. doi: 10.1371/journal.pntd.0003864

Casas, M., Torres, J. L., Bown, D. N., Rodriguez, M. H., and Arredondo-Jimenez, J. I. (1998). Selective and conventional house-spraying of DDT and bendiocarb against Anopheles pseudopunctipennis in southern Mexico. *J. Am. Mosq. Control Assoc.* 14, 410–420. PMID: 10084135

Casida, J. E., and Quistad, G. B. (1998). Golden age of insecticide research: past, present, or future? *Annu. Rev. Entomol.* 43, 1–16. doi: 10.1146/annurev.ento.43.1.1

Castello, J. A., and Gil Rivas, M. J. (1980). Proposal for a predator for the destruction of Triatoma infestans: Tarentola mauritanica. *Medicina (B Aires)* 40, 673–677. PMID: 22167700

Chagas, C. (1909). Nova tripanozomiaze humana: estudos sobre a morfolojia e o ciclo evolutivo do Schizotrypanum cruzi n. gen., n. sp., ajente etiolojico de nova entidade morbida do homem. *Mem. Inst. Oswaldo Cruz* 1, 159–218. doi: 10.1590/s0074-0276190900020008

Chan, Y. C., Chan, K. L., and Ho, B. C. (1971). *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore city: distribution and density. *Bull. World Health Organ.* 44, 617–627. PMID: 5316745

Chandra, G., Bhattacharjee, I., Chatterjee, S. N., and Ghosh, A. (2008a). Mosquito control by larvivorous fish. *Indian J. Med. Res.* 127, 13–27. PMID: 18316849

Chandra, G., Mandal, S. K., Ghosh, A. K., Das, D., Banerjee, S. S., and Chakraborty, S. (2008b). Biocontrol of larval mosquitoes by *Acilius sulcatus* (Coleoptera: Dytiscidae). *BMC Infect. Dis.* 8:138. doi: 10.1186/1471-2334-8-138

Chapman, H. C. (1974). Biological control of mosquito larvae. *Annu. Rev. Entomol.* 19, 33–59. doi: 10.1146/annurev.en.19.010174.000341

Chapman, H. C., Woodard, D. B., and Petersen, J. J. (1967). Pathogens and parasites of Louisiana Culicidae and Chaoboridae. *Proc. Annu. Meet. N. J. Mosq. Exterm. Assoc.* 54, 54–60.

Charlwood, J. D., Dagoro, H., and Paru, R. (1985). Blood-feeding and resting behaviour in the Anopheles punctulatus Donitz complex (Diptera: Culicidae) from coastal Papua New Guinea. *Bull. Entomol. Res.* 75, 463–476. doi: 10.1017/S0007485300014577

Chatterjee, S. N., Ghosh, A., and Chandra, G. (2007). Eco-friendly control of mosquito larvae by Brachytron pratense nymph. *J. Environ. Health* 69, 44–48. PMID: 17450955

Chaumeau, V., Kajeechiwa, L., Kulabkeeree, T., Sawasdichai, S., Haohankhunnatham, W., Inta, A., et al. (2022). Outdoor residual spraying for malaria vector-control in Myanmar: a cluster randomized controlled trial. *PLoS One* 17:e0274320. doi: 10.1371/journal.pone.0274320

Chen, S.-M., Dumler, J. S., Bakken, J. S., and Walker, D. H. (1994). Identification of a granulocytotropic Ehrlichia species as the etiologic agent of human diseases. *J. Clin. Microbiol.* 32, 589–595. doi: 10.1128/jcm.32.3.589-595.1994

Cheong, W. H., Mak, J. W., Naidu, S., and Mahadevan, S. (1981). Armigeres subalbatus incriminated as an important vector of the dog heartworm *Dirofilaria immitis* and the bird Cardiofilaria in urban Kuala Lumpur. *Southeast Asian J. Trop. Med. Public Health* 12, 611–612. PMID: 6124044

Chevalier, V., Mondet, B., Diaite, A., Lancelot, R., Fall, A., and Ponçon, N. (2004). Exposure of sheep to mosquito bites: possible consequences for the transmission risk of Rift Valley fever in Senegal. *Med. Vet. Entomol.* 18, 247–255. doi: 10.1111/j.0269-283X.2004.00511.x

Chiang, G. L., Cheong, W. H., Samarawickrema, W. A., Mak, J. W., and Kan, S. K. (1984). Filariasis in Bengkoka peninsula, Sabah, Malaysia: vector studies in relation to the transmission of filariasis. *Southeast Asian J. Trop. Med. Public Health* 15, 179–189. PMID: 6150551

Chomel, B. B. (2009). "Zoonoses" in *Encyclopedia of microbiology*. ed. M. Schaechter. *3rd* ed (Davis, CA: University of California, Elsevier Inc.), 820–829.

Chouin-Carneiro, T., Ant, T. H., Herd, C., Louis, F., Failloux, A. B., and Sinkins, S. P. (2019). Wolbachia strain wAlbA blocks Zika virus transmission in *Aedes aegypti*. *Med. Vet. Entomol.* 34, 116–119. doi: 10.1111/mve.12384

Clark, T. B., Kellen, W. R., Fukuda, T., and Lindegren, J. E. (1968). Field and laboratory studies on the pathogenicity of the fungus *Beauveria bassiana* to three genera of mosquitoes. *J. Invertebr. Pathol.* 11, 1–7. doi: 10.1016/0022-2011(68)90047-5

Clark, T. B., Kellen, W. R., Lindergren, J. E., and Sanders, R. D. (1966). Phytium sp. pathogenic to mosquito larvae. *J. Invertebr. Pathol.* 8, 351–354. doi: 10.1016/0022-2011(66)90049-8

Collins, L. E., and Blackwell, A. (2000). The biology of Toxorhynchites mosquitoes and their potential as biocontrol agents. *Biocontrol News Information* 21, 105–116.

Connal, A. (1921). Observations on filaria in Chrysops from West Africa. *Trans. R. Soc. Trop. Med. Hyg.* 14, 108–109. doi: 10.1016/S0035-9203(21)90176-7

Connor, M. E. (1922). Notes on the use of fresh water fish as consumers of mosquito larvae in containers used in the home. *Am. J. Public Health (NY)* 12, 193–194. doi: 10.2105/ajph.12.3.193

Cordaux, R., Michel-Salzat, A., and Bouchon, D. (2001). Wolbachia infection in crustaceans: novel hosts and potential routes for horizontal transmission. *J. Evol. Biol.* 14, 237–243. doi: 10.1046/j.1420-9101.2001.00279.x

Corliss, J. O. (1954). The literature on Tetrehymena: its history, growth, and recent trends. *J. Protozool.* 1, 156–169. doi: 10.1111/j.1550-7408.1954.tb00809.x

Corliss, J. O. (1960). Tetrahymena chironomini sp. nov., a ciliate from midge larvae and the current status of facultative parasitism in the genus Tetrahymena. *Parasitology* 50, 111–153. doi: 10.1017/s00311 82000025245

Couper, L. I., Yang, Y. Y., Yang, F. X. F., and Swei, A. (2020). Comparative vector competence of north American Lyme disease vectors. *Parasit. Vectors* 13:29. doi: 10.1186/s13071-020-3893-x

Crainey, J. L., Wilson, M. D., and Post, R. J. (2010). Phylogenetically distinct Wolbachia gene and pseudogene sequences obtained from the African onchocerciasis vector Simulium squamosum. *Int. J. Parasitol.* 40, 569–578. doi: 10.1016/j.ijpara.2009.10.017

Cunha, M. S., Tubaki, R. M., de Menezes, R. M. T., Pereira, M., Caleiro, G. S., Coelho, E., et al. (2020). Possible non-sylvatic transmission of yellow fever between non-human primates in São Paulo city, Brazil, 2017-2018. *Sci. Rep.* 10:15751. doi: 10.1038/s41598-020-72794-x

Curtis, C. F. (1976). "Testing systems for the genetic control of mosquitoes," in: *XV International Congress of Entomology*. 106–116.

Curtis, C. F., Brookes, G. D., Grover, K. K., Krishnamurthy, B. S., Laven, H., Rajagopalan, L., et al. (1982). A field trial on genetic control of Culex p. fatigans by release of integrated strain IS-31B. *Entomol. Exp. Appl.* 31, 181–190. doi: 10.1111/j.1570-7458.1982.tb03133.x

da Rocha, J. A., de Oliveira, S. B., Povoa, M. M., Moreira, L. A., and Krettli, A. U. (2008). Malaria vectors in areas of plasmodium falciparum epidemic transmission in the Amazon region, Brazil. *Am. J. Trop. Med. Hyg.* 78, 872–877. doi: 10.4269/ajtmh.2008.78.872

Dame, D. A., Woodward, D. B., Ford, H. R., and Weidhaas, D. E. (1964). Field behavior of sexually sterile *Anopheles quadrimaculatus* males. *Mosq. News* 24, 6–16.

Das, B. P., Sharma, S. N. L. K., Lal, S., and Saxena, V. K. (2005). First time detection of Japanese encephalitis virus antigen in dry and unpreserved mosquito Culex tritaeniorhynchus Giles, 1901, from Karnal district of Hayana state of India. *J. Commun. Dis.* 37, 131–133. PMID: 16749277

Davidson, G., Odetoyinbo, J. A., Colussa, B., and Coz, J. (1970). A field attempt to assess the mating competitiveness of sterile males produced by crossing two member species of the Anopheles gambiae complex. *Bull. World Health Organ.* 42, 55–67. PMID: 5309520

Davies, F. (1975). Observations on the epidemiology of Rift Valley fever in Kenya. *J. Hyg. (Lond.)* 75, 219–230. doi: 10.1017/s0022172400047252

Davies, T. G. E., Field, L. M., Usherwood, P. N. R., and Williamson, M. S. (2007). DDT, pyrethrins, pyrethroids and insect sodium channels. *IUBMB Life* 59, 151–162. doi: 10.1080/15216540701352042

Davies, F. G., and Highton, R. B. (1980). Possible vectors of Rift Valley fever in Kenya. *Trans. R. Soc. Trop. Med. Hyg.* 74, 815–816. doi: 10.1016/0035-9203(80)90213-8

Davis, E. L., Prada, J., Reimer, L. J., and Hollingsworth, T. D. (2021). Modelling the impact of vector control on lymphatic filariasis programs: current approaches and limitations. *Clin. Infect. Dis.* 72, S152–S157. doi: 10.1093/cid/ciab191

de Valdez, M. R. W., Nimmo, D., Betz, J., Gong, H., James, A. A., Alphey, L., et al. (2011). Genetic elimination of dengue vector mosquitoes. *Proc. Natl. Acad. Sci. U. S. A.* 108, 4772–4775. doi: 10.1073/pnas.1019295108

de Wispelaere, M., Despres, P., and Choumet, V. (2017). European *Aedes albopictus* and *Culex pipiens* are competent vectors for Japanese encephalitis virus. *PLoS Negl. Trop. Dis.* 11:e0005294. doi: 10.1371/journal.pntd.0005294

Demers, J., Bewick, S., Calabrese, J., and Fagan, W. F. (2018). Dynamic modelling of personal protection control strategies for vector-borne disease limits the role of diversity amplification. *J. R. Soc. Interface* 15:20180166. doi: 10.1098/rsif.2018.0166

Deredec, A., Godfray, H. C. J., and Burt, A. (2011). Requirements for effective malaria control with homing endonuclease genes. *Proc. Natl. Acad. Sci. U. S. A.* 108, E874–E880. doi: 10.1073/pnas.1110717108

Dias, J. C. (2007). Southern cone initiative for the elimination of domestic populations of *Triatoma infestans* and the interruption of transfusional Chagas disease: historical aspects, present situation, and perspectives. *Mem. Inst. Oswaldo Cruz* 102, 11–18. doi: 10.1590/S0074-02762007005000092

Dick, G. W. A. (1952). Zika virus. II. Pathogenicity and physical properties. *Trans. R. Soc. Trop. Med. Hyg.* 46, 521–534. doi: 10.1016/0035-9203(52)900436

Dinesh, D. S., Kumar, V., Kesari, S., and Das, P. (2014). Mites & spiders act as biological control agent to sand flies. *Asian Pac. J. Trop. Dis.* 4, S463–S466. doi: 10.1016/S2222-1808(14)60491-6

Dodson, B. L., Hughes, G. L., Paul, O., Matacchiero, A. C., Kramer, L. D., and Rasgon, J. L. (2014). Wolbachia enhances West Nile virus (WNV) infection in the mosquito *Culex tarsalis*. *PLoS Negl. Trop. Dis.* 8:e2965. doi: 10.1371/journal.pntd.0002965

Doherty, R. L., Carley, J. G., Mackerras, M. J., and Marks, E. N. (1963). Studies of arthropod-borne virus infections in Queensland. III. Isolation and characterization of virus strains from wild-caught mosquitoes in North Queensland. *Aus. J. Exp. Biol. Med. Sci.* 41, 17–39. doi: 10.1038/icb.1963.2

Dolan, G., ter Kuile, F. O., Jacoutot, V., White, N. J., Luxemburger, C., Malankirii, L., et al. (1993). Bed nets for the prevention of malaria and anaemia in pregnancy. *Trans. R. Soc. Trop. Med. Hyg.* 87, 620–626. doi: 10.1016/0035-9203(93)90262-o

Domrow, R., and Derrick, E. H. (1964). Ixodes holocyclus, the man-biting tick in S.E. Queensland. *Aus. J. Sci.* 27, 234–236.

Donald, C. L., Siriyasatien, P., and Kohl, A. (2020). Toxorhynchites species: a review of current knowledge. *Insects* 11:747. doi: 10.3390/insects11110747

Dong, Y., Simões, M. L., Marois, E., and Dimopoulos, G. (2018). CRISPR/Cas9-mediated gene knockout of Anopheles gambiae FREP1 suppresses malaria parasite infection. *PLoS Pathog.* 14:e1006898. doi: 10.1371/journal.ppat.1006898

Donnelly, J., and Peirce, M. A. (1975). Experiments on the transmission of *Babesia divergens* to cattle by the tick *Ixodes ricinus*. *Int. J. Parasitol.* 5, 363–367. doi: 10.1016/0020-7519(75)90085-5

Dorta, D. M., Vasuki, V., and Rajavel, A. (1993). Evaluation of organophosphorus and synthetic pyrethroid insecticides against six vector mosquito species. *Rev. Saúde Públ.* 27, 391–397. doi: 10.1590/s0034-89101993000600001

Durden, L. A. (2019). "Lice (Phthiraptera)" in *Medical and veterinary entomology*. eds. G. R. Mullen and L. A. Durden (GA, United States: Academic Press), 79–106.

Dutra, H. L. C., Rocha, M. N., Dias, F. B. S., Mansur, S. B., Caragata, E. P., and Moreira, L. A. (2016). Wolbachia blocks currently circulating Zika virus isolates in Brazillian *Aedes aegypti* mosquitoes. *Cell Host Microbe* 19, 771–774. doi: 10.1016/j.chom.2016.04.021

Economidis, P. S. (1995). Endangered freshwater fishes of Greece. *Biol. Conserv.* 72, 201–211. doi: 10.1016/0006-3207(94)00083-3

Economidis, P. S., Dimitriou, E., Pagoni, R., Michaloudi, E., and Natsis, L. (2000). Introduced and translocated fish species in the inland waters of Greece. *Fish. Manag. Ecol.* 7, 239–250. doi: 10.1046/j.1365-2400.2000.00197.x

Edeson, J. F. B., and Wilson, T. (1964). The epidemiology of filariasis due to *Wuchereria bancrofti* and *Brugia malayi*. *Annu. Rev. Entomol.* 9, 245–268. doi: 10.1146/annurev.en.09.010164.001333

Emmons, R. W. (1988). "Reoviridae: the Orbiviruses (Colorado tick fever)" in *Laboratory diagnosis of infectious diseases principle and practice*. eds. K. Araki, A. Galloway and K. Taguchi (New York: Springer New York), 375–383.

Fairhurst, R. M., and Dondorp, A. M. (2016). Artemisinin-resistant Plasmodium falciparum malaria. *Microbiol. Spectr.* 4, 409–429. doi: 10.1128/microbiolspec.EI10-0013-2016

Fatih, F. A., Staines, H. M., Siner, A., Ahmed, M. A., Woon, L. C., Pasini, E. M., et al. (2013). Susceptibility of human plasmodium knowlesi infections to anti-malarials. *Malar. J.* 12:425. doi: 10.1186/1475-2875-12-425

Fayolle, S., Bertrand, C., Logez, M., and Franquet, É. (2015). Does mosquito control by Bti spraying affect the phytoplankton community? A 5-year study in Camargue temporary wetlands (France). *Ann. Limnol. – Int. J. Limnol.* 51, 189–198. doi: 10.1051/limn/2015013

Fenner, F. (1946). The epidemiology of North Queensland tick typhus: natural mammalian hosts. *Med. J. Aus.* 2, 666–668. doi: 10.5694/j.1326-5377.1946.tb34592.x

Ferroni, E., Jefferson, T., and Gachelin, G. (2012). Angello celli and research on the prevention of malaria in Italy a century ago. *J. R. Soc. Med.* 105, 35–40. doi: 10.1258/jrsm.2011.11k049

Fischer, P., Bamuhiiga, J., and Büttner, D. W. (1997). Occurrence and diagnosis of *Mansonella streptocerca* in Uganda. *Acta Trop.* 63, 43–55. doi: 10.1016/s0001-706x(96)00607-9

Fischhoff, I. R., Burtis, J. C., Keesing, F., and Ostfeld, R. S. (2018). Tritophic interactions between a fungal pathogen, a spider predator, and the blacklegged tick. *Ecol. Evol.* 8, 7824–7834. doi: 10.1002/ece3.4271

Fletcher, M., Teklehaimanot, A., and Yemane, G. (1992). Control of mosquito larvae in the port city of Assam by an indigenous larvivorous fish, *Aphanius dispar*. *Acta Trop.* 52, 155–166. doi: 10.1016/00 01-706x(92)90032-s

Floridis, J., McGuinness, S. L., Kurucz, N., Burrow, J. N., Baird, R., and Francis, J. R. (2018). Murray Valley encephalitis virus: an ongoing cause of encephalitis in Australia's north. *Trop. Med. Infect. Dis.* 3:49. doi: 10.3390/tropicalmed3020049

Florio, L., and Miller, M. S. (1948). Epidemiology of Colorado tick fever. *Am. J. Public Health Nations Health* 38, 211–213. doi: 10.2105/ajph.38.2.211

Florio, L., Miller, M. S., and Mugrage, E. R. (1950). Colorado tick fever: isolation of the virus from *Dermacentor andersoni* in nature and laboratory study of the transmission of the virus in the tick. *J. Immunol.* 64, 257–263. doi: 10.4049/jimmunol.64.4.257

Florio, L., Stewart, M. O., and Mugrage, E. R. (1944). The experimental transmission of Colorado tick fever. *J. Exp. Med.* 80, 165–188. doi: 10.1084/jem.80.3.165

Focks, D. A., Sackett, S. R., Dame, D. A., and Bailey, D. L. (1983). Toxorhynchites rutilus rutilus (Diptera: Culicidae): field studies on dispersal and oviposition in the context of the biocontrol of urban container-breeding mosquitoes. *J. Med. Entomol.* 20, 383–390. doi: 10.1093/jmedent/20.4.383

Focks, D. A., Sackett, S. R., Dame, D. A., and Bailey, D. L. (1985). Effect of weekly releases of *Toxorhynchites amboinensis* (Doleschall) on *Aedes aegypti* (L.) (Diptera: Culicidae) in New Orleans, Louisiana. *J. Econ. Entomol.* 78, 622–626. doi: 10.1093/jee/78.3.622

Franz, G. (2002). Recombination between homologous autosomes in medfly (*Ceratitis capitata*) males: type-1 recombination and the implications for the stability of genetic sexing strains. *Genetica* 116, 73–84. doi: 10.1023/a:1020911725724

Franz, G. (2005). "Genetic sexing strains in Mediterranean fruit fly, an example for other species amenable to large-scale rearing for the sterile insect technique" in *Sterile insect technique: Principles and practice in area-wide integrated pest management*. eds. V. A. Dyck, J. Hendrichs and A. S. Robinson (The Netherlands: Springer)

Fu, G., Lees, R. S., Nimmo, D., Aw, D., Jin, L., Gray, P., et al. (2010). Female-specific flightless phenotype for mosquito control. *Proc. Natl. Acad. Sci. U. S. A.* 107, 4550–4554. doi: 10.1073/pnas.10 00251107

Gabiane, G., Yen, P., and Failoux, A. (2022). Aedes mosquitoes in the emerging threat of urban yellow fever transmission. *Rev. Med. Virol.* 32:e2333. doi: 10.1002/rmv.2333

Ganguly, S., and Mukhopadhayay, S. K. (2008). Tick-borne ehrlichiosis infection in human beings. *J. Vector Borne Dis.* 45, 273–280.

Gerberich, J. B., and Laird, M. (1985). "Larvivorous fish in the biocontrol of mosquitoes, with a selected bibliography of recent literature" in *Integrated mosquito control methodologies*. eds. M. Laird and J. W. Miles, vol. *2*. Biocontrol and other innovative components and future directions, (London: Academic Press), 47–76.

Ghebreyesus, T. A., Haile, M., Witten, K. H., Getachew, A., Yohannes, M., Lindsay, S. W., et al. (2000). Household risk factors for malaria among children in the Ethiopian highlands. *Trans. R. Soc. Trop. Med. Hyg.* 94, 17–21. doi: 10.1016/s0035-9203(00)90424-3

Gillette, B. (1988). Controlling mosquitoes biologically. *Bioscience* 38, 80–83. doi: 10.2307/1310 610

Ginsberg, H. S., Lebrun, R. A., Heyer, K., and Zhioua, E. (2002). Potential nontarget effects of Metarhizium anisopliae (Deuteromycetes) used for biological control of ticks (Acari: Ixodidae). *Environ. Entomol.* 31, 1191–1196. doi: 10.1603/0046-225X-31.6.1191

Glaser, L. C. (1999) in *"organophosphates and carbamate pesticides," in Field manual of wildlife diseases: general field procedures and diseases of birds*. eds. M. Friend and J. C. Franson (Reston, VA: US Geological Survey), 287–294.

Goodman, A. L., Lockhart, S. R., Lysen, C. B., Westblade, L. F., Burnham, C. A., and Burd, E. M. (2018). Two cases of fungal keratitis caused by *Metarhizium anisopliae*. *Med. Mycol. Case Rep.* 21, 8–11. doi: 10.1016/j.mmcr.2018.03.003

Grace, D., Mutua, F., Ochungo, P., Kruska, R., Jones, K., Brierley, L., et al. (2012). "Mapping of poverty and likely zoonoses hotspots" in *Zoonoses project 4. Report to Department for International Development* (Nairobi, Kenya: ILRI).

Graham, D. H. (1939). Mosquito life in the Auckland district. Report of Auckland mosquito research committee on an investigation made by David H. Graham. *Trans. R. Soc. N. Z* 69, 210–244.

Gray, J. S., von Stedingk, L.-V., Gurtelschmid, M., and Granstrom, M. (2002). Transmission studies on Babesia microti in Ixodes ricinus ticks and gerbils. *J. Clin. Microbiol.* 40, 1259–1263. doi: 10.1128/JCM.40.4.1259-1263.2002

Griffin, L. F., and Knight, J. M. (2012). A review of the role of fish as biological control agents of disease vector mosquitoes in mangrove forests: reducing human health risks while reducing environmental risk. *Wetl. Ecol. Manag.* 20, 243–252. doi: 10.1007/s11273-012-9248-4

Grover, K. K., Suguna, S. G., Uppal, D. K., Singh, K. R. P., Ansari, M. A., Curtis, C. F., et al. (1976). Field experiments on the competitiveness of males of three genetic control systems of Aedes aegypti. *Entomol. Exp. Appl.* 20, 8–18. doi: 10.1111/j.1570-7458.1976.tb02611.x

Gu, J.-B., Dong, Y.-Q., Peng, H.-J., and Chen, X.-G. (2010). A recombinant AeDNA containing the insect-specific toxin, BmK IT1, displayed an increasing pathogenicity on Aedes albopictus. *Am. J. Trop. Med. Hyg.* 83, 614–623. doi: 10.4269/ajtmh.2010.10-0074

Gubler, D. J. (1988). "Dengue" in *Epidemiology of arthropod-borne viral diseases*. ed. T. P. Monath (Boca Raton: CRC Press Inc.), 223–260.

Gubler, D. J., Kuno, G., and Markoff, L. (2007). "Flaviviruses" in *Fields Virology*. eds. D. M. Knipe and P. M. Howley. *5th* ed (Philadelphia: Lippincott, Williams and Wilkins), 1153–1252.

Guerbouj, S., Chemkhi, J., and Kaabi, B. (2007). Natural infection of Phlebotomus (Larroussius) langeroni (Diptera: Psychodidae) with Leishmania infantum in Tunisia. *Trans. R. Soc. Trop. Med. Hyg.* 101, 372–377. doi: 10.1016/j.trstmh.2006.07.007

Gupta, D., Bhattacharjee, O., Mandal, D., Sen, M. K., Dey, D., Dasgupta, A., et al. (2019). CRISPR-Cas9 system: a new-fangled dawn in gene editing. *Life Sci.* 232:116636. doi: 10.1016/j.lfs.2019.116636

Gurycová, D. (1998). First isolation of Francisella tularensis subsp. tularensis in Europe. *Eur. J. Epidemiol.* 14, 797–802. doi: 10.1023/a:1007537405242

Haddow, A. J. (1969). The natural history of yellow fever in Africa. *Proc. R. Soc. Edinb. B. Biol. Sci.* 70, 191–227. doi: 10.1017/S0080455X00001338

Hadjinicolaou, J., and Betzios, B. (1973). *Gambusia fish as a means of mosquito control of anopheles sacharovi in Greece*. Geneva, Switzerland: World Health Organization.

Hadole, P., and Vankhede, G. (2013). Spiders for eradication of vector-borne diseases. *Indian Soc. Arachnol.* 1, 59–62.

Hall, R. A., Broom, A. K., Smith, D. W., and Mackenzie, J. S. (2002). "The ecology and epidemiology of Kunjin virus" in *Japanese encephalitis and West Nile viruses*. eds. J. S. Mackenzie, A. D. T. Barrett and V. Deubel (Berlin, Heidelberg: Springer), 253–269.

Hall, R. A., Scherret, J. H., and Mackenzie, J. S. (2006). Kunjin virus: an Australian variant of West Nile? *Ann. N. Y. Acad. Sci.* 951, 153–160. doi: 10.1111/j.1749-6632.2001.tb02693

Hancock, R. G., Boyd, T., MacFadden, S., Sowders, A., Foster, W. A., and Lounibos, L. P. (2022). Mosquitoes eating mosquitoes: how *Toxorhynchites amboinensis*, *Psorophora ciliata*, and *Sabethes cyaneus* (Diptera: Culicidae) capture prey? *Ann. Entomol. Soc. Am.* 115, 461–471. doi: 10.1093/aesa/saac017

Harbach, R. E. (2007). The Culicidae (Diptera): a review of taxonomy, classification and phylogeny. *Zootaxa* 1668, 591–638. doi: 10.11646/zootaxa.1668.1.28

Hargrove, J. W. (2003). *Tsetse eradication: Sufficiency, necessity and desirability*. University of Edinburgh, UK: DFID Animal Health Programme, Centre for Tropical Veterinary Medicine.

Harris, A. F., McKemey, A. R., Nimmo, D., Curtis, Z., Black, I., Morgan, S. A., et al. (2012). Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nat. Biotechnol.* 30, 828–830. doi: 10.1038/nbt.2350

Harris, A. F., Nimmo, D., McKemey, A. R., Kelly, N., Scaife, S., Donnelly, C. A., et al. (2011). Field performace of engineered male mosquitoes. *Nat. Biotechnol.* 29, 1034–1037. doi: 10.1038/nbt.2019

Hassan, S. M., Dipeolu, O. O., Amoo, A. O., and Odhiambo, T. R. (1991). Predation on livestock ticks by chickens. *Vet. Parasitol.* 38, 199–204. doi: 10.1016/0304-4017(91)90129-j

Hassanain, M. A., Garhy, M. F. E., Abdel-Ghaffar, F. A., El-Sharaby, A., and Abdel Megeed, K. N. (1997). Biological control studies of soft and hard ticks in Egypt. *Parasitol. Res.* 83, 209–213. doi: 10.1007/s00 4360050235

Headrick, D. R. (2014). Sleeping sickness epidemics and colonial responses in east and Central Africa, 1900-1940. *PLoS Negl. Trop. Dis.* 8:e2772-e. doi: 10.1371/journal.pntd.0002772

Hedges, L. M., Brownlie, J. C., O'Neill, S. L., and Johnson, K. N. (2008). Wolbachia and virus protection in insects. *Science* 322:702. doi: 10.1126/science.1162418

Hendy, A., Krüger, A., Pfarr, K., De Witte, J., Kibweja, A., Mwingira, U., et al. (2018). The blackfly vectors and transmission of Onchocerca volvulus in Mahenge, south eastern Tanzania. *Acta Trop.* 181, 50–59. doi: 10.1016/j.actatropica.2018.01.009

Henke, M. O., de Hoog, G. S., Gross, U., Zimmermann, G., Kraemer, D., and Weig, M. (2002). Human deep tissue infection with an entomopathogenic Beauveria species. *J. Clin. Microbiol.* 40, 2698–2702. doi: 10.1128/JCM.40.7.2698-2702.2002

Higa, Y. (2011). Dengue vectors and their spatial distribution. *Trop. Med. Health* 39, 17–27. doi: 10.2149/tmh.2011-S04

Hii, J. L. K., Kan, S., Foh, C. K., and Chan, M. K. C. (1984). Anopheles (Cellia) balabacensis Baisas is a vector of Wuchereria bancrofti in Sabah, Malaysia. *Trans. R. Soc. Trop. Med. Hyg.* 78, 281–282. doi: 10.1016/0035-9203(84)90302-x

Hill, E. F. (1995). "Organophosphorus and carbamate pesticides" in *Handbook of ecotoxicology*. eds. D. H. Hoffman, B. A. Rattner, G. A. J. Burton and J. J. Cairns (Boca Raton, Florida: Lewis Publishers), 243–274.

Hoermann, A., Habtewold, T., Selvaraj, P., Del Corsano, G., Capriotti, P., Inghilterra, M. G., et al. (2022). Gene drive mosquitoes can aid malaria elimination by retarding plasmodium sporogonic development. *Sci. Adv.* 8:eabo1733. doi: 10.1126/sciadv.abo1733

Hoffman, A., and Turelli, M. (1997). "Cytoplasmic incompatibility in insects" in *Influential passengers. Inherited microorganisms and arthropod reproduction*. eds. S. L. O'Neill, A. Hoffman and J. H. Werren (Oxford: Oxford University Press), 42–80.

Hoffmann, A. A., Montgomery, B. L., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P. H., Muzzi, F., et al. (2011). Successful establishment of Wolbachia in Aedes populations to suppress dengue transmission. *Nature* 476, 454–457. doi: 10.1038/nature10356

Hotta, S. (1998). Dengue vector mosquitoes in Japan: the role of Aedes albopictus and Aedes aegypti in the 1942-1944 dengue epidemics of Japanese main islands. *Med. Entomol. Zool.* 49, 267–274. doi: 10.7601/mez.49.267

Howard, A. F. V., Zhou, G., and Omlin, F. X. (2007). Malaria mosquito control using edible fish in western Kenya: preliminary findings of a controlled study. *BMC Public Health* 7:199. doi: 10.1186/1471-2458-7-199

Huang, Y.-J. S., Higgs, S., and Vanlandingham, D. L. (2017). Biological control strategies for mosquito vectors of arboviruses. *Insects* 8:21. doi: 10.3390/insects8010021

Isenhart, F. R., and DeSante, D. F. (1985). Observations of scrub jays cleaning ectoparasites from black-tailed deer. *Condor* 87, 145–147. doi: 10.2307/1367147

Ismail, I., Notananda, V., and Schepens, J.Organization, W.H (1975). Studies on malaria and responses of Anopheles balabacensis balabacensis and Anopheles minimus to DDT residual spraying in Thailand. *Acta Trop.* 32, 206–231. PMID: 1984

Iturbe-Ormaetxe, I., Walker, E. C., and O'Neill, S. L. (2011). Wolbachia and the biological control of mosquito-borne diseases. *EMBO Rep.* 12, 508–518. doi: 10.1038/embor.2011.84

Jackson, C. H. N. (1941). The analysis of a tsetse fly population. *Ann. Eugenics* 10, 332–369. doi: 10.1111/j.1469-1809.1940.tb02259.x

Jackson, C. H. N. (1943). The analysis of a tsetse fly population II. *Ann. Eugenics* 12, 176–205. doi: 10.1111/j.1469-1809.1943.tb02322.x

Jackson, C. H. N. (1948). The analysis of a tsetse fly population III. *Ann. Eugenics* 14, 91–108. doi: 10.1111/j.1469-1809.1947.tb02381.x

Jaipieam, S., Visuthismajarn, P., Sutheravut, P., Siriwong, W., Thoumsang, S., Borjan, M., et al. (2009). Organophosphate pesticide residues in drinking water from artesian wells and health risk assessment of agricultural communities, Thailand. *Hum. Ecol. Risk. Assess.* 15, 1304–1316. doi: 10.1080/108070309 03306984

Jeffries, C. L., and Walker, T. (2016). Wolbachia biocontrol strategies for arboviral diseases and the potential influences of resident Wolbachia strains in mosquitoes. *Curr. Trop. Med. Rep.* 3, 20–25. doi: 10.1007/s40475-016-0066-2

Jehle, J. A., Blissard, G. W., Bonning, B. C., Cory, J. S., Herniou, E. A., Rohrmann, G. F., et al. (2006). On the classification and nomenclature of baculoviruses: a proposal for revision. *Arch. Virol.* 151, 1257–1266. doi: 10.1007/s00705-006-0763-6

Jeyaprakasam, N. K., Liew, J. W. K., Low, V. L., Wan Sulaiman, W. Y., and Vythilingam, I. (2020). Plasmodium knowlesi infecting humans in Southeast Asia: What's next? *PLoS Negl. Trop. Dis.* 14:e0008900. doi: 10.1371/journal.pntd.0008900

Jiram, A. I., Vythilingam, I., NoorAzian, Y. M., Yusof, Y. M., Azahari, A. H., and Fong, M.-Y. (2012). Entomologic investigation of plasmodium knowlesi vectors in Kuala Lipis, Pahang, Malaysia. *Malar. J.* 11, 213–222. doi: 10.1186/1475-2875-11-213

Johnson, B. K. (1988). "O'nyong nyong virus disease" in *The arboviruses: epidemiology and ecology*. ed. T. P. Monath, vol. *III* (Florida: CRC Press), 217–223.

Johnson, R. M., and Rasgon, J. L. (2018). Densonucleosis viruses ('densoviruses') for mosquito and pathogen control. *Curr. Opin. Insect Sci.* 28, 90–97. doi: 10.1016/j.cois.2018.05.009

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., et al. (2008). Global trends in emerging infectious diseases. *Nature* 451, 990–993. doi: 10.1038/nature06536

Juliano, S. A., and Lawton, J. H. (1990). The relationship between competition and morphology II: experiments on co-occurring Dytiscid beetles. *J. Anim. Ecol.* 59, 831–848. doi: 10.2307/5017

Jurberg, J., and Galvão, C. (2006). "Biology, ecology, and systematics of Triatominae (Heteroptera, Reduviidae), vectors of Chagas disease, and implications for human health" in *Hug the bug, for love of true bugs*. ed. W. Rabitsch (Austria: OÖ Landes-Kultur GmbH), 1096–1116.

Kageyama, D., Nishimura, G., Hoshizaki, S., and Ishikawa, Y. (2002). Feminizing Wolbachia in an insect, *Ostrinia furnacalis* (Lepidoptera: Crambidae). *Heredity (Edinb.)* 88, 444–449. doi: 10.1038/sj.hdy.6800077

Kamareddine, L. (2012). The biological control of the malaria vector. *Toxins(Basel)* 4, 748–767. doi: 10.3390/toxins4090748

Kambris, Z., Cook, P. E., Phuc, H. K., and Sinkins, S. P. (2009). Immune activation by life-shortening Wolbachia and reduced filarial competence in mosquitoes. *Science* 326, 134–136. doi: 10.1126/science.1177531

Kanjanopas, K., Choochote, W., Jitpakdi, A., Suvannadabba, S., Loymak, S., Chungpivat, S., et al. (2001). Brugia malayi in a naturally infected cat from Narathiwat province, southern Thailand. *Southeast Asian J. Trop. Med. Public Health* 32, 585–587. PMID: 11944722

Karesh, W. B., Dobson, A., Lloyd-Smith, J. O., Lubroth, J., Dixon, M. A., Bennett, M., et al. (2012). Ecology of zoonoses: natural and unnatural histories. *Lancet* 380, 1936–1945. doi: 10.1016/S0140-6736(12)61678-X

Katargina, O., Geller, J., Alekseev, A., Dubinina, H., Efremova, G., Mishaeva, N., et al. (2012). Identification of *Anaplasma phagocytophilum* in tick populations in Estonia, the European part of Russia and Belarus. *Clin. Microbiol. Infect.* 18, 40–46. doi: 10.1111/j.1469-0691.2010.03457.x

Kaushal, J., Khatri, M., and Arya, S. K. (2021). A treatise on organophosphate pesticide pollution: current strategies and advancements in their environmental degradation and elimination. *Ecotoxicol. Environ. Saf.* 207:111483. doi: 10.1016/j.ecoenv.2020.111483

Kay, B. H., Fanning, I. D., and Carley, J. G. (1984). The vector competence of Australian Culex annulirostris with Murray Valley encephalitis and Kunjin viruses. *Aus. J. Exp. Biol. Med. Sci.* 62, 641–650. doi: 10.1038/icb.1984.61

Kay, B. H., Lyons, S. A., Holt, J. S., Holynska, M., and Russell, B. M. (2002). Point source inoculation of Mesocyclops (Copepoda: Cyclopidae) gives widespread control of Ochlerotatus and Aedes (Diptera: Culicidae) immatures in service manholes and pits in North Queensland, Australia. *J. Med. Entomol.* 39, 469–474. doi: 10.1603/0022-2585-39.3.469

Kelly-Hope, L., Paulo, R., Thomas, B., Brito, M., Unnasch, T. R., and Molyneux, D. (2017). Loa loa vectors Chrysops spp.: perspectives on research, distribution, bionomics, and implications for elimination of lymphatic filariasis and onchocerciasis. *Parasit. Vectors* 10:172. doi: 10.1186/s13071-017-2103-y

Kementerian Kesihatan Malaysia (2022). Kenyataan media Ketua Pengarah Kesihatan-situasi semasa demam denggi di Malaysia Minggu Epidemiologi 49/2022 (4-10 Disember 2022). Available at: https://www. moh.gov.my/index.php/database_stores/store_view_page/17/2244 (Accessed December 14, 2022).

Kendie, F. A. (2020). Potential biological control agents against mosquito vector in the case of larvae stage: a review. *World News Nat. Sci.* 28, 34–50.

Killeen, G. F. (2014). Characterizing, controlling and eliminating residual malaria transmission. *Malar. J.* 13:330. doi: 10.1186/1475-2875-13-330

Kim, K. C., and Merritt, R. W. (1987). *Black flies: Ecology, population management, and annonated world list*. Pennsylvania, USA: The Pennsylvania State University.

Kittayapong, P., Baisley, K. J., Baimai, V., and O'Neill, S. L. (2000). Distribution and diversity of Wolbachia infections in southeast Asian mosquitoes (Diptera: Culicidae). *J. Med. Entomol.* 37, 340–345. doi: 10.1093/jmedent/37.3.340

Kleine, F. K. (1915). Die Übertragung von Filarien durch Chrysops. *Z. Hyg. Infekt.* 80, 345–349. doi: 10.1007/BF02174495

Kleinschmidt, I., Bradley, J., Knox, T. B., Mnzava, A. P., Kafy, H. T., Mbogo, C. M., et al. (2018). Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: a WHO-coordinated, prospective, international, observational cohort study. *Lancet Infect. Dis.* 18, 640–649. doi: 10.1016/S1473-3099(18)30172-5

Kohls, G. M. (1947). Vectors of Rickettsial diseases. *Ann. Intern. Med.* 26:713. doi: 10.7326/0003-4819-26-5-713

Koskinioti, P., Augustinos, A. A., Carvalho, D. O., Misbah-ul-Haq, M., Pillwax, G., de la Fuente, L. D., et al. (2021). Genetic sexing strains for the population suppression of the mosquito vector Aedes aegypti. *Philos. Trans. R. Soc. B. Biol. Sci.* 376:20190808. doi: 10.1098/rstb.2019.0808

Kozuch, O., and Nosek, J. (1971). Transmission of tick-borne encephalitis (TBE) virus by *Dermacentor marginatus* and *D. reticulatus* ticks. *Acta Virol.* 15:334. PMID: 4398089

Kugeler, K. J., Mead, P. S., Janusz, A. M., Staples, J. E., Kubota, K. A., Chalcraft, L. G., et al. (2009). Molecular epidemiology of Francisella tularensis in the United States. *Clin. Infect. Dis.* 48, 863–870. doi: 10.1086/597261

Kulkarni, M. A., Berrang-Ford, L., Buck, P. A., Drebot, M. A., Lindsay, L. R., and Ogden, N. H. (2015). Major emerging vector-borne zoonotic diseases of public health importance in Canada. *Emerg. Microbes Infect.* 4:e33, 1–7. doi: 10.1038/emi.2015.33

Kumar, R., and Hwang, J.-S. (2006). Larvicidal efficiency of aquatic predators: a perspective for mosquito biocontrol. *Zool. Stud.* 45, 447–466.

Kumar, N. P., Sabesan, S., Krishnamoorthy, K., and Jambulingam, P. (2012). Detection of Chikungunya virus in wild populations of Aedes albopictus in Kerala state, India. *Vector Borne Zoonotic Dis.* 12, 907–911. doi: 10.1089/vbz.2012.0969

Kumar, A., Sharma, V. P., Sumodan, P. K., and Thavaselvam, D. (1998). Field trials of biolarvicide bacillus thuringiensis var. israelensis strain 164 and the larvivorous fish Aplocheilus blocki against Anopheles stephensi for malaria control in Goa, India. *J. Am. Mosq. Control Assoc.* 14, 457–462. PMID: 10084141

Kurucz, N., Whelan, P. I., Jacups, S. P., Broom, A. K., and Melville, L. F. (2005). Rainfall, mosquito vector numbers and seroconversions in sentinel chickens to Murray Valley encephalitis virus in the Northern Territory. *Arbovirus Res. Aus.* 9, 188–192.

Kushwaha, A. K., Scorza, B. M., Singh, O. P., Rowton, E., Lawyer, P., Sundar, S., et al. (2022). Domestic mammals as reservoirs for Leishmania donovani on the Indian subcontinent: possibility and consequences on elimination. *Transbound. Emerg. Dis.* 69, 268–277. doi: 10.1111/tbed.14061

Kwenti, T. E. (2017). "Biological control of parasites" in *Natural remedies in the fight against parasites*. eds. H. Khater, M. Govindarajan and G. Benelli (London: IntechOpen)

Labuda, M., and Randolph, S. E. (1999). Survival strategy of tick-borne encephalitis virus: cellular basis and environmental determinants. *Zent. bl. Bakteriol.* 289, 513–524. doi: 10.1016/s09348840(99)800 05-x

Lacey, L. A. (2007). Bacillus thuringiensis serovariety israelensis and *Bacillus sphaericus* for mosquito control. *J. Am. Mosq. Control Assoc.* 23, 133–163. doi: 10.2987/8756-971X(2007)23[133,BTSI AB]2.0.CO;2

Lacey, L. A., and Inman, A. (1985). Efficacy of granular formulations of bacillus thuringiensis (H-14) for the control of anopheles larvae in rice fields. *J. Am. Mosq. Control Assoc.* 1, 38–42. PMID: 3880212

Laine, M., Luukainen, R., and Toivanen, A. (2004). Sindbis virus and other alphaviruses as a cause of human arthritic disease. *J. Intern. Med.* 256, 457–471. doi: 10.1111/j.1365-2796.2004.01413.x

Lambrechts, L., Scott, T. W., and Gubler, D. J. (2010). Consequences of the expanding global distribution of Aedes albopictus for dengue virus transmission. *PLoS Negl. Trop. Dis.* 4:e646. doi: 10.1371/journal.pntd.0000646

Lantova, L., Svobodova, M., and Volf, P. (2011). Effects of Psychodiella sergenti (Apicomplexa, Eugregarinorida) on its natural host Phlebotomus sergenti (Diptera, Psychodidae). *J. Med. Entomol.* 48, 985–990. doi: 10.1603/me11018

Lantova, L., and Volf, P. (2012). The development of Psychodiella sergenti (Apicomplexa: Eugregarinorida) in Phlebotomus sergenti (Diptera: Psychodidae). *Parasitology* 139, 726–734. doi: 10.1017/S0031182011002411

Lantova, L., and Volf, P. (2014). Mosquito and sand fly gregarines of the genus Ascogregarina and Psychodiella (Apicomplexa: Eugregarinorida, Aseptatorina) – overview of their taxonomy, life cycle, host specificity, and pathogenicity. *Infect. Genet. Evol.* 28, 616–627. doi: 10.1016/j.meegid.2014.04.021

Lara Oya, A., Medialdea Hurtado, M. E., Rojo Martín, M. D., Pérez, A. A., Alastruey-Izquierdo, A., Casas, C. M., et al. (2016). Fungal keratitis due to *Beauveria bassiana* in a contact lenses wearer and review of published reports. *Mycopathologia* 181, 745–752. doi: 10.1007/s11046-016-0027-2

Lardeux, F. (1992). Biological control of Culicidae with the copepod Mesocyclops asperconis and larvivorous fish (Poeciliidae) in a village of French Polynesia. *Med. Vet. Entomol.* 6, 9–15. doi: 10.1111/j.1365-2915.1992.tb00028.x

Lardeux, F., Riviere, F., Sechan, Y., and Kay, B. H. (1992). Release of Mesocyclops aspericornis (Copepoda) for control of larval Aedes polynesiensis (Diptera: Culicidae) in land crab burrows on an atoll of French Polynesia. *J. Med. Entomol.* 29, 571–576. doi: 10.1093/jmedent/29.4.571

Lau, Y. L., Lee, W. C., Chen, J. H., Zhong, Z., Jian, J. B., Amir, A., et al. (2016). Draft genomes of Anopheles cracens and Anopheles maculatus: comparison of simian malaria and human malaria vectors in peninsular Malaysia. *PLoS One* 11:e0157893. doi: 10.1371/journal.pone.0157893

Lau, Y. L., Lee, W. C., Xia, J., Zhang, G., Razali, R., Anwar, A., et al. (2015). Draft genome of *Brugia pahangi*: high similarity between *B. pahangi* and *B. malayi*. *Parasit. Vectors* 8:451. doi: 10.1186/s13071-015-1064-2

Laven, H. (1967). Eradication of Culex pipiens fatigans through cytoplasmic incompatibility. *Nature* 216, 383–384. doi: 10.1038/216383a0

Lawrence, P. O. (2004). "*Simulium* spp., vectors of Onchocerca volvulus: life cycle and control" in *Encyclopedia of entomology*. ed. J. L. Capinera, vol. *2010* (Dordrecht: Springer)

Le Prince, J. A., and Orenstein, A. J. (1916). *Mosquito control in Panama: the eradication of malaria and yellow fever in Cuba and Panama*. New York/London: Putnam.

Lee, W. C., Cheong, F. W., Amir, A., Lai, M. Y., Tan, J. H., Phang, W. K., et al. (2022). Plasmodium knowlesi: the game changer for malaria eradication. *Malar. J.* 21:140. doi: 10.1186/s12936-022-04131-8

Lee, W. C., Chin, P. W., Lau, Y. L., Chin, L. C., Fong, M. Y., Yap, C. J., et al. (2013). Hyperparasitaemic human plasmodium knowlesi infection with atypical morphology in peninsular Malaysia. *Malar. J.* 12:88. doi: 10.1186/1475-2875-12-88

Lee, W. C., Russell, B., Lee, B., Chu, C. S., Phyo, A. P., Sriprawat, K., et al. (2021). Plasmodium falciparum rosetting protects schizonts against artemisinin. *EBioMedicine* 73:103680. doi: 10.1016/j.ebiom.2021.103680

Lerdthusnee, K., Khuntirat, B., Leepitakrat, W., Tanskul, P., Monkanna, T., Khlaimanee, N., et al. (2003). Scrub typhus: vector competence of Leptotrombidium chiangraiensis chiggers and transmission efficacy and isolation of Orientia

tsutsugamushi. *Ann. N. Y. Acad. Sci.* 990, 25–35. doi: 10.1111/j.1749-6632-2003.tb07333.x

Leung, S., Windbichler, N., Wenger, E. A., Bever, C. A., and Selvaraj, P. (2022). Population replacement gene drive characteristics for malaria elimination in a range of seasonal transmission settings: a modelling study. *Malar. J.* 21:226. doi: 10.1186/s12936-022-04242-2

Levy, R., and Miller, T. W. Jr. (1977). Experimental release of Romanomermis culicivorax (Mermithidae: Nematoda) to control mosquitoes breeding in Southwest Florida. *Mosq. News* 37, 483–486.

Lewis, D., and Young, E. R. (1980). The transmission of a human strain of *Babesia divergens* by Ixodes ricinus ticks. *J. Parasitol.* 66, 359–360. doi: 10.2307/3280841

Liew, J. W. K., Mohd Bukhari, F. D., Jeyaprakasam, N. K., Phang, W. K., Vythilingam, I., and Lau, Y. L. (2021). Natural plasmodium inui infections in humans and Anopheles cracens mosquito, Malaysia. *Emerg. Infect. Dis.* 27, 2700–2703. doi: 10.3201/eid2710.210412

Lindsay, S. W., Emerson, P. M., and Charlwood, J. D. (2002). Reducing malaria by mosquito-proofing houses. *Trends Parasitol.* 18, 510–514. doi: 10.1016/s1471-4922(02)02382-6

Lo Re, V. R., Occi, J. L., and MacGregor, R. R. (2004). Identifying the vector of Lyme disease. *Am. Fam. Physician* 69, 1935–1937.

Lockhart, J. M., Davidson, W. R., Stallknecht, D. E., Dawson, J. E., and Little, S. E. (1997). Natural history of *Ehrlichia chafeensis* (Rickettsiales: Ehrlichieae) in the piedmont physiographic province of Georgia. *J. Parasitol.* 83, 887–894. doi: 10.2307/3284284

Lofgren, C. S., Dame, D. A., Breeland, S. G., Weidhaas, D. E., Jeffery, G., Kaiser, R., et al. (1974). Release of chemosterilized males for the control of Anopheles albimanus in El Salvador: III. Field methods and population control. *Am. J. Trop. Med. Hyg.* 23, 288–297. doi: 10.4269/AJTMH.1974.23.288

Louca, V., Lucas, M. C., Green, C., Majambere, S., Fillinger, U., and Lindsay, S. W. (2009). Role of fish as predators of mosquito larvae on the floodplain of the Gambia river. *J. Med. Entomol.* 46, 546–556. doi: 10.1603/033.046.0320

Louis, J. P., and Albert, J. P. (1988). Malaria in the Republic of Djibouti. Strategy for control using a biological antilarval campaign: indigenous larvivorous fishes (*Aphanius dispar*) and bacterial toxins. *Med. Trop.* 48, 127–131.

Lounibos, L. P. (1980). The bionomics of three sympatric Eretmapodites (Diptera: Culicidae) at the Kenya coast. *Bull. Entomol. Res.* 70, 309–320. doi: 10.1017/S0007485300007598

Loyola, E. G., Rodriguez, M. H., Gonzalez, L., Arredondo, J. I., Bown, D. N., and Vaca, M. A. (1990). Effect of indoor residual spraying of DDT and bendiocarb on the feeding patterns of Anopheles pseudopunctipennis in Mexico. *J. Am. Mosq. Control Assoc.* 6, 635–640.

Loyola, E. G., Vaca, M. A., Bown, D. N., Perez, E., and Rodriguez, M. H. (1991). Comparative use of bendiocarb and DDT to control Anopheles pseudopunctipennis in a malarious area of Mexico. *Med. Vet. Entomol.* 5, 233–242. doi: 10.1111/j.1365-2915.1991.tb00546.x

Lundkvist, E., Landin, J., Jackson, M., and Svensson, C. (2003). Diving beetles (Dytiscidae) as predators of mosquito larvae (Culicidae) in field experiments and in laboratory tests of prey preference. *Bull. Entomol. Res.* 93, 219–226. doi: 10.1079/BER2003237

Macfie, J. W. S., and Corson, J. F. (1922). A new species of filarial larva found in the skin of natives in the Gold Coast. *Ann. Trop. Med. Parasitol.* 16, 465–471. doi: 10.1080/00034983.1922.11684341

Mackenzie, J. S., Lindsay, M. D., Coelen, R. J., Broom, A. K., Hall, R. A., and Smith, D. W. (1994). Arboviruses causing human disease in the Australasian zoogeographic region. *Arch. Virol.* 136, 447–467. doi: 10.1007/BF01321074

Mahesh Kumar, P., Murugan, K., Kovendan, K., Panneerselvam, C., Prasanna Kumar, K., Amerasan, D., et al. (2012). Mosquitocidal activity Solanum xanthocarpum fruit extract and copepod Mesocyclops thermocyclopoides for the control of dengue vector Aedes aegypti. *Parasitol. Res.* 111, 609–618. doi: 10.1007/s00436-012-2876-z

Mains, J. W., Kelly, P. H., Dobson, K. L., Petrie, W. D., and Dobson, S. L. (2019). Localized control of Aedes aegypti (Diptera: Culicidae) in Miami, FL, via inundative releases of Wolbachia-infected male mosquitoes. *J. Med. Entomol.* 56, 1296–1303. doi: 10.1093/jme/tjz051

Mandal, S., Ghosh, A., Bhattacharjee, I., and Chandra, G. (2008). Biocontrol efficacy of odonate nymphs against larvae of the mosquito, Culex quinquefasciatus say, 1823. *Acta Trop.* 106, 109–114. doi: 10.1016/j.actatropica.2008.02.002

Männikkö, N. (2011). Etymologia: Francisella tularensis. *Emerg. Infect. Dis.* 17:799. doi: 10.3201/eid1705.ET1705

Manrique-Saide, P., Ibanez-Bernal, S., Delfin-Gonzalez, H., and Parra Tabla, V. (1998). Mesocyclops longisetus effects on survivorship of Aedes aegypti immature stages in car tyres. *Med. Vet. Entomol.* 12, 386–390. doi: 10.1046/j.1365-2915.1998.00126.x

Manson, P. (1891). The filaria sanguinis hominis major and minor, two new species of haematozoa. *Lancet* 137, 4–8. doi: 10.1016/S0140-6736(02)15666-2

Marchette, N. J., Garcia, R., and Rudnick, A. (1969). Isolation of Zika virus from Aedes aegypti mosquitoes in Malaysia. *Am. J. Trop. Med. Hyg.* 18, 411–415. doi: 10.4269/ajtmh.1969.18.411

Marshall, I. D. (1988). "Murray Valley and Kunjin encephalitis" in *The arboviruses: epidemiology and ecology*. ed. T. P. Monath, vol. *III* (Florida: CRC Press), 151–190.

Marten, G. G., Astaiza, R., Suarez, M. F., Monje, C., and Reid, J. W. (1989). Natural control of larval Anopheles albimanus (Diptera: Culicidae) by the predator Mesocyclops (Copepoda: Cyclopoida). *J. Med. Entomol.* 26, 624–627. doi: 10.1093/jmedent/26.6.624

Marten, G. G., Nguyen, M., and Ngo, G. (2000). Copepod predation on Anopheles quadrimaculatus larvae in rice fields. *J. Vector Ecol.* 25, 1–6.

Marten, G. G., and Reid, J. W. (2007). Cyclopoid copepods. *J. Am. Mosq. Control Assoc.* 23, 65–92. doi: 10.2987/8756971X(2007)23[65,CC]2.0.CO;2

Martinez-Sanchez, A., Camacho, A. D., Quintero-Martinez, M. T., and Alejandre-Aguilar, R. (2007). Effect of ectoparasitic Pimeliaphilus plumifer mites (Acari: Pterygosomatidae) on Meccus pallidipennis (Hemiptera: Reduviidae) and several other Chagas' disease vectors under laboratory conditions. *Exp. Appl. Acarol.* 42, 139–149. doi: 10.1007/s10493-007-9079-9

Mather, T. N., Piesman, J., and Spielman, A. (1987). Absence of spirochaetes (*Borrelia burgdorgferi*) and piroplasms (*Babesia microti*) in deer ticks (*Ixodes dammini*) parasitized by chalcid wasps (*Hunterellus hookeri*). *Med. Vet. Entomol.* 1, 3–8. doi: 10.1111/j.1365-2915.1987.tb00317.x

Matsuo, N. (2019). Discovery and development of pyrethroid insecticides. *Proc. Jpn. Acad. B. Phys. Biol. Sci.* 95, 378–400. doi: 10.2183/pjab.95.027

Maurin, M., Pelloux, I., Brion, J. P., Del Banõ, J.-N., and Picard, A. (2011). Human tularemia in France, 2006-2010. *Clin. Infect. Dis.* 53, e133–e141. doi: 10.1093/cid/cir612

McCray, E. M. Jr., Fay, R. W., and Schoof, H. F. (1970). The bionomics of Lankesteria culicis and Aedes aegypti. *J. Invertebr. Pathol.* 16, 42–53. doi: 10.1016/0022-2011(70)90203-X

McDade, J. E., and Newhouse, V. F. (1986). Natural history of rickettsia rickettsii. *Annu. Rev. Microbiol.* 40, 287–309. doi: 10.1146/annurev.mi.40.100186.001443

McDade, J. E., Shepard, C. C., Redus, M. A., Newhouse, V. F., and Smith, J. D. (1980). Evidence of rickettsia prowazekii infection in the United States. *Am. J. Trop. Med. Hyg.* 29, 277–284. doi: 10.4269/ajtmh.1980.29.277

McInnis, D., Tam, S., Grace, C., and Miyashita, D. (1994). Populations suppression and sterility rates induced by variable sex-ratio, terile insect releases of Ceratitis capitata (Diptera, Tephritidae) in Hawaii. *Ann. Entomol. Soc. Am.* 87, 231–240. doi: 10.1093/aesa/87.2.231

Mellanby, K. (1992). *The DDT story*. Surrey, UK: British Crop Protection Council (BCPC).

Menon, P. K., and Rajagopalan, P. K. (1978). Control of mosquito breeding in wells by using *Gambusia affinis* and *Aplocheilus blochii* in Pondicherry town. *Indian J. Med. Res.* 68, 927–933. PMID: 582034

Mitchell, C. J., Chen, P. S., and Chapman, H. C. (1974). Exploratory trials utilizing a mermithid nematode as a control agent for Culex mosquitoes in Taiwan (China). *Taiwan Yi Xue Hui Za Zhi* 73, 241–254. PMID: 4531476

Mogi, M., and Chan, K. L. (1996). Predatory habits of dipteran larvae inhabiting nepenthes pinchers. *Raffles Bull. Zool.* 44, 233–245.

Molloy, D. P., and Jamnback, H. (1977). A larval black fly control field trial using mermithid parasites and its cost implications. *Mosq. News* 37, 104–108.

Moore, C. G., and Mitchell, C. J. (1997). Aedes albopictus in the United States: ten-year presence and public health implications. *Emerg. Infect. Dis.* 3, 329–334. doi: 10.3201/eid0303.970309

Mooring, M. S., and Mundy, P. J. (1996). Factors influencing host selection by yellow-billed oxpeckers at Matoba national park, Zimbabwe. *Afr. J. Ecol.* 34, 177–188. doi: 10.1111/j.1365-2028.1996.008-89008.x

Moreau, R. (1933). The food of the red-billed oxpecker, Buphagus erythrorhynchus (Stanley). *Bull. Entomol. Res.* 24, 325–335. doi: 10.1017/S000748530003162X

Moreira, L. A., Iturbe-Ormaetxe, I., Jeffery, J. A., Lu, G., Pyke, A. T., Hedges, L. M., et al. (2009a). A Wolbachia symbiont in Aedes aegypti limits infection with dengue, chikungunya and plasmodium. *Cells* 139, 1268–1278. doi: 10.1016/j.cell.2009.11.042

Moreira, L. A., Saig, E., Turley, A. P., Ribeiro, J. M., O'Neill, S. L., and McGraw, E. A. (2009b). Human probing behavior of Aedes aegypti when infected with a life-shortening strain of Wolbachia. *PLoS Negl. Trop. Dis.* 3:e568. doi: 10.1371/journal.pntd.0000568

Motabar, M. (1978). *Larvivorous fish, Gambusia affinis: a review*. Geneva: World Health Organization.

Mourya, D. T., Singh, D. K., Yadav, P., Gokhale, M. D., Barde, P. V., Narayan, N. B., et al. (2003). Role of gregarine parasite Ascogregarina culicis (Apicomplexa: Lecudinicae) in the maintenance of Chikungunya virus in vector mosquito. *J. Eukaryot. Microbiol.* 50, 379–382. doi: 10.1111/j.1550-7408.2003.tb00153.x

Mousson, L., Zouache, K., Arias-Goeta, C., Raquin, V., Mavingui, P., and Failloux, A. B. (2012). The native Wolbachia symbionts limit transmission of dengue virus in Aedes albopictus. *PLoS Negl. Trop. Dis.* 6:e1989. doi: 10.1371/journal.pntd.0001989

Mukhopadhyay, J., Ghosh, K., and Braigh, H. R. (2000). Identification of cutaneous leishmaniasis vectors, *Phlebotomus papatasi* and *P. duboscqi* using random amplified polymorphic DNA. *Acta Trop.* 76, 277–283. doi: 10.1016/s0001-706x(00)00130-3

Müller, M., and Schlagenhauf, P. (2014). Plasmodium knowlesi in travellers, update 2014. *Int. J. Infect. Dis.* 22, 55–64. doi: 10.1016/j/ijid.2013.12.016

Mulyaningsih, B., Umniyati, S. R., Hadisusanto, S., and Edyansyah, E. (2019). Study on vector mosquito of zoonotic Brugia malayi in Musi Rawas, south Sumatera, Indonesia. *Vet. World* 12, 1729–1734. doi: 10.14202/vetworld.2019.1729-1734

Mundy, P. J., and Cook, A. W. (1975). Observation of the yellow-billed oxpecker Buphagus africanus in northern Nigeria. *Ibis* 117, 504–506. doi: 10.1111/j/1474-919X.1975.tb04243.x

Murdock, W. W., Scott, M. A., and Ebsworth, P. (1984). Effects of the general predator, Notonecta (Hemiptera) upon a fresh water community. *J. Anim. Ecol.* 53, 791–808. doi: 10.2307/4660

Murphy, F. A. (1998). Emerging zoonoses. *Emerg. Infect. Dis.* 4, 429–435. doi: 10.3201/eid0403.98 0324

Murphy, A., Rajahram, G. S., Jilip, J., Maluda, M., William, T., Hu, W., et al. (2020). Incidence and epidemiological features of dengue in Sabah, Malaysia. *PLoS Negl. Trop. Dis.* 14:e0007504. doi: 10.1371/journal.pntd.0007504

Muslim, A., Fong, M. Y., Mahmud, R., Lau, Y. L., and Sivanandam, S. (2013). Armigeres subalbatus incriminated as a vector of zoonotic Brugia pahangi filariasis in suburban Kuala Lumpur, Peninsular Malaysia. *Parasit. Vectors* 6:219. doi: 10.1186/1756-3305-6-219

Mylonakis, E. (2001). When to suspect and how to monitor babesiosis. *Am. Fam. Physician* 63, 1969–1974.

Najera, J. A., Gonzalez-Silva, M., and Alonso, P. (2011). Some lessons for the future from the global malaria eradication Programme (1955-1969). *PLoS Med.* 8:e1000412. doi: 10.1371/journal.pmed.100 0412

Nazni, W. A., Hoffmann, A. A., NoorAfizah, A., Cheong, Y. L., Mancini, M. V., Golding, N., et al. (2019). Establishment of Wolbachia strain wAlbB in Malaysian populations of Aedes aegypti for dengue control. *Curr. Biol.* 29, 4241–4248.e5. doi: 10.1016/j.cub.2019.11.007

Ndava, J., Llera, S. D., and Manyanga, P. (2018). The future of mosquito control: the role of spiders as biological control agents: a review. *Int. J. Mosq. Res.* 5, 6–11.

Negri, I., Pellecchia, M., Mazzoglio, P. J., Patetta, A., and Alma, A. (2006). Feminizing Wolbachia in Zyginidia pullula (Insecta, Hemiptera), a leafhopper with an XX/XO sex-determination system. *Proc. R. Soc. B Biol. Sci.* 273, 2409–2416. doi: 10.1098/rspb.2006.3592

Nilsson, A. N., and Savensson, B. W. (1994). Dytiscid predators and culicid prey in two boreal snowmelt pools differing in temperature and duration. *Ann. Zool. Fenn.* 31, 365–376.

Nilsson, A. N., and Soderstrom, O. (1988). Larval consumption rates, interspecific predation and local guild composition of egg-overwintering Agabus (Coleoptera: Dytiscidae) species in vernal ponds. *Oecologia* 76, 131–137. doi: 10.1107/BF00379611

Nitatpattana, N., Apiwathnasorn, C., Barbazan, P., Leemingsawat, S., Yoksan, S., and Gonzales, J.-P. (2005). First isolation of Japanese encephalitis from Culex quinquefasciatus in Thailand. *Southeast Asian J. Trop. Med. Public Health* 36, 875–878.

Niyas, K., Abraham, R., Unnikrishnan, R., Mathew, T., Nair, S., Manakkadan, A., et al. (2010). Molecular characterization of Chikungunya virus isolates from clinical samples and adult Aedes albopictus mosquitoes emerged from larvae from Kerala, South India. *Virol. J.* 7:189. doi: 10.1186/1743-422X-7-189

Njiokou, F., Laveissière, C., Simo, G., Nkinin, S., Grébaut, P., Cuny, G., et al. (2006). Wild fauna as a probable animal reservoir for Trypanosoma brucei gambiense in Cameroon. *Infect. Genet. Evol.* 6, 147–153. doi: 10.1016/j.meegid.2005.04.003

Novak, R. J., Gubler, D., and Underwood, D. (1986). Evaluation of slow-release formulations of temephos (abate) and bacillus thuringiensis var. israelensis for the control of Aedes aegypti in Puerto Rico. *J. Am. Mosq. Control Assoc.* 1, 449–453.

Nunthanid, P., Roongruanchai, K., Wongkamchai, S., and Sarasombath, P. T. (2020). Case report: periorbital filariasis caused by *Brugia malayi*. *Am. J. Trop. Med. Hyg.* 103, 2336–2338. doi: 10.4269/ajtmh.20-0853

O'Leary, S., and Adelman, Z. N. (2020). CRISPR/Cas9 knockout of female-biased genes AeAct-4 or myo-fem in ae. Aegypti results in a flightless phenotype in female, but not male mosquitoes. *PLoS Negl. Trop. Dis.* 14:e0008971. doi: 10.1371/journal.pntd.0008971

Ohashi, N., Inayoshi, M., Kitamura, K., Kawamori, F., Kawaguchi, D., Nishimura, Y., et al. (2005). Anaplasma phagocytophilum-infected ticks, Japan. *Emerg. Infect. Dis.* 11, 1780–1783. doi: 10.3201/eid1111.050407

Okamoto, K. W., and Amarasekare, P. (2012). The biological control of disease vectors. *J. Theor. Biol.* 309, 47–57. doi: 10.1016/j.jtbi.2012.05.020

O'Neill, S. L., Hoffman, A., and Werren, J. H. (1997). *Influential passengers: inherited microorganisms and arthropod reproduction*. Oxford: Oxford University Press.

Orozco, M. M., Enriquez, G. F., Alvarado-Otegui, J. A., Cardinal, M. V., Schijman, A. G., Kitron, U., et al. (2013). New sylvatic hosts of Trypanosoma cruzi and their reservoir competence in the humid Chaco of Argentina: a longitudinal study. *Am. J. Trop. Med. Hyg.* 88, 872–882. doi: 10.4269/ajtmh.12-0519

Paily, K. P., and Balaraman, K. (2000). Susceptibility of ten species of mosquito larvae to the parasitic nematode Romanonermis iyengari and its development. *Med. Vet. Entomol.* 14, 426–429. doi: 10.1046/j.1365-2915.2000.00263.x

Panteleev, D., Goriacheva, I. I., Andrianov, B. V., Reznik, N. L., Lazebnyi, O. E., and Kulikov, A. M. (2007). The endosymbiotic bacterium Wolbachia enhances the nonspecific resistance to insect pathogens and alters behavior of Drosophila melanogaster. *Genetika* 43, 1277–1280.

Parker, A. G. (2005). "Mass-rearing for sterile insect release" in *Sterile insect technique: Principles and practice in area-wide integrated pest management*. eds. V. A. Dyck, J. Hendrichs and A. S. Robinson (The Netherlands: Springer)

Parker, R. R., Spencer, R. R., and Francis, E. (1924). Tularemia: XI. Tularemia infection in ticks of the species Dermacentor andersoni stiles in the Bitterrot Valley. *Mont. Public Health Rep.* 39, 1057–1073. doi: 10.2307/4577151

Patterson, R. S., Lowe, R. E., Smittle, B. J., Dame, D. A., Boston, M. D., and Cameron, A. L. (1977). Release of radiosterilized males to control Culex pipiens quinquefasciatus (Diptera: Culicidae). *J. Med. Entomol.* 14, 299–304. doi: 10.1093/jmedent/14.3.299

Patterson, R. S., Weidhaas, D. E., Ford, H. R., and Lofgren, C. S. (1970). Suppression and elimination of an island population of Culex pipiens quinquefasciatus with sterile males. *Science* 168, 1368–1370. doi: 10.1126/science.168.3937.1368

Paula, A. R., Carolino, A. T., Paula, C. O., and Samuels, R. I. (2011a). The combination of the entomopathogenic fungus Metarhizium anisopliae with the insecticide imidacloprid increases virulence against the dengue vector Aedes aegypti (Diptera: Culicidae). *Parasit. Vectors* 4:8. doi: 10.1186/1756-3 305-4-8

Paula, A. R., Carolino, A. T., Silva, C. P., and Samuels, R. I. (2011b). Susceptibility of adult female Aedes aegypti (Diptera: Culicidae) to the entomopathogenic fungus Metarhizium anisopliae is modified following blood feeding. *Parasit. Vectors* 4:91. doi: 10.1186/1756-3305-4-91

Peng, Y. F., Song, J. Z., Tian, G. Z., Xue, Q. L., Ge, F. X., Yang, J. L., et al. (1998). Field evaluations of Romanomermis yunanensis (Nematoda: Mermithidae) for control of Culicinae mosquitoes in China. *Fundam. Appl. Nematol.* 21, 227–232.

Petersen, J. J., Hoy, J. B., and O'Berg, A. G. (1972). Preliminary field tests with Reesimermis nielseni against mosquito larvae in California rice fields. *Calif. Vector Views* 19, 47–50.

Petersen, J. J., and Willis, O. R. (1972). Results of preliminary field applications of Reesimermis nielseni (Mermithidae: Nematoda) to control mosquito larvae. *Mosq. News* 32, 312–316.

Petersen, J. J., and Willis, O. R. (1974). Diximermis peterseni (Nematoda: Mermithidae): a potential biocontrol agent of Anopheles mosquito larvae. *J. Invertebr. Pathol.* 24, 20–23. doi: 10.1016/0022-2011(74)90158-X

Pfitzner, W. P., Beck, M., Weitzel, T., and Becker, N. (2015). The role of mosquitoes in the diet of adult dragon and damselflies (Odonata). *J. Am. Mosq. Control Assoc.* 31, 187–189. doi: 10.2987/14-6476R

Pham, X. D., Otsuka, Y., Suzuki, H., and Takaoka, H. (2001). Detection of Orientia tsutsugamushi (Rickettsiales: Rickettsiaceae) in unengorged chiggers (Acari: Trombiculidae) from Oita prefecture, Japan by nested polymerase chain reaction. *J. Med. Entomol.* 38, 308–311. doi: 10.1603/0022-2585-38.2.308

Phuc, H. K., Andreasen, M. H., Burton, R. S., Vass, C., Epton, M. J., Pape, G., et al. (2007). Late-acting dominant lethal genetic systems and mosquito control. *BMC Biol.* 5:11. doi: 10.1186/1741-7007-5-11

Pilossof, R. (2016). A brief history of tsetse control methods in Zimbabwe and possible effects of climate change on their distribution. *Int. J. Afr. Dev.* 4, 88–101.

Plantan, T., Howitt, M., Kotze, A., and Gaines, M. (2012). Feeding preferences of the red-billed oxpecker, Buphagus erythrorhynchus: a parasitic mutualist? *Afr. J. Ecol.* 51, 325–336. doi: 10.1111/aj e.12042

Poinar, G. O. (2018). *Nematodes for biological control of insects*. Boca Raton: CRC Press.

Poinar, G. O., and Camino, N. B. (1986). *Strelkovimermis spiculatus* n. sp. (Mermithidae: Nematoda) parasitizing Aedes albifasciatus mac. (Culicidae: Diptera) in Argentina. *J. Nematol.* 18, 317–319.

Pollitzer, R. (1954). *Plague*. Geneva: World Health Organization.

Popovici, J., Moreira, L. A., Poinsignon, A., Iturbe-Ormaetxe, I., McNaughton, D., and O'Neill, S. L. (2010). Assessing key safety concerns of a Wolbachia-based strategy to control dengue transmission by Aedes mosquitoes. *Mem. Inst. Oswaldo Cruz* 105, 957–964. doi: 10.1590/s0074-02762010000800002

Poulin, B., and Lefebvre, G. (2018). Perturbation and delayed recovery of the reed invertebrate assemblage in Camargue marshes sprayed with bacillus thuringiensis israelensis. *Insect Sci.* 25, 542–548. doi: 10.1111/1744-7917.12416

Poulin, B., Tétrel, C., and Lefebvre, G. (2022). Impact of mosquito control operations on waterbirds in a Camargue nature reserve. *Wetl. Ecol. Manag.* 30, 1049–1064. doi: 10.1007/s11273-021-09834-4

Pramasivan, S., Ngui, R., Jeyaprakasam, N. K., Liew, J. W. K., Low, V. L., Mohamed Hassan, N., et al. (2021). Spatial distribution of plasmodium knowlesi cases and their vectors in Johor, Malaysia: in light of human malaria elimination. *Malar. J.* 20:426. doi: 10.1186/s12936-021-03963-0

Pritchard, G. (1964). The prey of adult dragonflies in northern Alberta. *Can. Entomol.* 96, 821–825. doi: 10.4039/Ent96821-6

Promdonkoy, B., and Ellar, D. J. (2003). Investigation of the pore-forming mechanism of a cytolytic delta-endotoxin from bacillus thuringiensis. *Biochem. J.* 374, 255–259. doi: 10.1042/BJ20030437

Prusty, A. K., Meena, D. K., Mohapatra, S., Panikkar, P., Das, P., Gupta, S. K., et al. (2015). Synthetic pyrethroids (type II) and freshwater fish culture: perils and mitigations. *Int. Aquat. Res.* 7, 163–191. doi: 10.1007/s40071-015-0106-x

Quiroz-Martinez, H., and Rodriguez-Castro, A. (2007). Aquatic insects as predators of mosquito larvae. *J. Am. Mosq. Control Assoc.* 23, 110–117. doi: 10.2987/8756-971X(2007)23[110:AIAPOM]2.0.CO;2

Raghavendra, K., Sharma, P., and Dash, A. P. (2008). Biological control of mosquito populations through frogs: opportunities & constraints. *Indian J. Med. Res.* 128, 22–25. PMID: 18820354

Ramlee, S., Rashid, N., and Azirun, M. S. (2022). Odonata nymphs as potential biocontrol agent of mosquito larvae in Malaysia. *Southeast Asian J. Trop. Med. Public Health* 53, 426–435.

Rasgon, J. L., and Scott, T. W. (2004). An initial survey for Wolbachia (Rickettsiales: Rickettsiaceae) infections in selected California mosquitoes (Diptera: Culicidae). *J. Med. Entomol.* 41, 255–257. doi: 10.1603/0022-2585-41.2.255

Rasgon, J. L., Styer, L. M., and Scott, T. W. (2003). Wolbachia-induced mortality as a mechanism to modulate pathogen transmission by vector arthropods. *J. Med. Entomol.* 40, 125–132. doi: 10.1603/002 2-2585-40.2.125

Rassi, A. Jr., Rassi, A., and Marin-Neto, J. A. (2010). Chagas disease. *Lancet* 375, 1388–1402. doi: 10.1016/S0140-6736(10)60061-X

Rawlins, S. C., Clark, G. G., and Martinez, R. (1991). Effects of single introduction of Toxorhynchites moctezuma upon Aedes aegypti on a Caribbean Island. *J. Am. Mosq. Control Assoc.* 7, 7–10.

Resnik, D. B. (2014). Ethical issues in field trials of genetically modified disease-resistant mosquitoes. *Dev. World Bioeth.* 14, 37–46. doi: 10.1111/dewb.12011

Resnik, D. B. (2017). Field trials of genetically modified mosquitoes and public health ethics. *Am. J. Bioeth.* 17, 24–26. doi: 10.1080/15265161.2017.1353170

Reynolds, D. G. (1972). Experimental introduction of a microsporidian into awild population of Culex pipiens fatigans Wied. *Bull. World Health Organ.* 46, 807–812.

Rigau-Perez, J. G., and Gubler, D. J. (1997). "Surveillance for dengue and dengue hemorrhagic fever" in *Dengue and dengue hemorrhagic fever*. eds. D. J. Gubler and G. Kono (London, United Kingdom: CAB International), 405–423.

Rijal, S., Sundar, S., Mondal, D., Das, P., Alvar, J., and Boelaert, M. (2019). Eliminating visceral leishmaniasis in South Asia: the road ahead. *BMJ* 364:k5224. doi: 10.1136/bmj.k5224

Ritchie, S. A., Rapley, L. P., and Benjamin, S. (2010). Bacillus thuringiensis var. israelensis (Bti) provides residual control of Aedes aegypti in small containers. *Am. J. Trop. Med. Hyg.* 82, 1053–1059. doi: 10.4269/ajtmh.2010.09-0603

Ritchie, S. A., Townsend, M., Paton, C. J., Callahan, A. G., and Hoffman, A. A. (2015). Application of wMelPop Wolbachia strain to crash local populations of Aedes aegypti. *PLoS Negl. Trop. Dis.* 9:e0003930. doi: 10.1371/journal.pntd.0003930

Riviere, F., and Thirel, R. (1981). Predation of the copepod Mesocyclops leuckatipilosa (Crustacea) on the larvae of Aedes (Stegomyia) aegypti and ae. (St.) polynesiensis (Diptera: Culicidae): preliminary trials of its use as a biological agent. *Entomophaga* 26, 427–439. doi: 10.1007/BF02374717

Robert, L. I., Perich, M. J., Schlein, Y., Jacobson, R. L., Wirtz, R. A., Lawyer, P. G., et al. (1997). Phlebotomine sand fly control using bait-fed adults to carry the larvicide Bacillus sphaericus to the larval habitat. *J. Am. Mosq. Control Assoc.* 13, 140–144.

Roberts, D. R., and Alecrim, W. D. (1991). Behavioral response of Anopheles darlingi to DDT-sprayed house walls in Amazonia. *Bull. Pan Am. Health Organ.* 25, 210–217.

Roekring, S., and Smith, D. R. (2010). Induction of apoptosis in densovirus infected Aedes aegypti mosquitoes. *J. Invertebr. Pathol.* 104, 239–241. doi: 10.1016/j.jip.2010.04.002

Rohani, A., Fakhriy, H. A., Suzilah, I., Zurainee, M. N., Wan Najdah, W. M. A., Ariffin, M. M., et al. (2020). Indoor and outdoor residual spraying of a novel formulation of deltamethrin K-Othrine® (Polyzone) for the control of simian malaria in Sabah, Malaysia. *PLoS ONE.* 15:e0230860. doi: 10.1371/journal.pone.0230860

Rohani, A., Lokman, H. S., Hassan, A. R., Chan, S. T., Ong, Y. F., Abdullah, A. G., et al. (1999). Bionomics of Anopheles balabacensis Baisas, the principal malaria vectors in Ranau. *Sabah. Trop. Biomed.* 16, 1–8.

Rosen, L., and Gubler, D. J. (1974). The use of mosquitoes to detect and propagate dengue virus. *Am. J. Trop. Med. Hyg.* 23, 1153–1160. doi: 10.4269/ajtmh.1974.23.1153

Ross, P. A., Wiwatanaratanabutr, I., Axford, J. K., White, V. L., Endersby-Harshman, N. M., and Hoffman, A. A. (2017). Wolbachia infections in Aedes aegypti differ markedly in their response to cyclical heat stress. *PLoS Pathog.* 13:e1006006. doi: 10.1371/journal.ppat.1006006

Rupp, H. R. (1996). Adverse assessments of Gambusia affinis: an alternate view for mosquito control practitioners. *J. Am. Mosq. Control Assoc.* 12, 155–166.

Samish, M., and Glazer, I. (2001). Entomopathogenic nematodes for the biocontrol of ticks. *Trends Parasitol.* 17, 368–371. doi: 10.1016/s1471-4922(01)01985-7

Sanad, M. M., Shamseldean, M. S. M., Elgindi, A.-E. Y., and Gaugler, R. (2013). Host penetration and emergence patterns of the mosquito-parasitic mermithids Romanonermis iyengari and Strelkovimermis spiculatus (Nematoda: Mermithidae). *J. Nematol.* 45, 30–38. PMID: 23589657

Sanad, M. M., Sun, J. S., Shamseldean, M. S. M., Wang, Y., and Gaugler, R. (2017). Superparasitism and population regulation of the mosquito-parasitic mermithid nematodes Romanomermis iyengari and Strelkovimermis spiculatus. *J. Nematol.* 49, 168–176. doi: 10.21307/jofnem-2017-078

Sang, R., Arum, S., Chepkorir, E., Mosomtai, G., Tigoi, C., Sigei, F., et al. (2017). Distribution and abundance of key vectors of Rift Valley fever and other arboviruses in two ecologically distinct counties in Kenya. *PLoS Negl. Trop. Dis.* 11:e0005341. doi: 10.1371/journal.pntd.0005341

Sankaralingam, A., and Venkatesan, P. (1989). Larvicidal properties of water bug Diplonychus indicus Venkatesan & Rao and its use in mosquito control. *Indian J. Exp. Biol.* 27, 174–176. PMID: 2807408

Santamarina Mijares, A., and Perez Pacheco, R. (1997). Reduction of mosquito larval densities in natural sites after introduction of Romanomermis culicivorax (Nematoda: Mermithidae) in Cuba. *J. Med. Entomol.* 34, 1–4. doi: 10.1093/jmedent/34.1.1

Schaper, S. (1999). Evaluation of Costa Rican copepods (Crustacea: Eudecapoda) for larval Aedes aegypti control with special reference to *Mesocyclops thermocyclopoides*. *J. Am. Mosq. Control Assoc.* 15, 510–519. PMID: 10612615

Schiller, A., Allen, M., Coffey, J., Fike, A., and Carballo, F. (2019). Updated methods for the production of Toxorhynchites rutilus septentrionalis (Diptera, Culicidae) for use as biocontrol agent against container breeding pest mosquitoes in Harris County, Texas. *J. Insect Sci.* 19:8. doi: 10.1093/jisesa/iez011

Scholte, E. J., Knols, B. G. J., Samson, R. A., and Takken, W. (2004). Entomopathogenic fungi for mosquito control: a review. *J. Insect Sci.* 4:19. doi: 10.1093/jis/4.1.19

Scholte, E.-J., Takken, W., and Knols, B. G. J. (2007). Infection of adult Aedes aegypti and ae. Albopictus mosquitoes with the entomopathogenic fungus Metarhizium anisopliae. *Acta Trop.* 102, 151–158. doi: 10.1016/j.actatropica.2007.04.011

Scola, B. L., Bandi, C., and Raoult, D. (2015). "Wolbachia" in *Bergey's manual of systematics of archaea and bacteria*. eds. W. B. Whitman, F. Rainey, P. Kampfer, M. Trujillo, J. Chun and P. DeVoset al (Hoboken, NJ: John Wiley & Sons, Inc), 1–12.

Scott, W. N. (1966). Tsetse control by game destruction. *Oryx* 8, 244–248. doi: 10.1017/S0030605300 004816

Scott, T. W., Naksathit, A., Day, J. F., Kittayapong, P., and Edman, J. D. (1997). A fitness advantage for Aedes aegypti and the viruses it transmits when females feed only on human blood. *Am. J. Trop. Med. Hyg.* 57, 235–239. doi: 10.4269/ajtmh.1997.57.235

Sebastian, A., Sein, M. M., Thu, M. M., and Corbet, P. S. (1990). Suppression of Aedes aegypti (Diptera: Culicidae) using augmentative release of dragonfly larvae (Odonata: Libellulidae) with community participation in Yangon, Myanmar. *Bull. Entomol. Res.* 80, 223–232. doi: 10.1017/S00074853000134 68

Sebastian, A., Thu, M. M., Kyaw, M., and Sein, M. M. (1980). The use of dragonfly nymphs in the control of Aedes aegypti. *Southeast Asian J. Trop. Med. Public Health* 11, 104–107.

Secundio, N. F. C., Araujo, M. S. S., Oliveira, G. H. B., Massara, C. L., Carvalho, O. S., Lanfredi, R. M., et al. (2002). Preliminary description of a new entomoparasitic nematode infecting Lutzomyia longipalpis sand fly, the vector of visceral leishmaniasis in the New World. *J. Invertebr. Pathol.* 80, 35–40. doi: 10.1016/s0022-2011(02)00046-0

Selvarajan, R., and Kakkassery, F. K. (2019). Predatory potential of water bugs against the filarial vector Culex quinquefasciatus say. *Indian J. Entomol.* 81:40. doi: 10.5958/0974-8172.2019.00035.X

Seo, H. J., Kim, H. C., Klein, T. A., Ramey, A. M., Lee, J. H., Kyung, S. G., et al. (2013). Molecular detection and genotyping of Japanese encephalitis virus in mosquitoes during a 2010 outbreak in the Republic of Korea. *PLoS One* 8:e55165. doi: 10.1371/journal.pone.0055165

Serebrovsky, A. S. (1940). On the possibility of a new method for the control of insect pests. *Zool. Zhurnal* 19, 618–630.

Sexton, D. J., Dwyer, B., Kemp, R., and Graves, S. (1991). Spotted fever group rickettsial infections in Australia. *Rev. Infect. Dis.* 13, 876–886. doi: 10.1093/clinids/13.5.876

Shaalan, E. A.-S., and Canyon, D. V. (2009). Aquatic insect predators and mosquito control. *Trop. Biomed.* 26, 223–261. PMID: 20237438

Shaalan, E. A.-S., Canyon, D. V., Muller, R., Younes, M. W. F., Abdel-Wahab, H., and Mansour, A.-H. (2007). A mosquito predator survey in Townsville, Australia, and an assessment of *Diplonychyus* sp. and *Anisops* sp. predatorial capacity against Culex annulirostris mosquito immatures. *J. Vector Ecol.* 32, 16–21. doi: 10.3376/1081-1710(2007)32[16:ampsit]2.0.co;2

Sharma, V. P. (1976). Elimination of aziridine residues from chemosterilized mosquitoes. *Nature* 261:135. doi: 10.1038/261135a0

Sharma, V. P., Patterson, R. S., LaBrecque, G. C., and Singh, K. R. P. (1976). Three field releases with chemosterilized Culex pipiens fatigans Wied in Delhi villages. *J. Commun. Dis.* 8, 18–27.

Shirai, A., Tanskul, P. L., and Andre, R. G. (1981). Rickettsia tsutsugamushi strains found in chiggers collected in Thailand. *Southeast Asian J. Trop. Med. Public Health* 12, 1–6.

Shirato, K., Miyoshi, H., Kariwa, H., and Takashima, I. (2005). Detection of West Nile virus and Japanese encephalitis virus using real-time PCR with a probe common to both viruses. *J. Virol. Methods* 126, 119–125. doi: 10.1016/j.jviromet.2005.02.001

Shore, H. (1961). O'nyong-nyong fever: an epidemic virus disease in East Africa. III. Some clinical and epidemiological observations in the northern province of Uganda. *Trans. R. Soc. Trop. Med. Hyg.* 55, 361–373. doi: 10.1016/0035-9203(61)90106-7

Short, N. J., and Norval, R. A. I. (1982). Tick predation by shrews in Zimbabwe. *J. Parasitol.* 68:1052. doi: 10.2307/3281090

Siler, J. F., Hall, M. W., and Hitchens, A. (1926). Dengue: its history, epidemiology, mechanism of transmission, etiology, clinical manifestations, immunity, and prevention. *Philipp. J. Sci.* 29, 1–304.

Singh, B., and Daneshvar, C. (2013). Human infections and detection of plasmodium knowlesi. *Clin. Microbiol. Rev.* 26, 165–184. doi: 10.1128/CMR.00079-12

Singh, R. K., Dhiman, R. C., and Singh, S. P. (2003). Laboratory studies on the predatory potential of dragon-fly nymphs on mosquito larvae. *J. Commun. Dis.* 35, 96–101. PMID: 15562955

Singh, N., Mishra, J., Singh, R., and Singh, S. (2013). Animal reservoirs of visceral leishmaniasis in India. *J. Parasitol.* 99, 64–67. doi: 10.1645/GE-3085.1

Sinka, M. E., Bangs, M. J., Manguin, S., Chareonviriyaphap, T., Patil, A. P., Temperley, W. H., et al. (2011). The dominant anopheles vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic précis. *Parasit. Vectors* 4:89. doi: 10.1186/1756-3305-4-89

Sinka, M. E., Bangs, M. J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., et al. (2012). A global map of dominant malaria vectors. *Parasit. Vectors* 5:69. doi: 10.1186/1756-3305-5-69

Sinka, M. E., Rubio-Palis, Y., Manguin, S., Patil, A. P., Temperley, W. H., Gething, P. W., et al. (2010). The dominant anopheles vectors of human malaria in the Americas: occurrence data, distribution maps and bionomic précis. *Parasit. Vectors* 3:72. doi: 10.1186/1756-3305-3-72

Sjöstedt, A. (2007). Tularemia: history, epidemiology, pathogen physiology, and clinical manifestations. *Ann. N. Y. Acad. Sci.* 1105, 1–29. doi: 10.1196/annals.1409.009

Smithuis, F. M., Kyaw, M. K., Phe, U. O., van der Broek, I., Katterman, N., Rogers, C., et al. (2013). Entomological determinants of insecticide-treated bed net effectiveness in Western Myanmar. *Malar. J.* 12:364. doi: 10.1186/1475-2875-12-364

Soh, S., Ho, S. H., Seah, A., Ong, J., Dickens, B. S., Tan, K. W., et al. (2021). Economic impact of dengue in Singapore from 2010 to 2020 and the cost-effectiveness of Wolbachia interventions. *PLoS Glob. Public Health* 1:e0000024. doi: 10.1371/journal.pgph.0000024

Soper, F. L., and Wilson, D. B. (1943). *Anopheles gambiae in Brazil 1930 to 1940*. New York: Rockfeller Foundation.

Soumare, M. K. F., and Cilek, J. E. (2011). The effectiveness of Mesocyclops longisetus (Copepoda) for the control of container-inhabiting mosquitoes in residential environments. *J. Am. Mosq. Control Assoc.* 27, 376–383. doi: 10.2987/11-6129.1

Sousa, C. A., Pinto, J., Almeida, P. G., Ferreira, C., Do Rosário, V. E., and Charlwood, J. D. (2001). Dogs as a favored host choice of Anopheles gambiae sensu stricto (Diptera: Culicidae) of São Tomé, West Africa. *J. Med. Entomol.* 38, 122–125. doi: 10.1603/0022-2585-38.1.122

Spielman, A. (1976). Human babesiosis on Nantucket Island: transmission by nymphal Ixodes ticks. *Am. J. Trop. Med. Hyg.* 25, 784–787. doi: 10.4269/ajtmh.1976.25.784

Stabler, R. M. (1952). Parasitism of mosquito larvae by mermithids (Nematoda). *J. Parasitol.* 38, 130–132. doi: 10.2307/3273830

Steere, A. C. (2001). Lyme disease. *N. Engl. J. Med.* 345, 115–125. doi: 10.1056/NEJM20010712345 0207

Steffan, W. A., and Evenhuis, N. L. (1981). Biology of Toxorhynchites. *Annu. Rev. Entomol.* 26, 159–181. doi: 10.1146/annurev.en.26.010181.001111

Steverding, D. (2017). The history of leishmaniasis. *Parasit. Vectors* 10:82. doi: 10.1186/s13071-017-2028-5

Steyn, J., Fourie, I., Steyl, J., Williams, J., Stivaktas, V., Botha, E., et al. (2020). Zoonotic alphaviruses in fatal and neurologic infections in wildlife and nonequine domestic animals, South Africa. *Emerg. Infect. Dis.* 26, 1182–1191. doi: 10.3201/eid2606.191179

Stouthamer, R., Breeuwer, J. A., and Hurst, G. D. (1999). Wolbachia pipientis: microbial manipulator of arthropod reproduction. *Annu. Rev. Microbiol.* 53, 71–102. doi: 10.1146/annurev.micro.53.1.71

Subramaniam, J., Murugan, K., Panneerselvam, C., Kovendan, K., Madhiyazhagan, P., Kumar, P. M., et al. (2015). Eco-friendly control of malaria and arbovirus vectors using the mosquito fish Gambusia affinis and ultra-low dosages of Mimusops elengi-synthesized silver nanoparticles: towards an integrative approach. *Environ. Sci. Pollut. Res.* 22, 20067–20083. doi: 10.1007/s11356-015-5253-5

Sucharit, S., Surathin, K., and Shrestha, S. R. (1989). Vectors of Japanese encephalitis virus (JEV): species complexes of the vectors. *Southeast Asian J. Trop. Med. Public Health* 20, 611–621. PMID: 2576966

Suchman, E. L., Kononko, A., Plake, E., Doehling, M., Kleker, B., Black, W. C., et al. (2006). Effects of AeDNV infection on Aedes aegypti lifespan and reproduction. *Biol. Control* 39, 465–473. doi: 10.1016/j.biocontrol.2006.05.001

Sukhacheva, G. A. (1996). Study of the natural diet of adult dragonflies using an immunological method. *Odonatologica* 25, 397–403.

Sulaiman, I. (1992). Infectivity and pathogenicity of Ascogregarina culicinis (Eugregarinida: Lecudinidae) to Aedes aegypti (Diptera: Culicidae). *J. Med. Entomol.* 29, 1–4. doi: 10.1093/jmedent/29.1.1

Swaminath, C. S., Short, H. E., and Anderson, L. A. P. (1942). Transmission of Indian kala-azar to man by the bite of P. argentipes. *Indian J. Med. Res.* 123, 473–477.

Swei, A., Couper, L. I., Coffey, L. L., Kapan, D., and Bennett, S. (2020). Patterns, drivers, and challenges of vector-borne disease emergence. *Vector Borne Zoonotic Dis.* 20, 159–170. doi: 10.1089/vbz.201 8.2432

Szewczyk, B., de Souza, M. L., de Castro, M. E. B., Moscardi, M. L., and Moscardi, F. (2011). "Baculovirus biopesticides" in *Pesticides – formulations, effects, fate*. ed. M. Stoytcheva (Rijeka, Croatia: IntechOpen), 25–36.

Szewczyk, B., Rabalski, L., Krol, E., Sihler, W., and de Souza, M. L. (2009). Baculovirus biopesticides-a safe alternative to chemical protection of plants. *J. Biopest.* 2, 209–216.

Tabashnik, B. E. (1992). Evaluation of synergism among bacillus thuringiensis toxins. *Appl. Environ. Microbiol.* 58, 3343–3346. doi: 10.1128/aem.58.10.3343-3346.1992

Tan, C. H., Vythilingam, I., Matusop, A., Chan, S. T., and Singh, B. (2008). Bionomics of Anopheles latens in Kapit, Sarawak, Malaysian Borneo in relation to the transmission of zoonotic simian malaria parasite plasmodium knowlesi. *Malar. J.* 7:52. doi: 10.1186/1475-2875-7-52

Tangena, J.-A. A., Hendriks, C. M. J., Devine, M., Tammaro, M., Trett, A. E., Williams, I., et al. (2020). Indoor residual spraying for malaria control in sub-saharan Africa 1997 to 2017: an adjusted retrospective analysis. *Malar. J.* 19:150. doi: 10.1186/s12936-020-03216-6

Taylor, R. M., and Hurlbut, H. S. (1953). Isolation of West Nile virus from Culex mosquitoes. *J. Egypt. Med. Assoc.* 36, 199–208.

Taylor, L. H., Latham, S. M., and Woolhouse, M. E. (2001). Risk factors for human disease emergence. *Philos. Trans. R. Soc. B. Biol. Sci.* 356, 983–989. doi: 10.1098/rstb.2001.0888

Tetreau, G., Stalinski, R., David, J.-P., and Després, L. (2013). Monitoring resistance to bacillus thuringiensis subsp. israelensis in the field by performing bioassays with each cry toxin separately. *Mem. Inst. Oswaldo Cruz* 108, 894–900. doi: 10.1590/0074-0276130155

Thatheyus, A. J., and Selvam, A. D. G. (2013). Synthetic pyrethroids: toxicity and biodegradation. *Appl. Ecol. Environ. Sci.* 1, 33–36. doi: 10.12691/aees-1-3-2

Thomas, M. B. (2018). Biological control of human disease vectors: a perspective on challenger and opportunities. *Biocontrol(Dordr)* 63, 61–69. doi: 10.1007/s10526-017-9815-y

Tijsse-Klasen, E., Braks, M., Scholte, E. J., and Sprong, H. (2011). Parasites of vectors-Ixodiphagus hookeri and its Wolbachia symbionts in ticks in the Netherlands. *Parasit. Vectors* 4, 228–234. doi: 10.1186/1756-3305-4-228

Troha, K., Urbancic, N. B., Korva, M., Avsic-Zupanc, T., Battelino, S., and Vozel, D. (2022). Vector-borne tularemia: a re-emerging cause of cervical lymphadenopathy. *Trop. Med. Infect. Dis.* 7:189. doi: 10.3390/tropicalmed7080189

Tsuda, Y., Kobayashi, J., Nambanya, S., Miyagi, I., Toma, T., Phompida, S., et al. (2002). An ecological survey of dengue vector mosquitos in central Lao PDR. *Southeast Asian J. Trop. Med. Public Health* 33, 63–67.

Tucker, D. L., Beresford, C. H., Sigler, L., and Rogers, K. (2004). Disseminated Beauveria bassiana infection in a patient with acute lymphoblastic leukemia. *J. Clin. Microbiol.* 42, 5412–5414. doi: 10.1128/JCM.42.11.5412-5414.2004

Turelli, M. (2010). Cytoplasmic incompatibility in populations with overlapping generations. *Evolution* 64, 232–241. doi: 10.1111/j.1558-5646.2009.00822.x

Ujváry, I. (2010). "Pest control agents from natural products" in *Hayes' handbook of pesticide toxicology*. ed. R. Krieger. *3rd* ed (United States: Academic Press), 119–229.

Ulrich, J. N., Beier, J. C., Devine, G. J., and Hugo, L. E. (2016). Heat sensitivity of wMel Wolbachia during Aedes aegypti development. *PLoS Negl. Trop. Dis.* 10:e0004873. doi: 10.1371/journal/pnt d.0004873

Utzinger, J., Tozan, Y., and Singer, B. H. (2001). Efficacy and cost-effectiveness of environmental management for malaria control. *Tropical Med. Int. Health* 6, 677–687. doi: 10.1046/j.1365-3156.200 1.00769.x

Valentine, M. J., Murdock, C. C., and Kelly, P. J. (2019). Sylvatic cycles of arboviruses in non-human primates. *Parasit. Vectors* 12:463. doi: 10.1186/s13071-019-3732-0

van den Berg, H., da Silva Bezerra, H. S., Al-Eryani, S., Chanda, E., Nagpal, B. N., Knox, T. B., et al. (2021a). Recent trends in global insecticide use for disease vector control and potential implications for resistance management. *Sci. Rep.* 11:23867. doi: 10.1038/s41598-021-03367-9

van den Berg, H., Kelly-Hope, L. A., and Lindsay, S. W. (2013). Malaria and lymphatic filariasis: the case for integrated vector management. *Lancet Infect. Dis.* 13, 89–94. doi: 10.1016/S1473-3099(12)70 148-2

van den Berg, H., Velayudhan, R., and Yadav, R. S. (2021b). Management of insecticides for use in disease vector control: lessons from six countried in Asia and the Middle East. *PLoS Negl. Trop. Dis.* 15:e0009358. doi: 10.1371/journal.pntd.0009358

van den Berg, H., Zaim, M., Yadav, R. S., Soares, A., Ameneshewa, B., Mnzava, A. P., et al. (2012). Global trends in the use of insecticides to control vector-borne diseases. *Environ. Health Perspect.* 120, 577–582. doi: 10.1289/ehp.1104340

van den Bosch, R., Messenger, P. S., and Gutierrez, A. P. (1982). "The history and development of biological control" in *An introduction to biological control*. eds. R. Bosch, P. S. Messenger and A. P. Gutierrez (US: Springer), 21–36.

van den Hurk, A. F., Hall-Mendelin, S., Pyke, A. T., Frentiu, F. D., McElroy, K., Day, A., et al. (2012). Impact of Wolbachia on infection with chikungunya and yellow fever viruses in the mosquito vector Aedes aegypti. *PLoS Negl. Trop. Dis.* 6:e1892. doi: 10.1371/journal.pntd.0001892

van den Hurk, A. F., Montgomery, B. L., Northill, J. A., Smith, I. L., Zborowski, P., Ritchie, S. A., et al. (2006). Short report: the first isolation of Japanese encephalitis virus from mosquitoes collected from mainland Australia. *Am. J. Trop. Med. Hyg.* 75, 21–25. doi: 10.4269/ajtmh.2006.75.21

van Schalkwyk, D. A., Moon, R. W., Blasco, B., and Sutherland, C. J. (2017). Comparison of the susceptibility of plasmodium knowlesi and plasmodium falciparum to antimalarial agents. *J. Antimicrob. Chemother.* 72, 3051–3058. doi: 10.1093/jac/dkx279

van Someren, V. D. (1951). The red-billed oxpecker and its relation to stock in Kenya. *East Afr. Agric. J.* 17, 1–11. doi: 10.1080/03670074.1951.11664775

Vatandoost, H. (2021). Dragonflies as an important aquatic predator insect and their potential for control of vectors for different diseases. *J. Mar. Sci.* 3, 13–20. doi: 10.30564/jms.v3i3.3121

Venkatesan, P., and Jeyachandra, C. M. (1985). Estimation of mosquito predation by the water bug Diplonychus indicus Venkatesan & Rao. *Indian J. Exp. Biol.* 23, 227–229.

Vershini, R. A., and Kanagappan, M. (2014). Effect of quantity of water on the feeding efficiency of dragonfly nymph – Bradynopyga geminate (Rambur). *J. Entomol. Zool. Stud.* 2, 249–252.

Vinogradov, D. D., Sinev, A. Y., and Tiunov, A. V. (2022). Predators as control agents of mosquito larvae in micro-reservoirs (review). *Inland Water Biol.* 15, 39–53. doi: 10.1134/S1995082922010138

Vorou, R. (2016). Zika virus, vectors, reservoirs, amplifying hosts, and their potential to spread worldwide: what we know and what we should investigate urgently. *Int. J. Infect. Dis.* 48, 85–90. doi: 10.1016/j.ijid.2016.05.014

Vreysen, M. J. B., Saleh, K., Mramba, F., Parker, A., Feldmann, U., Dyck, V. A., et al. (2014). Sterile insects to enhance agricultural development: the case of sustainable tsetse eradication on Unguja Island, Zanzibar, using an area-wide integrated pest management approach. *PLoS Negl. Trop. Dis.* 8:e2857. doi: 10.1371/journal.pntd.0002857

Vu, S. N., Nguyen, T. Y., Kay, B. H., Marten, G. G., and Reid, J. W. (1998). Eradication of Aedes aegypti from a village in Vietnam, using copepods and community participation. *Am. J. Trop. Med. Hyg.* 59, 657–660. doi: 10.4269/ajtmh.1998.59.657

Vu, S. N., Nguyen, T. Y., Tran, V. P., Truong, U. N., Le, Q. M., Le, V. L., et al. (2005). Elimination of dengue by community programs using Mesocyclops (Copepoda) against Aedes aegypti in Central Vietnam. *Am. J. Trop. Med. Hyg.* 72, 67–73. doi: 10.4269/ajtmh.2005.72.67

Vythilingam, I. (2012). Plasmodium knowlesi and Wuchereria bancrofti: their vectors and challenges for the future. *Front. Physiol.* 3:115. doi: 10.3389/fphys.2012.00115

Vythilingam, I., Chua, T. H., Liew, J. W. K., O'Mannin, B., and Ferguson, H. M. (2021). The vectors of plasmodium knowlesi and other simian malarias Southeast Asia: challenges in malaria elimination. *Adv. Parasitol.* 113, 131–189. doi: 10.1016/bs.apar.2021.08.005

Vythilingam, I., and Hii, J. (2013). "Simian malaria parasites: special emphasis on plasmodium knowlesi and their anopheles vectors in Southeast Asia" in *Anopheles mosquitoes-New insights into malaria vectors*. ed. S. Manguin (London: Intech Open), 487–510.

Vythilingam, I., Keokenchan, K., Phommakot, S., Nambanya, S., and Inthakone, S. (2001). Preliminary studies of anopheles mosquitos in eight provinces in Lao PDR. *Southeast Asian J. Trop. Med. Public Health* 32, 83–87.

Vythilingam, I., Lim, Y. A. L., Venugopalan, B., Ngui, R., Leong, C. S., Wong, M. L., et al. (2014). Plasmodium knowlesi malaria an emerging public health problem in Hulu Selangor, Selangor Malaysia (2009-2013): epidemiologic and entomologic analysis. *Parasit. Vectors* 7, 436–449. doi: 10.1186/175 6-3305-7-436

Vythilingam, I., Oda, K., Chew, T. K., Mahadevan, S., Vijayamalar, B., Morita, K., et al. (1995). Isolation of Japanese encephalitis virus from mosquitoes collected in Sabak Bernam, Selangor, Malaysia in 1992. *J. Am. Mosq. Control Assoc.* 11, 94–98.

Vythilingam, I., Oda, K., Mahadevan, S., Abdullah, G., Thim, C. S., Hong, C. C., et al. (1997). Abundance, parity, and Japanese encephalitis virus infection of mosquitoes (Diptera: Culicidae) in Sepang district, Malaysia. *J. Med. Entomol.* 34, 257–262. doi: 10.1093/jmedent/34.3.257

Vythilingam, I., Oda, K., Tsuchie, H., Mahadevan, S., and Vijayamalar, B. (1994). Isolation of Japanese encephalitis virus from Culex sitiens mosquitoes in Selangor, Malaysia. *J. Am. Mosq. Control Assoc.* 10, 228–229. PMID: 8965071

Vythilingam, I., Sidavong, B., Chan, S., Phonemixay, T., Vanisaveth, V., Sisoulad, P., et al. (2005). Epidemiology of malaria in Attapeu Province, Lao PDR in relation to entomological parameters. *Trans. R. Soc. Trop. Med. Hyg.* 99, 833–839. doi: 10.1016/j.trstmh.2005.06.012

Vythilingam, I., Tan, C., Asmad, M., Chan, S., Lee, K., and Singh, B. (2006). Natural transmission of plasmodium knowlesi to humans by Anopheles latens in Sarawak, Malaysia. *Trans. R. Soc. Trop. Med. Hyg.* 100, 1087–1088. doi: 10.1016/j.trstmh.2006.02.006

Vythilingam, I., Wan-Sulaiman, W. Y., and Jeffery, J. (2013). "Vectors of malaria and Filariasis in Southeast Asia: a changing scenario" in *Parasites and their vectors-a special focus on Southeast Asia*. eds. Y. A. Lim and I. Vythilingam (Vienna: Springer), 57–75.

Walker, T., Johnson, P. H., Moreira, L. A., Iturbe-Ormaetxe, I., Frentiu, F. D., McMeniman, C. J., et al. (2011). The wMel Wolbachia strain blocks dengue and invades caged Aedes aegypti populations. *Nature* 476, 450–453. doi: 10.1038/nature10355

Walker, K., and Lynch, M. (2007). Contributions of anopheles larval control to malaria suppression in tropical Africa: review of achievements and potential. *Med. Vet. Entomol.* 21, 2–21. doi: 10.1111/j.1365-2915.2007.00674.x

Walter, G., and Weber, G. (1981). A study on the transmission (transstadial, transovarial) of Babesia microti, strain Hannover i, in its tick vector, Ixodes ricinus. *Tropenmed Parasitol* 32, 228–230.

Walton, W. E. (2007). Larvivorous fish including Gambusia. *J. Am. Mosq. Control Assoc.* 23, 184–220. doi: 10.2987/8756-971X(2007)23[184:LFIG]2.0.CO;2

Wang, Z. Y., He, K. L., Zhang, F., Lu, X. S., and Babendreier, D. (2014). Mass rearing and release of Trichogramma for biological control of insect pests of corn in China. *Biol. Control* 68, 136–144. doi: 10.1016/j.biocontrol.2013.06.015

Wang, Z. Z., Liu, Y. Q., Shi, M., Huang, J. H., and Chen, X. X. (2019). Parasitoid wasps as effective biological control agents. *J. Integr. Agric.* 18, 705–715. doi: 10.1016/S2095-3119(18)62078-7

Warburg, A., and Pimenta, P. F. (1995). A cytoplasmic polyhedrosis virus in the phlebotomine sand fly Lutzomyia longipalpis. *Med. Vet. Entomol.* 9, 211–213. doi: 10.1111/j.1365-2915.1995.tb00182.x

Warren, M., and Wharton, R. (1963). The vectors of simian malaria: identity, biology, and geographical distribution. *J. Parasitol.* 49, 892–904. doi: 10.2307/3275715

Watsons, M. (1921). *The prevention of malaria in the federated Malay states*. Liverpool: The Liverpool School of Tropical Medicine

Weeks, P. (1999). Interaction between red-billed oxpeckers, Buphagus erythrorhynchus and domestic cattle, Bos taurus, in Zimbabwe. *Anim. Behav.* 58, 1253–1259. doi: 10.1006/anbe.1999.1265

Wei, W., Shao, D., Huang, X., Li, J., Chen, H., Zhang, Q., et al. (2006). The pathogenicity of mosquito densovirus (C6/36DNV) and its interaction with dengue virus type II in Aedes albopictus. *Am. J. Trop. Med. Hyg.* 75, 1118–1126. doi: 10.4269/ajtmh.2006.75.1118

Weidhaas, D. E., Schidmt, C. H., and Seabrook, E. L. (1962). Field studies on the release of sterile males for the control of Anopheles quadrimaculatus. *Mosq. News* 22, 283–291.

Weitzel, T., Silva-de la Fuente, M. C., Martínez-Valdebenito, C., Stekolnikov, A. A., Pérez, C., Pérez, R., et al. (2022). Novel vector of scrub typhus in sub-Antarctic Chile: evidence from human exposure. *Clin. Infect. Dis.* 74, 1862–1865. doi: 10.1093/cid/ciab748

Weng, M. H., Lien, J. C., Wang, Y. M., Lin, C. C., Lin, H. C., and Chin, C. (1999). Isolation of Japanese encephalitis virus from mosquitoes collected in northern Taiwan between 1995 and 1996. *J. Microbiol. Immunol. Infect.* 32, 9–13.

Werner, D., and Pont, A. C. (2003). Dipteran predators of Simuliid blackflies: a worldwide review. *Med. Vet. Entomol.* 17, 115–132. doi: 10.1046/j.1365-2915.2003.00431.x

Werren, J. H. (1997). Biology of Wolbachia. *Annu. Rev. Entomol.* 42, 587–609. doi: 10.1146/annure v.ento.42.1.587

Werren, J. H., Baldo, L., and Clark, M. E. (2008). Wolbachia: master manipulators of invertebrate biology. *Nat. Rev. Microbiol.* 6, 741–751. doi: 10.1038/nrmicro1969

Werren, J. H., Wan, Z., and Li, R. G. (1995). Evolution and phylogeny of Wolbachia: reproductive parasites of arthropods. *Proc. R. Soc. Lond. B Biol. Sci.* 261, 55–63. doi: 10.1098/rspb.1995.0117

Wheeler, C. M. (1946). Control of typhus in Italy 1943-1944 by use of DDT. *Am. J. Public Health* 36, 119–129. doi: 10.2105/ajph.36.2.119

WHO (2012). *Handbook for integrated vector management*. Geneva, Switzerland: World Health Organization.

WHO (2013). *Lymphatic filariasis: A handbook of practical entomology for national lymphatic filariasis elimination programs*. Italy: WHO.

WHO (2015). Chagas disease in Latin America: an epidemiological update based on 2010 estimates. *World Health Organ. Weekly Epidemiol. Record* 90, 33–44.

Wilke, A. B. B., Chase, C., Vasquez, C., Carvajal, A., Medina, J., Petrie, W. D., et al. (2019). Urbanization creates diverse aquatic habitats for immature mosquitoes in urban areas. *Sci. Rep.* 9:15335. doi: 10.1038/s41598-019-51787-5

Wilkerson, R. C., Linton, Y. M., and Strickman, D. (2021). *Mosquitoes of the world*, Vol *1*. Baltimore: John Hopkins University Press.

Wilson, A. L., Courtenay, O., Kelly-Hope, L. A., Scott, T. W., Takken, W., Torr, S. J., et al. (2020). The importance of vector control for the control and elimination of vector-borne diseases. *PLoS Negl. Trop. Dis.* 14:e0007831. doi: 10.1371/journal.pntd.0007831

Wilson, M. L., Levine, J. F., and Spielman, A. (1985). Mice as reservoirs of the Lyme disease spirochete. *Am. J. Trop. Med. Hyg.* 34, 355–360. doi: 10.4269/ajtmh.1985.34.355

Windbichler, N., Papathanos, P. A., Catteruccia, F., Ranson, H., Burt, A., and Crisanti, A. (2007). Homing endonuclease mediated gene targeting in Anopheles gambiae cells and embryos. *Nucleic Acid Res.* 35, 5922–5933. doi: 10.1093/nar/gkm632

Wiwatanaratanabutr, I., and Kittayapong, P. (2009). Effects of crowding and temperature on Wolbachia infection density among life cycle stages of Aedes albopictus. *J. Invertebr. Pathol.* 102, 220–224. doi: 10.1016/j.jip.2009.08.009

Wong, M. L., Chua, T. H., Leong, C. S., Khaw, L. T., Fornace, K., Wan Yusoff, W. S., et al. (2015). Seasonal and spatial dynamics of the primary vector of plasmodium knowlesi within a major transmission focus in Sabah, Malaysia. *PLoS Negl. Trop. Dis.* 9:e0004135. doi: 10.1371/journal.pntd.0004135

Woolhouse, M. E., and Gowtage-Sequeria, S. (2005). Host range and emerging and reemerging pathogens. *Emerg. Infect. Dis.* 11, 1842–1847. doi: 10.3201/eid1112.050997

World Mosquito Program (2022). Global Progress [online]. Available at: https://www.worldmosquito program.org/en/global-progress (Accessed November 13, 2022).

Wu, D., Johnson, J. J., and Federici, B. A. (1994). Synergism of mosquitocidal toxicity between CytA and CryIBD proteins using inclusions produced from cloned genes of bacillus thuringiensis. *Mol. Microbiol.* 13, 965–972. doi: 10.1111/j.1365-2958.1994.tb00488.x

Wu, M., Sun, L. V., Vamathevan, J., Riegler, M., Deboy, R., Brownlie, J. C., et al. (2004). Phylogenomics of the reproductive parasite Wolbachia pipientis wMel: a streamlined genome overrun by mobile genetic elements. *PLoS Biol.* 2:e69:E69. doi: 10.1371/journal.pbio.0020069

Wu, W. K., and Tesh, R. B. (1989). Experimental infection of old and New World phlebotomine sandflies (Diptera: Psychodidae) with Ascogregarina chagasi (Eugregarinida: Lecudinidae). *J. Med. Entomol.* 26, 237–242. doi: 10.1093/jmedent/26.4.237

Xi, Z., Khoo, C. C. H., and Dobson, S. L. (2005). Wolbachia establishment and invasion in an Aedes aegypti laboratory population. *Science* 310, 326–328. doi: 10.1126/science.1117607

Ximena, J. N., Robert, R. J., and Godfrey, S. (2005). Use of anopheles-specific prey-capture behaviour by the small juveniles of Evarcha culicivora, a mosquito-eating jumping spider. *J. Arachnol.* 33, 541–548. doi: 10.1636/05-3.1

Yeni, D. K., Büyük, F., Ashraf, A., and Shah, M. S. U. D. (2021). Tularemia: a re-emerging tick-borne infectious disease. *Folia Microbiol.* 66, 1–14. doi: 10.1007/s12223-020-00827-z

Young, P. R., Ng, L. F. P., Hall, R. A., Smith, D. W., and Johansen, C. A. (2014). "Arbovirus infections" in *Manson's tropical infectious diseases*. eds. J. Farrar, T. Junghanss, D. Lalloo, P. J. Hotez, G. Kang and N. J. White. *23rd* ed (Philadelphia: Elsevier/Saunders Ltd), 129–161.

Zahedi, M., and White, G. B. (1994). Filaria vector competence of some anopheles species. *Trop. Med. Parasitol.* 45, 27–32. PMID: 8066378

Zakaria, N. D., and Avoi, R. (2022). Prevalence and risk factors for positive lymphatic filariasis antibody in Sabah, Malaysia: a cross-sectional study. *Trans. R. Soc. Trop. Med. Hyg.* 116, 369–374. doi: 10.1093/trstmh/trab132

Zhang, J. L., and Qiao, C. L. (2002). Novel approaches for remediation of pesticide pollutants. *Int. J. Environ. Pollut.* 18, 423–433. doi: 10.1504/IJEP.2002.002337

Zhao, J., Song, R., Li, H., Zheng, Q., Li, S., Liu, L., et al. (2022). New formulation to accelerate the degradation of pesticide residues: composite nanoparticles of imidacloprid and 24-epibrassinolide. *ACS Omega.* 7, 29027–29037. doi: 10.1021/acsomega.2c02820

Zheng, X., Zhang, D., Li, Y., Yang, C., Wu, Y., Liang, X., et al. (2019). Incompatible and sterile insect techniques combined eliminate mosquitoes. *Nature* 572, 56–61. doi: 10.1038/s41586-019-1407-9

Zhioua, E., Lebrun, R. A., Ginsberg, H. S., and Aeschlimann, A. (1995). Pathogeneticity of Steinertma carpocapsae and S. glaseri (Nematoda: Steinernematidae) to Ixodes scapularis (Acari: Ixodidae). *J. Med. Entomol.* 32, 900–905. doi: 10.1093/jmedent/32.6.900

Zoppi de Roa, E., Gordon, E., Montiel, E., Delgado, L., Berti, J., and Ramos, S. (2002). Association of cyclopoid copepods with the habitat of the malaria vector Anopheles aquasalis in the peninsula of Paria, Venezuela. *J. Am. Mosq. Control Assoc.* 18, 47–51. PMID: 11998930

Zuharah, W. F., Fadzly, N., Yusof, N. A., and Dieng, H. (2015). Risky behaviors: effects of Toxorhynchites splendens (Diptera: Culicidae) predator on the behavior of three mosquito species. *J. Insect Sci.* 15:128. doi: 10.1093/jisesa/iev115

frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 06 April 2023
DOI 10.3389/fmicb.2023.1156061



OPEN ACCESS

EDITED BY
Wenn-Chyau Lee,
University of Malaya,
Malaysia

REVIEWED BY
Danilo Ciccone Miguel,
State University of Campinas,
Brazil
Sueli Fumie Yamada-Ogatta,
State University of Londrina,
Brazil

*CORRESPONDENCE
Narissara Jariyapan
✉ narissara.j@chula.ac.th

SPECIALTY SECTION
This article was submitted to Infectious Agents and Disease, a section of the journal Frontiers in Microbiology

RECEIVED 01 February 2023
ACCEPTED 15 March 2023
PUBLISHED 06 April 2023

CITATION
Mano C, Kongkaew A, Tippawangkosol P, Somboon P, Roytrakul S, Pescher P, Späth GF, Uthaipibull C, Tantiworawit A, Siriyasatien P and Jariyapan N (2023) Amphotericin B resistance correlates with increased fitness *in vitro* and *in vivo* in *Leishmania* (*Mundinia*) *martiniquensis*. *Front. Microbiol.* 14:1156061.
doi: 10.3389/fmicb.2023.1156061



# Amphotericin B resistance correlates with increased fitness *in vitro* and *in vivo* in *Leishmania* (*Mundinia*) *martiniquensis*

Chonlada Mano[1], Aphisek Kongkaew[2], Pongsri Tippawangkosol[1], Pradya Somboon[1], Sittiruk Roytrakul[3], Pascale Pescher[4], Gerald F. Späth[4], Chairat Uthaipibull[5], Adisak Tantiworawit[6], Padet Siriyasatien[7] and Narissara Jariyapan[7]*

[1]Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, [2]Animal House Unit, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, [3]National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Pathum Thani, Thailand, [4]Institut Pasteur, INSERM U1201, Université Paris Cité, Unité de Parasitologie Moléculaire et Signalisation, Paris, France, [5]Thailand Center of Excellence for Life Sciences (TCELS), Bangkok, Thailand, [6]Department of Internal Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, [7]Center of Excellence in Vector Biology and Vector-Borne Disease, Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Amphotericin B (AmpB) deoxycholate is the available first-line drug used to treat visceral leishmaniasis caused by *Leishmania* (*Mundinia*) *martiniquensis*, however, some cases of AmpB treatment failure have been reported in Thailand. Resistance to drugs is known to affect parasite fitness with a potential impact on parasite transmission but still little is known about the effect of resistance to drugs on *L. martiniquensis*. Here we aimed to gain insight into the fitness changes occurring after treatment failure or *in vitro*-induced resistance to AmpB. *L. martiniquensis* parasites isolated from a patient before (LSCM1) and after relapse (LSCM1-6) were compared for *in vitro* and *in vivo* fitness changes together with an *in vitro* induced AmpB-resistant parasite generated from LSCM1 parasites (AmpBRP2i). Results revealed increased metacyclogenesis of the AmpBPR2i and LSCM1-6 strains (AmpB-resistant strains) compared to the LSCM1 strain and increased fitness with respect to growth and infectivity. The LSCM1-6 and AmpBRP2i strains were present in mice for longer periods compared to the LSCM1 strain, but no clinical signs of the disease were observed. These results suggest that the AmpB-resistant parasites could be more efficiently transmitted to humans and maintained in asymptomatic hosts longer than the susceptible strain. The asymptomatic hosts therefore may represent "reservoirs" for the resistant parasites enhancing transmission. The results in this study advocate an urgent need to search and monitor for AmpB-resistant *L. martiniquensis* in patients with relapsing leishmaniasis and in asymptomatic patients, especially, in HIV/*Leishmania* coinfected patients.

KEYWORDS

***Leishmania*, *Leishmania martiniquensis*, leishmaniasis, fitness, Amphotericin B, drug resistance, relapse, Thailand**

# Introduction

*Leishmania* parasites cause a group of anthropozoonoses called leishmaniases, which are important neglected tropical diseases. Metacyclic promastigotes, the infectious form of the parasite, are inoculated to mammalian hosts during the blood meal of infected insect vectors, where they are efficiently phagocytosed by cells of the mononuclear phagocytic system, notably macrophages. There, promastigotes differentiate into amastigote forms whose intracellular replication will ultimately cause the immunopathologies associated with the different forms of leishmaniasis, which include cutaneous, mucocutaneous, and visceral leishmaniases (VL). Their clinical spectrum ranges from asymptomatic infection or self-limiting cutaneous lesions to lethal disseminated infections depending on the species of *Leishmania* parasite and the host's immune response. *L. martiniquensis* is a newly emerging causative agent of human leishmaniasis first reported from Martinique Island (French West Indies) (Desbois et al., 2014) and later found in many countries including Thailand. Most human cases present clinical features of VL, however, HIV-coinfected patients also develop disseminated cutaneous leishmaniasis (Pothirat et al., 2014; Chiewchanvit et al., 2015). In the absence of vaccines against human leishmaniasis, chemotherapy is still the only alternative to tackle the disease despite the emergence of drug resistance (World Health Organization, 2022).

Amphotericin B deoxycholate is a widely used antifungal and antiprotozoal compound. It binds to ergosterol molecules presenting in the membrane of fungi or trypanosomatids, which causes depolarization of the membrane and alters membrane permeability towards cations, water, and glucose molecules resulting in ions leakage and ultimately leading to cell death. This drug has been introduced for the treatment of VL in antimonial unresponsive patients from Bihar in India, but two clinical AmpB-resistant *Leishmania donovani* strains have already been reported (Srivastava et al., 2011; Purkait et al., 2012). Most leishmaniasis cases in Thailand are treated with AmpB (Jariyapan et al., 2018) but recurrence of the disease after treatment has been reported in some cases for both immunocompetent and immunocompromised patients, including HIV-infected individuals (Osatakul et al., 2014; Siriyasatien et al., 2016).

Resistance phenotype to overcome drug treatment often comes with fitness changes, fitness being defined as a complex integrated skill that allows microorganisms to successfully replicate in a defined environment (Natera et al., 2007). *Leishmania* parasites are highly adaptive microorganisms as they must survive and replicate within different environments depending on their hosts (insect and mammal) to be transmitted. In this context, the ability of *Leishmania* parasites to resist the activity of an antileishmanial drug is shown to have an impact on their fitness in one or both hosts which can vary depending on the drug and the parasite species. Differences in parasites viability, growth, metacyclic promastigote generation, and infectivity in laboratory animals have been reported for different species or strains of *Leishmania* parasites resistant to different anti-leishmanial drugs (García-Hernández et al., 2015; Hendrickx et al., 2015; Turner et al., 2015). Experimentally generated *L. donovani* strains that are resistant to single anti-leishmanial drugs (AmpB, miltefosine (MIL), paromomycin (PMM), and trivalent antimony ($Sb^{III}$)) or to drug combinations (AmB-MIL, AmB-PMM, AmB-$Sb^{III}$, MIL-PMM, and $Sb^{III}$-PMM) present a higher promastigote survival rate in conditions of starvation, a higher tolerance to heat shock and pH stress, and an increased survival rate for *in vitro* macrophage infections compared to their corresponding wild type (García-Hernández et al., 2015). MIL-resistant *L. major* populations generated *in vitro* using stepwise selection exhibited a similar growth rate and response to stress as the wild-type parasites but, despite the enhancement of metacyclogenesis these parasites show virulence attenuation *in vitro* and *in vivo* infection assays and decrease survival rates in the natural sandfly vector (Turner et al., 2015). In contrast, comparative phenotypic analysis of a matched pair of an *L. donovani* PMM-susceptible, WT parent strain, and its derived PMM-resistant strain revealed no impact of the PMM-resistance phenotype on parasite fitness regarding promastigote growth, metacyclogenesis, and *in vitro* and *in vivo* infectivity (Hendrickx et al., 2015).

So far, no data on the fitness of drug-resistant *L. martiniquensis*, a member of the new subgenus *Mundinia*, are available. We have reported three cases of leishmaniasis caused by *L. martiniquensis* in northern Thailand (Pothirat et al., 2014; Chiewchanvit et al., 2015) that showed relapse of the disease after the first treatment with AmpB. We successfully isolated the parasites from bone marrow samples and/or skin biopsy samples collected from a patient before treatment (LSCM1) and after relapse (LSCM1-6) and could generate an *in vitro*-induced AmpB-resistant strain (AmpBRP2i). Their respective fitness phenotypes were analyzed by comparing (i) *in vitro* promastigote growth, (ii) differentiation into infectious metacyclic forms in culture (metacyclogenesis), (iii) *in vitro* infectivity and multiplication in mouse peritoneal exudate macrophages (PEMs), and (iv) *in vivo* infectivity in BALB/c mice. We uncovered *in vitro* and *in vivo* increased fitness that correlated with the AmpB resistance of *L. martiniquensis*. This information reveals an important, latent public health threat that calls for an in-depth epidemiological survey and molecular analysis of the underlying drug resistance and fitness mechanisms.

# Materials and methods

## Ethics statement

The study was approved by the ethics committee of the Faculty of Medicine, Chulalongkorn University (COA No. 467/2021), and approval to use mice was obtained from the Ethics Committee on Animal Use of the Laboratory Animal Center, Chiang Mai University, Chiang Mai, Thailand (COA No. 2562/MC-0009).

## Parasite strain and culture

*Leishmania martiniquensis* parasites, LSCM1 (MHOM/TH/2012/LSCM1, wild-type), and LSCM1-6 (MHOM/TH/2017/LSCM1-6, relapse) strains were used in this study. *Leishmania martiniquensis* LSCM1 was obtained from a VL patient with no known underlying immunodeficiency. After being treated with AmpB (1 mg/kg/day) for 21 days at the first admission, the patient was in remission (Pothirat et al., 2014). However, relapse occurred about 1 year after the first treatment. Over 5 years, the patient had been given at least six courses

of the treatment with AmpB. In 2017, 5 years after the first relapse we successfully isolated and cultured *L. martiniquensis* LSCM1-6 parasites from bone marrow aspirate.

To avoid loss of parasite virulence, the parasite strains, LSCM1 and LSCM1-6, were inoculated in BALB/c mice and recovered from the liver or spleen of the infected mice 4 weeks post-infection. Isolated parasites were then cultured for two to three passages in sterile Schneider's Insect medium (SIM) (Sigma-Aldrich, St Louis, MO, United States), pH 6.8 supplemented with 10% (v/v) heat-inactivated fetal bovine serum (hiFBS) (Life Technologies-Gibco, Grand Island, NY, United States). Cultured parasites (approximately $1 \times 10^7$ cells/mL) were cryopreserved in 7.5% (v/v) glycerol in the culture medium and stored in liquid nitrogen. Cryopreserved promastigotes were used for this study, and the maximum passage number used was seven after cryopreservation. For routine cultivation, parasites were maintained in the SIM complete medium supplemented with 25 μg/mL gentamycin sulfate (Sigma-Aldrich, St Louis, MO, United States) at 26°C. Promastigotes were sub-passaged to a fresh medium every 4 days to maintain the growth and viability of the parasites.

## Drug

AmpB was purchased from Gibco (Life Technologies-Gibco, Grand Island, NY, United States) as a 250 μg/mL solution solubilized in sodium deoxycholate. The stock solution of AmpB was stored at −20°C and used within 12 months.

## Animal

Eight to twelve-week-old inbred male BALB/c mice (*Mus musculus*), purchased from Nomura Siam International Co., Ltd., Bangkok, Thailand, weighing approximately 20 g were used. Mice were housed in the animal facilities at the Laboratory Animal Center, Chiang Mai University under standard conditions of temperature (25 ± 2°C), 12 h light/dark cycle, and fed with a standard pellet diet and water *ad libitum*.

## Isolation of mouse peritoneal exudate macrophages

The isolation of PEMs was performed as described by Zhang et al. (2008) with some modifications. Eight to twelve-week-old female BALB/c mice were injected *via* an intraperitoneal route with 1 mL of 3% (w/v) Brewer thioglycolate (Himedia, India) solution in PBS. PEMs were harvested after 48 h by peritoneal lavage with 2% hiFBS-RPMI1640 ice-cold medium (GE Healthcare Life Science-HyClone, South Logan, UT, United States) containing 1% penicillin–streptomycin (PenStrep; Sigma, United Kingdom). PEMs were collected by centrifugation (×500 g, 4°C, 10 min) and then resuspended in RPMI medium containing 10% (v/v) hiFBS. The viability of PEMs was estimated using trypan blue staining solution (Sigma-Aldrich, St Louis, MO, United States) in an improved Neubauer chamber (Precicolor, HBG, Germany) under light microscopy.

## *In vitro* drug susceptibility on promastigotes

Promastigote viability in the presence of the drug was evaluated using alamarBlue® assay (Thermo Fisher Scientific, MA, United States) and performed in flat-bottomed 96-well tissue-culture plates. Each well was filled with 50 μL of the logarithmic phase culture of promastigotes ($2 \times 10^6$ cells/mL) and incubated at 26°C for 1 h before adding AmpB. Parasites were exposed to 50 μL of AmpB over a range of concentrations in two-fold drug dilutions (0.0016–25.6 μg/mL). After 48 h of incubation, 10 μL of alamarBlue® reagent was added to each well and continuously incubated for 24 h. The concentration of resorufin in the parasite-drug mixture was measured using a spectrophotometer at a wavelength of 570 and 600 nm. The optical density in the absence of drugs was set as 100% control. Drug susceptibility was determined by calculating the half-maximal inhibitory concentration $IC_{50}$ values from the nonlinear concentration-response curves using GraphPad Prism version 9.1 software (Graphpad Software Inc., San Diego, CA, United States) and the results were expressed as the mean ± standard deviation (SD) of three independent experiments.

## Resistance selection on promastigotes

A stepwise process previously described by Al-Mohammed et al. (2005) and García-Hernández et al. (2012) was used to select an AmpB-resistant line from *L. martiniquensis* promastigotes (LSCM1) with some modifications. Briefly, the selection was initiated with promastigotes ($2 \times 10^6$ cells/mL) starting from 0.025 μg/mL of AmpB corresponding to half of the $IC_{50}$ value determined for the LSCM1 promastigote (0.05 μg/mL) to 1.0 μg/mL. After each selection step, log-phase (day 3) promastigotes ($2 \times 10^6$ cells/mL) were sub-passaged in the SIM medium supplemented with 20% (v/v) hiFBS and increasing concentrations of AmpB in stepwise increments. This experiment was carried out in flat-bottomed 24-well plastic tissue-culture plates (ThermoFisher Scientific, Jiangsu, China) with a final volume of 1 ml and the plates were maintained at 26°C. At each concentration of AmpB, the promastigotes were maintained until a growth rate was similar to the LSCM1 control culture. This process was applied until reaching the maximum concentration of the drug allowing parasite growth. Then, three single clones of the AmpB-resistant promastigotes, namely, AmpB-Resistant Promastigote clone 1 (AmpBRP1), AmpB-Resistant Promastigote clone 2 (AmpBRP2), and AmpB-Resistant Promastigote clone 3 (AmpBRP3), were selected from the *in vitro* derived AmpB-resistant line by limiting dilution to 1 cell/mL. The three resistant clones were sub-passaged in the SIM, pH 6.8 supplemented with 10% (v/v) hiFBS without AmpB for 20, 30, and 40 passages after the selection. For the 20th, 30th, and 40th passages the corresponding resistance indexes ($IC_{50}$ of each clone divided by $IC_{50}$ of the LSCM1) were calculated. The AmpBRP2 resistant clone that showed high stability of $IC_{50}$ value and resistance index at passage 40 was selected. To avoid the impact of the long-term culture of the selected clone (40 passages) on infectivity, the parasites with stationary phase promastigotes (at 120 h) were used to infect BALB/c mice for 4 weeks. As described above parasites were isolated from the liver or spleen of the infected mice and cultured and the $IC_{50}$ value and resistance index of the isolated parasites were determined

to check for stability of AmpB resistance before cryopreservation for further use.

## *In vitro* promastigote growth

The growth profile of the LSCM1, AmpBRP, and LSCM1-6 parasites was assessed by a direct counting method using an improved Neubauer chamber. To generate growth curves, stationary phase promastigotes were inoculated at exactly $1 \times 10^6$ cells/mL in 5 mL of the SIM complete medium and incubated at 26°C. Parasite density was determined every 24 h for 10 consecutive days (24 h to 240 h). The average promastigote density at each time point was calculated and used to draw the final growth curves using GraphPad Prism version 9.1 software. The doubling time was calculated during the exponential growth of the parasites, i.e., between 48 and 72 h of culture. All experiments were carried out in duplicate from three independent experiments. Results were expressed as mean ± SD.

## Morphological assessment for metacyclogenesis

Promastigote morphology was evaluated microscopically to assess metacyclogenesis. The cell body size (length and width) and flagellum lengths were measured. Promastigotes were considered metacyclic when the body length was ≤12.5 µm, body width ≤ 1.5 µm, and flagellum length > body length (Chanmol et al., 2019). Promastigotes of the LSCM1, AmpBRP, and LSCM1-6 were cultured in the SIM, at 26°C. From 72 h to 240 h of the cultivation, 10 µl of each promastigote suspension was stained with 10% (v/v) Giemsa solution. Images were acquired under Olympus CX41RF light microscope (Tokyo, Japan) at ×1,000 magnification and the cell body and flagellum lengths of ≥200 parasites were measured using DP2-SAL Firmware Ver.3.3.1.198, software. The percentage of metacyclic form was calculated at each time point of the culture. Results were expressed as mean ± SD and were based on three independent experiments in duplicates.

## *In vitro* infection and evaluation of intracellular amastigote multiplication

PEMs harvested from BALB/c mice were tested for cell viability using trypan blue. PEMs with cell viability above 95% were used. A total of $2.5 \times 10^5$ cells of PEMs in 500 µL RPMI medium with 10% (v/v) hiFBS were plated in round coverslips placed in 24-well tissue culture plates and incubated at 37°C and 5% $CO_2$ for 24 h. Nonadherent cells were washed out with a pre-warmed RPMI medium. To estimate the number of parasites for a ratio of 1:10 adherent cells was counted after the 24 h incubation. Then, the adherent cells were infected with the stationary phase promastigotes (at 120 h) of the LSCM1, AmpBRP, or the LSCM1-6 at the ratio of 1:10. Live/dead staining with trypan blue was used to correct for the variable number of dead promastigotes in the different cultures. Parasites with cell viability above 99% were used in this experiment. After 3 h of incubation, extracellular promastigotes were then removed by washing twice with a pre-warmed RPMI medium. Coverslips were fixed with absolute methanol for 10 s, Giemsa's-stained for 30 min, and visualized under the Olympus CX41RF light microscope (×1,000 magnification). To evaluate the level of infection, at least 200 macrophages were counted in 10 randomly selected microscopic fields in duplicate. The percentage of infected macrophages (infection rate) and the average number of intracellular parasites per macrophage were determined. In addition, the infection index was calculated by multiplying the percentage of infected macrophages by the average number of intracellular parasites per macrophage to account for the overall intracellular parasite burden.

Evaluation of amastigote multiplication was performed every 24 h from 24 h to 120 h post-infection using the same process. To allow comparison between the different strains, correction for the baseline infectivity was made based on the infection ratio at 24 h post-infection ($T_0$). The amastigote multiplication ratio was calculated from the average number of intracellular amastigotes at $T_x$ (the evaluated time after 24 h post-infection) divided by the average number of intracellular amastigotes at $T_0$ (Chanmol et al., 2019). Results were expressed as mean ± SD and based on three independent infection experiments, each performed in duplicate.

## *In vivo* infectivity

For each parasite strain, 42 BALB/c mice were used. Animals were intraperitoneally injected with $2 \times 10^7$ stationary phase promastigotes (at 120 h) resuspended in 200 µL of PBS. In the control group, six mice were injected with 200 µL of PBS. The infected mice were monitored weekly for cachexia, fatigue, ascites, scabs or skin lesions, hepatomegaly, and splenomegaly and their body weight was recorded using a balance (Sartorius TE313S Talent Analytical Balance, Sartorius AG, Goettingen, Germany). At 1-, 3-, 7-, 28-, 84-, and 168-days post infection (dpi), six animals from each group were sacrificed. In each animal, the liver, spleen, and bone marrow were collected separately under sterile conditions. The liver and spleen samples were weighed. Parasite burden in the liver, spleen, and bone marrow was determined by the impression smear method and limiting dilution assay.

For the impression smear method, parasite burden in each organ expressed as Leishman Donovan units (LDU) was calculated using the formula according to Stauber et al. (1958). The LDU values correspond to the number of amastigotes per 1,000 nucleated cells multiplied by the organ weight (g).

For limiting dilution assay, parasite burden was quantified in these tissues as previously described by Intakhan et al. (2020). The parasite load was calculated from the mean of reciprocal positive titers divided by the weight of the homogenized cross-section and calculated as the number of parasites per gram of organ. Genomic DNA from all samples was also extracted for the detection of *L. martiniquensis* DNA by PCR using 70IRD/70IRM primers for the 3′ untranslated region (3′-UTR) of the heat shock protein 70 (type I) gene (*HSP70-I*) (Jariyapan et al., 2021).

## Statistical analysis

All statistical analyses were performed using GraphPad Prism version 9.1 software. The statistical differences among the LSCM1, *in vitro* derived AmpB-resistant, and LSCM1-6 strains and between the

different time points within one group were evaluated using two-way ANOVA, followed by Bonferroni post-hoc comparison tests. The $IC_{50}$ values were established from the dose–response curves using the GraphPad Prism software. All experiments were performed in triplicate and considered statistically significant if the *p* value was <0.05.

## Results

### *In vitro* generation of AmpB-resistant clones

To select AmpB-resistant lines of *L. martiniquensis*, promastigotes of the LSCM1 strain were exposed to AmpB at a starting concentration of 0.025 μg/mL, which was stepwise increased to induce drug resistance (Figure 1). The final concentration of AmpB that did not affect the normal growth of the *in vitro* derived AmpB-resistant promastigotes was 1.0 μg/mL. Overall, the selection time lasted 260 days. Three single clones, AmpBRP1, AmpBRP2, and AmpBRP3, were selected from the *in vitro* derived AmpB-resistant line by limiting dilution to 1 cell/mL. In every ten *in vitro* passages the drug susceptibility of the selected AmpB-resistant clones was tested to assess the stability of AmpB resistance. The cutoff value for AmpB-resistant strains was set at 0.55 μg/ml. At passage 20, the $IC_{50}$ and resistance index of the AmpBRP clones were ranging from 1.23 to 1.59 μg/mL and from 22.4 to 28.9, respectively (Table 1). The $IC_{50}$ and the resistance index for the three selected clones slightly decreased (statistically insignificant) after being maintained in the drug-free medium for more than 20 passages. Both the $IC_{50}$ and resistance index of these AmpBRP clones at passages 20, 30, and 40 were higher than that of the LSCM1 input population (Table 1). Based on the highest resistance index observed at passage 40 (26.3), the AmpBRP2 clone was selected for further study.

To avoid the impact of the long-term culture on the infectivity of the AmpBRP2 clone, the parasites at passage 40 were used to infect BALB/c mice for 4 weeks. The stability of resistance to AmpB of the AmpBRP2 parasites recovered from the spleen of one infected mouse (further called AmpBRP2i) was evaluated. The $IC_{50}$ value and resistance index of the isolated AmpBRP2i were 0.191 ± 0.02 μg/mL and 3.7, respectively (Table 2), inferior to the values of the input parasites but still in the same range as the relapsed strain, LSCM1-6. The AmpBRP2i strain, therefore, was used for the *in vitro* and *in vivo* phenotypic comparisons with the susceptible wild-type parent strain, LSCM1, and the relapse strain, LSCM1-6 (Figure 1).

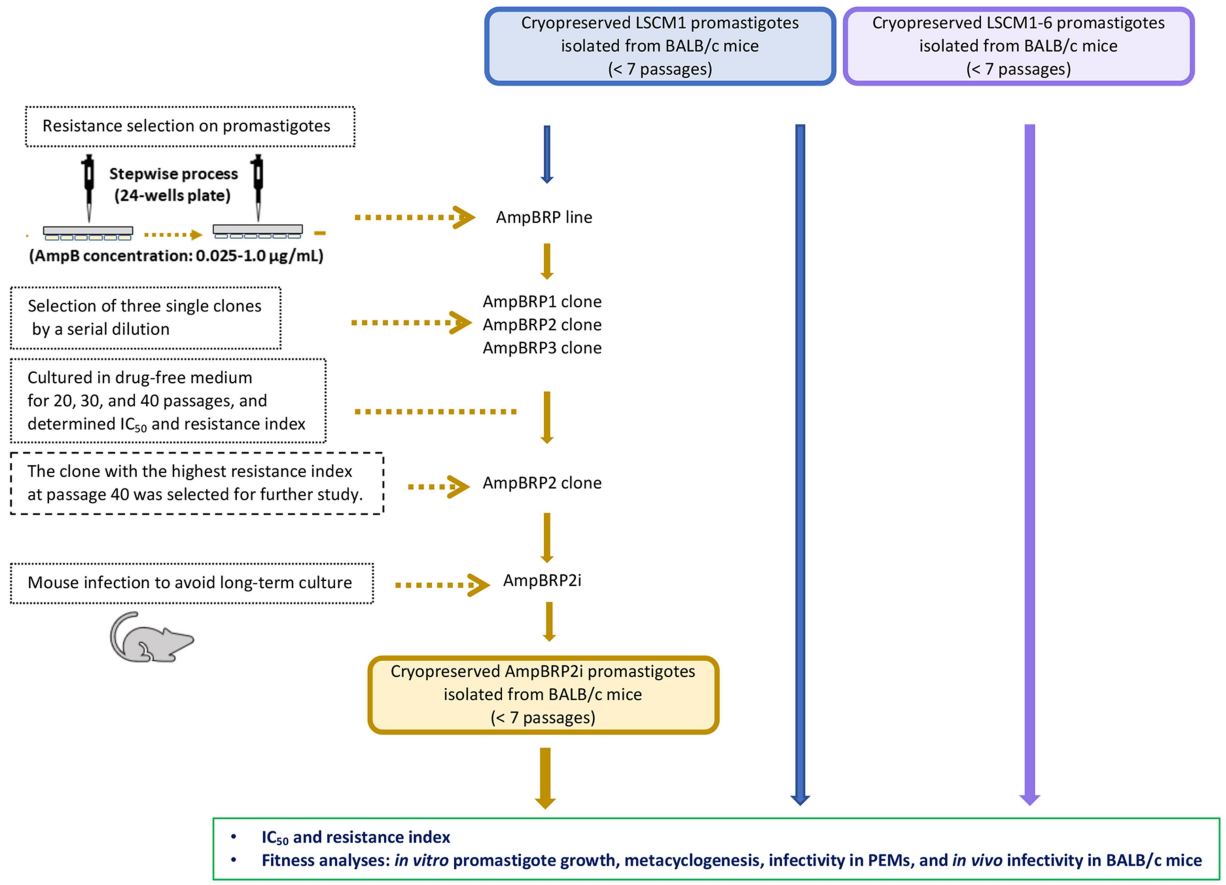


FIGURE 1
Schematic diagram of resistance selection for an AmpB-resistant clone on promastigotes and an overview of the comparative fitness of the LSCM1, AmpB-resistant clone, and LSCM1-6.

10.3389/fmicb.2023.1156061

TABLE 1 Promastigote susceptibility to AmpB for *L. martiniquensis* LSCM1, and *in vitro* derived AmpBRP clones after removal from drug pressure. Results are expressed as mean±SD based on three independent replicates.

| Parasite strain | Passages 20 | | Passages 30 | | Passages 40 | |
|---|---|---|---|---|---|---|
| | $IC_{50}$ (μg/mL) | Resistance index[a] | $IC_{50}$ (μg/mL) | Resistance index | $IC_{50}$ (μg/mL) | Resistance index |
| LSCM1 | 0.055 ± 0.02 | 1 | 0.054 ± 0.01 | 1 | 0.052 ± 0.01 | 1 |
| AmpBRP1 | 1.23 ± 0.22 | 22.4 | 1.17 ± 0.07 | 21.7 | 1.11 ± 0.08 | 21.3 |
| AmpBRP2 | 1.59 ± 0.01 | 28.9 | 1.49 ± 0.15 | 27.6 | 1.37 ± 0.29 | 26.3 |
| AmpBRP3 | 1.55 ± 0.01 | 28.2 | 1.52 ± 0.03 | 28.1 | 1.25 ± 0.17 | 24.0 |

[a]Resistance index = $IC_{50}$ of each AmpBRP clone ÷ $IC_{50}$ of LSCM1.

TABLE 2 Promastigote susceptibility to AmpB for the LSCM1, AmpBRP2i, and LSCM1-6 strains before and after mouse infection. Results are expressed as mean±SD based on three independent replicates.

| Parasite strain | Before mouse infection | | After the 1st mouse infection | | After the 2nd mouse infection (day 28) | |
|---|---|---|---|---|---|---|
| | $IC_{50}$ (μg/mL) | Resistance index | $IC_{50}$ (μg/mL) | Resistance index | $IC_{50}$ (μg/mL) | Resistance index |
| LSCM1 | 0.052 ± 0.01 | 1 | 0.052 ± 0.01 | 1 | 0.053 ± 0.01 | 1 |
| AmpBRP2i | 1.370 ± 0.29 | 26.3 | 0.191 ± 0.02 | 3.7 | 0.190 ± 0.02 | 3.6 |
| LSCM1-6 | 0.147 ± 0.03 | 2.8 | NA[a] | NA | 0.154 ± 0.08 | 2.9 |

[a]NA = not available.

## *In vitro* promastigote growth

LSCM1, AmpBRP2i, and LSCM1-6 strains were compared for their growth as promastigotes in the SIM complete medium at 26°C (Figure 2; Supplementary Table S1). Promastigotes of the LSCM1 reached their peak at 96 h ($4.56 \times 10^7$ cells/mL), and then started to decline to approximately $0.32 \times 10^7$ cells/mL at 240 h. AmpBRP2i promastigotes reached their peak and entered the stationary phase at 120 h with a density of $6.91 \times 10^7$ cells/mL, and then at 144 h the parasite concentration started to decline to $1.97 \times 10^7$ cells/mL at 240 h. LSCM1-6 promastigotes reached a plateau at 96 h ($5.54 \times 10^7$ cells/mL) and sustained the stationary phase until 144 h ($5.84 \times 10^7$ cells/mL) before the continuous decrease until the end of the experiment. The average promastigote densities of the AmpBRP2i and the LSCM1-6 were significantly greater than that of the LSCM1 from 120 h to 240 h or from 96 h to 240 h, respectively. However, the average promastigote density of the LSCM1-6 was significantly lower than that of the AmpBRP2i at 144, 168, and 216 h. In summary, the AmpB-resistant parasites reached a higher cell density than the parental strain LSCM1.

## Metacyclogenesis

Metacyclic promastigotes of the LSCM1, AmpBRP2i, and LSCM1-6 strains were evaluated based on morphological criteria (Figure 3A). The percentage of metacyclic promastigotes of the three strains was similar at 72 h (3.6–5.4%), increased by approximately 6-fold at 120 h (25.4–27%) and sustained for almost 3 days (Figure 3B; Supplementary Table S2). The number of LSCM1 metacyclic-like parasites reached a maximum at 120 h with 25.4% and then gradually dropped to 9.7% after 240 h of culture. For both AmpBRP2i and LSCM1-6, the percentage of metacyclic promastigotes reached its peak at 144 h with 27.6 and 28.1% respectively, and then decreased gradually to 12% at 240 h. Overall, the results demonstrate that the maximum rate of metacyclogenesis of the resistant strains, AmpBRP2i and LSCM1-6, was rather similar to that of the LSCM1 parental strain but the differences observed at 144 and 168 h of culture were, however, considered statistically significant.

## *In vitro* PEM infection and intracellular amastigote multiplication

PEMs recovered 48 h after injection of Brewer thioglycolate were infected at a ratio of 1:10 using stationary phase promastigotes collected after 120 h of culture when the average number of metacyclic forms was similar for all the parasites. At all-time points of the infection, the infection rate, average number of intracellular parasites per cell, and infection index of the AmpBRP2i and LSCM1-6 strains were significantly higher than those of the LSCM1 strain (Figures 4A–C; Supplementary Table S3). The lesser uptake (1.6-fold) of the LSCM1 parasites alone cannot account for differences in infectivity that we observed. However, after 24 h post-infection, the infection index and the amastigote multiplication ratio of all strains gradually declined until 120 h of infection (Figures 4C,D) showing that none of the parasites replicated as amastigotes in PEMs. The failure to obtain a robust and long-lasting infection may result from the nature of the PEMs we used in our assay. Indeed, (i) mouse macrophages may not be as permissive for *L. martiniquensis* replication, and (ii) the use of thioglycolate elicits inflammatory macrophages with increased phagocytic and respiratory burst capacity. In conclusion, in our experimental *in vitro* system, the AmpB-resistant parasites were initially more infectious than the initial LSCM1 with more infected cells, more parasites per cell, and a persisting infection but as the susceptible parasites they fell to replicate.

## *In vivo* infectivity

BALB/c mice were inoculated with $2 \times 10^7$ stationary phase culture promastigotes (120 h) of the LSCM1, AmpBRP2i, and LSCM1-6 strains, each composed of approximately 25% metacyclic promastigotes (Supplementary Table S2). Throughout the observation, no clinical signs of the disease including cachexia, fatigue, ascites, scabs or skin lesions, hepatomegaly, and splenomegaly, and no statistically significant differences in the weight of the body, liver, and spleen among the infected mice with the LSCM1, AmpBRP2i, and LSCM1-6 were observed (Figure 5). At all-time points, no parasites could be observed in any impression smear of any infected mice.

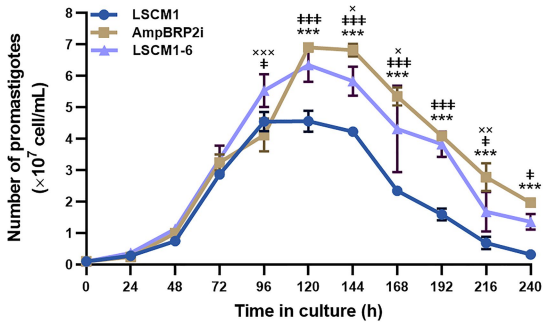


FIGURE 2
Growth curves of the *L. martiniquensis* LSCM1, AmpBRP2i, and LSCM1-6 promastigotes cultured in the SIM supplemented with 10% (v/v) hiFBS and 25μg/ml gentamycin sulfate, pH 6.8 at 26°C. All parasites were inoculated at 1x10$^6$ parasites from stationary growth phase/mL and were counted daily until day 10 (240h). Statistically significant differences between LSCM1 and AmpBRP2i are indicated as ***=$p$<0.001; LSCM1 and LSCM1-6 are indicated as ǂǂ=$p$<0.001, ǂ= $p$<0.01, and ǂ= $p$<0.05; AmpBPR2i and LSCM1-6 are indicated as ××× $p$<0.001 and ×× $p$<0.01, and × $p$<0.05.

Parasite burdens in the infected organs were quantified using a limiting dilution assay. At 1 and 3 dpi, no parasites were present in the culture of liver and spleen samples. After 7 dpi parasites were found in the cultures of the liver samples from five to six infected mice with a mean parasite burden of approximately $4.47 \times 10^2$ parasites/gram of liver, $7.08 \times 10^2$ parasites/gram of liver and $8.51 \times 10^2$ parasites/gram of liver from mice infected with LSCM1, AmpBRP2i and LSCM1-6 parasites, respectively. At 28 dpi, the number of mice with parasites in the liver remained stable. The parasite load after infection with the LSCM1 started to decrease to $1.70 \times 10^2$ parasites/gram of liver whilst in the livers of mice infected with the AmpB resistant parasites it slightly increased with $1.15 \times 10^3$ and $1.44 \times 10^3$ parasites/gram of organ for AmpBRP2i and LSCM1-6 parasites, respectively. Although the parasitic loads in the liver were low the difference between susceptible and resistant parasites is statistically significant. No parasites of any strains were isolated from the liver samples of the infected mice at 84 and 168 dpi (Figure 6A).

For the cultures of spleen samples, the LSCM1 parasites were recovered from infected mice sacrificed at 7 and 28 dpi with the parasitic load decreasing from $1.23 \times 10^2$ (5 out of 6 mice) to 46.77 parasites/gram of organ in half of the animals After 84 and 168 dpi LSCM1 parasites were not anymore detected. AmpBRP2i parasites were found in the spleen of the mice sacrificed at 7 to 84 dpi with a parasite burden around $1.70 \times 10^2$, $3.47 \times 10^2$, and $1.70 \times 10^2$parasites/gram of organ, respectively, in at least 3 to 5 mice. No AmpBRP2i parasites were observed in the samples at 168 dpi. For LSCM1-6, parasites were found in the cultures of the spleen samples collected at 7 to 168 dpi but the number of mice with parasites decreased over time with only half of them still carrying parasites in their spleen at the end of the experiment. Consequently, the parasite burden gradually decreased from $1.15 \times 10^3$ to $1.23 \times 10^2$ parasites/gram of organ (Figure 6B). No parasites were isolated from all bone marrow samples of the infected mice at all-time points.

Polymerase chain reactions on genomic DNA extracted from all liver spleen and bone marrow samples from the infected mice were

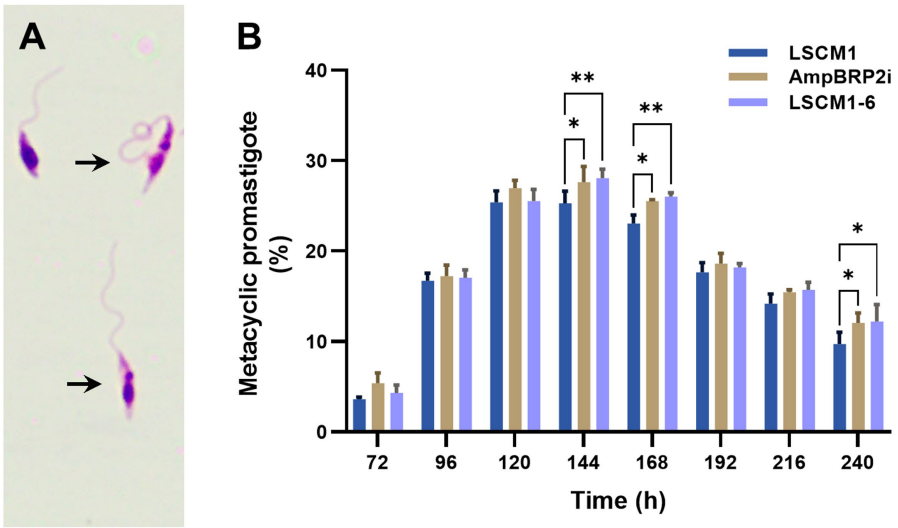


FIGURE 3
**(A)** Metacyclic promastigotes of *L. martiniquensis* (arrows). **(B)** Metacyclogenesis of the LSCM1, AmpBRP2i, and LSCM1-6 promastigotes. Morphometric analysis was conducted by microscopy using the criteria defined by Chanmol et al. (2019) from 72 to 240h of culture. Statistically significant differences among the three groups are indicated as follows: **=$p$<0.01; and *=$p$<0.05.

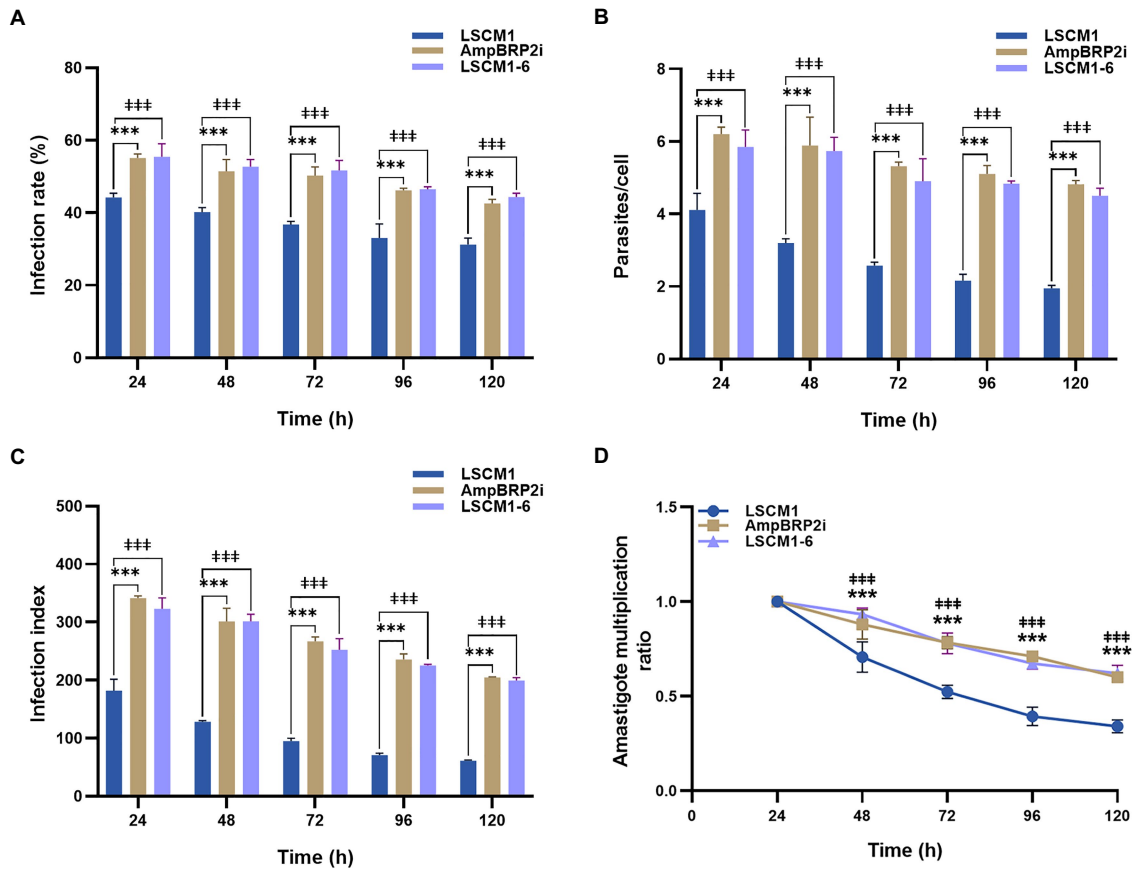


FIGURE 4
PEMs were infected using stationary phase promastigotes after 120h of culture at a multiplicity of infection (MOI)=1:10. **(A)** Percentage of infected cells measured from 24 to 120h post-infection. **(B)** Average number of parasites per macrophage. **(C)** Infection index calculated by multiplying the percentage of infected macrophages by the average number of parasites per macrophage. **(D)** Amastigote multiplication ratio calculated from the average number of intracellular amastigotes at $T_x$ divided by the average number of intracellular amastigotes at $T_0$ (Chanmol et al., 2019). Results are expressed as the means±SD from three different experiments run in duplicate. Statistically significant differences between LSCM1 and AmpBRP2i are indicated as ***=$p$<0.001; between LSCM1 and LSCM1-6 are indicated as ǂǂǂ=$p$<0.001.

performed. DNA of each parasite strain was detected in the same samples where parasites were quantified by the limiting dilution assay. No parasitic DNA could be detected in any of the bone marrow samples (Supplementary Figure S1).

The susceptibility to AmpB of the LSCM1, AmpBRP2i, and LSCM1-6 strains after the infection was tested. The $IC_{50}$ value and resistance index at 28 dpi were not affected with 0.053 ± 0.01 and 1, 0.19 ± 0.02 and 3.6, and 0.154 ± 0.08 and 2.9, respectively, indicating that the resistant phenotype is stable (Table 2).

Even though the mice model may not be the more suitable system for experimental infection with *L. martiniquensis*, our results suggest that AmpB-resistant parasites may survive longer in the animals.

## Discussion

Drug resistance in *Leishmania* parasites is frequently associated with molecular and fitness changes. AmpB resistance in *L. martiniquensis* and its consequences on the parasite phenotype is poorly documented especially in clinical samples due to the (i) recent identification of this new species, (ii) the small number of patients with a positive diagnosis of leishmaniasis and AmpB treatment failure, and (iii) the even smaller number of patients from whom parasites could be isolated before and after the relapse. We have reported three cases of leishmaniasis caused by *L. martiniquensis* in northern Thailand that showed relapse of the disease after the first treatment with AmpB (Pothirat et al., 2014; Chiewchanvit et al., 2015). We successfully isolated the parasites from a patient before treatment (LSCM1) and after relapse (LSCM1-6) and could generate an *in vitro*-induced AmpB-resistant clone (AmpBRP2) that was subsequently inoculated to mouse (AmpBRP2i). Here, we used phenotypic analyses to correlate AmpB resistance with fitness changes that could be relevant for parasite transmission or survival.

The inoculation in the mouse of the AmpBRP2 clone that was maintained for 40 *in vitro* passages led to the recovery of parasites (AmpBRP2i) with an 8-fold decrease in the $IC_{50}$ compared to the initial clone. Furthermore, the $IC_{50}$ value and resistance index were unchanged even after a second infection in mice revealing the stability of the phenotype. It suggests that the AmpBRP2i strain was a good representative for *in vitro*-generated AmpB-resistant strains and could

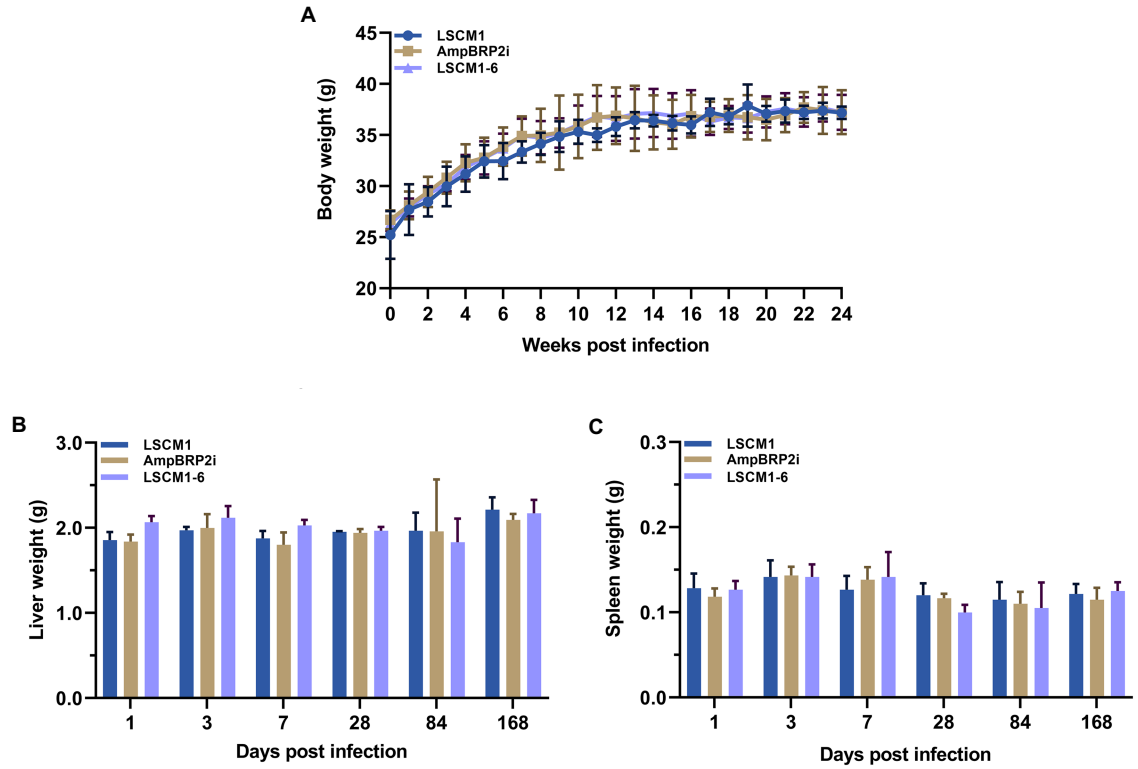


FIGURE 5
**(A)** Bodyweight of BALB/c mice infected with the *L. martiniquensis* LSCM1, AmpBRP2i, and LSCM1-6 stationary promastigotes. Animal weight was recorded every week. **(B,C)** Weight of liver and spleen collected from BALB/c mice infected with the *L. martiniquensis* LSCM1, AmpBRP2i, and LSCM1-6 stationary promastigotes.

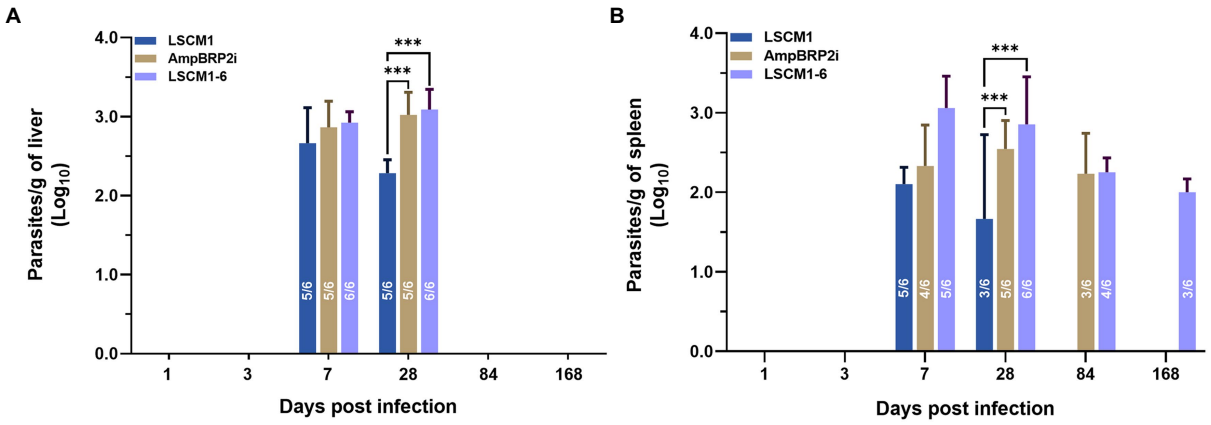


FIGURE 6
Parasite burdens in the liver and spleen samples of BALB/c mice infected with the *L. martiniquensis* LSCM1, AmpBRP2i, and LSCM1-6 stationary phase promastigotes at 1, 3, 7, 28, 84, and 168 dpi determined by limiting dilution assay. **(A)** Parasite load quantified from the liver of infected mice. **(B)** Parasite load quantified from the spleens of infected mice. Statistically significant differences among the three groups are indicated as follows: ***=$p<0.001$. The number inside the columns is the number of positive mice per total mice sacrificed in each group.

be used the same parameters to analyze the influence of drug resistance on the fitness of the resistant strain in order to compare with the wild-type LSCM1 and the relapse LSCM1-6 strains. The difference in the level of resistance between the AmpBRP2 and AmpBRP2i is not associated with the absence of drug pressure during the passage in the animal since AmpBRP2 was maintained *in vitro* in a drug-free medium for 40 passages before the injection to the mouse. *Leishmania* genomic plasticity may account for this result as drug resistance (reviewed by Rastrojo et al., 2018; Santi and Murta, 2022) and long-term culture (Dumetz et al., 2017; Prieto Barja et al., 2017)

has been associated with chromosome and gene copy number variations or down-regulation of genes. In AmpB-resistant *L. donovani* parasites generated *in vitro,* chromosome 29 for which many transcripts were overexpressed is co-amplified with chromosomes 5 and 26 (Rastrojo et al., 2018) previously associated with fast growth in culture (Dumetz et al., 2017; Prieto Barja et al., 2017). Conversely, inoculation to animals of trisomic parasites notably for chromosomes 5 and 26 resulted in a shift to disomic karyotype (Prieto Barja et al., 2017). Comparative genomic analysis of AmpBRP2 and AmpBRP2i would be needed to confirm this hypothesis. Such chromosome amplification may also explain the differences in growth that we observed between AmpB-resistant (AmpBRP2i and LSCM1-6) and the parental strain (LSCM1).

The naturally resistant strain (LSCM1-6) and the *in vitro*-induced AmpB-resistant clone after passage in the mouse (AmpBR2i) display the same trend for all the parameters we have tested including $IC_{50}$ suggesting that the molecular mechanisms underlying the resistance to the drug are if not similar at least converge to the same parasite phenotype. Pountain et al. (2019) have shown that AmpB *L. mexicana*-resistant lines could harbor mutation(s) in one or more genes from the sterol pathway. At least three genes in which mutations resulted in AmpB resistance in *L. mexicana* have been identified, i.e., genes involving (1) resistance-associated mutation of the C5DS, (2) specific sterol changes resulting from decreased expression of the C24SMT due to structural variation events at the genome level, and (3) loss of the miltefosine transporter (Pountain et al., 2019). Recently, Alpizar-Sosa et al. (2022) have reported the selection and characterization of fourteen independent lines of *L. mexicana* and one of *L. infantum* resistant to AmpB or its analog nystatin and demonstrated loss of heterozygosity derived from mutations in the C24SMT gene locus and changes in the C5DS gene. Single-cell DNA/RNAseq of our naturally resistant parasites (LSCM1-6) would help to characterize the molecular basis of AmpB resistance in the context of a treatment failure.

Drug-resistant parasites tend to be less infective, less virulent or display a decreased transmission potential (Hendrickx et al., 2015) or at best they present mixed fitness gain in mammal and sand fly hosts compared to WT strain (Hendrickx et al., 2020; Van Bockstal et al., 2020). In our study, in contrast, the fitness of the *L. martiniquensis* AmpB-resistant parasites (LSCM1-6 and AmpBPR2i) increased compared to the wild-type LSCM1. The AmpBRP2i and the LSCM1-6 grew at higher concentrations and produced more metacyclic forms than the LSCM1. The fitness of *Leishmania* parasites relates to their ability to successfully survive, reproduce/replicate, infect, and -be transmitted from the host to the vector and reciprocally (Natera et al., 2007). Metacyclogenesis is regarded as a contributor to the fitness of the parasite but the ability of metacyclic promastigotes themselves is an important factor supporting the successful infectivity of the parasites. However, immunopathology depends on the ability of metacyclic forms to undergo differentiation in replicating amastigotes and the genetic background/immune status of the host. BALB/c mouse model is a suitable model for the asymptomatic form of human leishmaniasis caused by *L. martiniquensis* whilst hamsters are symptomatic (Intakhan et al., 2020). In our study, when injected into BALB/c mice the AmpB-resistant parasites promoted a persisting infection, although at a low level, compared to the WT strain. This silent infection could greatly impact the level of transmission of *L. martiniquensis* AmpB-resistant parasites in the field provided that AmpB resistance does not affect infectivity in vectors.

In conclusion, our results provide indications that AmpB treatment of leishmaniasis caused by *L. martiniquensis* has the potential to generate parasites with increased fitness, not only in response to treatment (hence resistance) but also in terms of infectivity and transmission. In Thailand, at least 24.9% of HIV/*Leishmania* coinfected patients are asymptomatic and *L. martiniquensis* is one of the predominant species detected (Manomat et al., 2017). Since not only symptomatic leishmaniasis patients but also asymptomatic HIV/leishmaniasis patients play an important role as "reservoirs" in *Leishmania* transmission (Molina et al., 2020; Ibarra-Meneses et al., 2022), our results highlight the need to search and monitor for AmpB-resistant *L. martiniquensis* in both patients with symptomatic and asymptomatic leishmaniasis, mainly in HIV/*Leishmania* coinfected patients.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

## Ethics statement

The studies involving human participants were reviewed and approved by The ethics committee of the Faculty of Medicine, Chulalongkorn University (COA No. 467/2021). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. The animal study was reviewed and approved by The Ethics Committee on Animal Use of the Laboratory Animal Center, Chiang Mai University, Chiang Mai, Thailand (COA No. 2562/MC-0009).

## Author contributions

NJ and CM: conceptualization, methodology, formal analysis, and writing-original draft preparation. CM, AK, AT, and NJ: investigation. CM and NJ: visualization. NJ, SR, CU, PP, and GS: writing-review and editing. NJ, PT, PrS, PaS, and GS: funding acquisition. All authors contributed to the article and approved the submitted version.

## Funding

This work was supported by Ratchadapiseksompotch Fund Chulalongkorn University [grant number RCU_H_64_013_30] received by NJ; the Thailand Graduate Institute of Science and Technology (TGIST) and NSTDA [grant number TG-22-10-60-001D] received by NJ for CM; the Faculty of Medicine, Chiang Mai University [grant number 128–2562] received by PT; the Program Management Unit for Human Resources & Institutional Development, Research and Innovation - Chulalongkorn University [grant number B16F630071] received by PaS; the Thailand Science Research and

Innovation (TSRI) Fund [grant number CU_FRB640001_01_30_1] received by PaS and NJ; the French Ministry of Europe and Foreign Affairs (MEAE), the French Ministry of Higher Education, Research and Innovation (MESRI) and the Ministry of Higher Education, Science, Research and Innovation of Thailand (MHESI) - Franco-Thai Mobility Program/PHC SIAM 2021–2022 [grant number PHC SIAM 2021–2022] received by NJ and GS.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1156061/full#supplementary-material

## References

Al-Mohammed, H. I., Chance, M. L., and Bates, P. A. (2005). Production and characterization of stable amphotericin-resistant amastigotes and promastigotes of *Leishmania mexicana*. *Antimicrob. Agents Chemother.* 49, 3274–3280. doi: 10.1128/AAC.49.8.3274-3280.2005

Alpizar-Sosa, E. A., Ithnin, N. R. B., Wei, W., Pountain, A. W., Weidt, S. K., Donachie, A. M., et al. (2022). Amphotericin B resistance in *Leishmania mexicana*: alterations to sterol metabolism and oxidative stress response. *PLoS Negl. Trop. Dis.* 16:e0010779. doi: 10.1371/journal.pntd.0010779

Chanmol, W., Jariyapan, N., Somboon, P., Bates, M. D., and Bates, P. A. (2019). Axenic amastigote cultivation and *in vitro* development of *Leishmania orientalis*. *Parasitol. Res.* 118, 1885–1897. doi: 10.1007/s00436-019-06311-z

Chiewchanvit, S., Tovanabutra, N., Jariyapan, N., Bates, M. D., Mahanupab, P., Chuamanochan, M., et al. (2015). Chronic generalized fibrotic skin lesions from disseminated leishmaniasis caused by *Leishmania martiniquensis* in two patients from northern Thailand infected with HIV. *Br. J. Dermatol.* 173, 663–670. doi: 10.1111/bjd.13812

Desbois, N., Pratlong, F., Quist, D., and Dedet, J. P. (2014). *Leishmania* (*Leishmania*) *martiniquensis* n. sp. (Kinetoplastida: Trypanosomatidae), description of the parasite responsible for cutaneous leishmaniasis in Martinique Island (French West Indies). *Parasite* 21:12. doi: 10.1051/parasite/2014011

Dumetz, F., Imamura, H., Sanders, M., Seblova, V., Myskova, J., Pescher, P., et al. (2017). Modulation of aneuploidy in *Leishmania donovani* during adaptation to different *in vitro* and *in vivo* environments and its impact on gene expression. *MBio* 8, e00599–e00517. doi: 10.1128/mBio.00599-17

García-Hernández, R., Gómez-Pérez, V., Castanys, S., and Gamarro, F. (2015). Fitness of *Leishmania donovani* parasites resistant to drug combinations. *PLoS Negl. Trop. Dis.* 9:e0003704. doi: 10.1371/journal.pntd.0003704

García-Hernández, R., Manzano, J. I., Castanys, S., and Gamarro, F. (2012). *Leishmania donovani* develops resistance to drug combinations. *PLoS Negl. Trop. Dis.* 6:e1974. doi: 10.1371/journal.pntd.0001974

Hendrickx, S., Leemans, A., Mondelaers, A., Rijal, S., Khanal, B., Dujardin, J. C., et al. (2015). Comparative fitness of a parent *Leishmania donovani* clinical isolate and its experimentally derived paromomycin-resistant strain. *PLoS One* 10:e0140139. doi: 10.1371/journal.pone.0140139

Hendrickx, S., van Bockstal, L., Bulté, D., Mondelaers, A., Aslan, H., Rivas, L., et al. (2020). Phenotypic adaptations of *Leishmania donovani* to recurrent miltefosine exposure and impact on sand fly infection. *Parasit. Vectors* 13:96. doi: 10.1186/s13071-020-3972-z

Ibarra-Meneses, A. V., Corbeil, A., Wagner, V., Onwuchekwa, C., and Fernandez-Prada, C. (2022). Identification of asymptomatic *Leishmania* infections: a scoping review. *Parasit. Vectors* 15:5. doi: 10.1186/s13071-021-05129-y

Intakhan, N., Chanmol, W., Kongkaew, A., Somboon, P., Bates, M. D., Bates, P. A., et al. (2020). Experimental infection of *Leishmania* (*Mundinia*) *martiniquensis* in BALB/c mice and Syrian golden hamsters. *Parasitol. Res.* 119, 3041–3051. doi: 10.1007/s00436-020-06842-w

Jariyapan, N., Bates, M. D., and Bates, P. A. (2021). Molecular identification of two newly identified human pathogens causing leishmaniasis using PCR-based methods on the 3′ untranslated region of the heat shock protein 70 (type I) gene. *PLoS Negl. Trop. Dis.* 15:e0009982. doi: 10.1371/journal.pntd.0009982

Jariyapan, N., Daroontum, T., Jaiwong, K., Chanmol, W., Intakhan, N., Sor-Suwan, S., et al. (2018). *Leishmania* (*Mundinia*) *orientalis* n. sp. (Trypanosomatidae), a parasite from Thailand responsible for localized cutaneous leishmaniasis. *Parasit. Vectors* 11:351. doi: 10.1186/s13071-018-2908-3

Manomat, J., Leelayoova, S., Bualert, L., Tan-ariya, P., Siripattanapipong, S., Mungthin, M., et al. (2017). Prevalence and risk factors associated with *Leishmania* infection in Trang Province, southern Thailand. *PLoS Negl. Trop. Dis.* 11:e0006095. doi: 10.1371/journal.pntd.0006095

Molina, R., Jiménez, M., García-Martínez, J., San Martín, J. V., Carrillo, E., Sánchez, C., et al. (2020). Role of asymptomatic and symptomatic humans as reservoirs of visceral leishmaniasis in a Mediterranean context. *PLoS Negl. Trop. Dis.* 14:e0008253. doi: 10.1371/journal.pntd.0008253

Natera, S., Machuca, C., Padrón-Nieves, M., Romero, A., Díaz, E., and Ponte-Sucre, A. (2007). *Leishmania* spp.: proficiency of drug-resistant parasites. *Int. J. Antimicrob. Agents* 29, 637–642. doi: 10.1016/j.ijantimicag.2007.01.004

Osatakul, S., Mungthin, M., Siripattanapipong, S., Hitakarun, A., Kositnitikul, R., Naaglor, T., et al. (2014). Recurrences of visceral leishmaniasis caused by *Leishmania siamensis* after treatment with amphotericin B in a seronegative child. *Am. J. Trop. Med. Hyg.* 90, 40–42. doi: 10.4269/ajtmh.13-0398

Pothirat, T., Tantiworawit, A., Chaiwarith, R., Jariyapan, N., Wannasan, A., Siriyasatien, P., et al. (2014). First isolation of *Leishmania* from northern Thailand: case report, identification as *Leishmania martiniquensis* and phylogenetic position within the *Leishmania enriettii* complex. *PLoS Negl. Trop. Dis.* 8:e3339. doi: 10.1371/journal.pntd.0003339

Pountain, A. W., Weidt, S. K., Regnault, C., Bates, P. A., Donachie, A. M., Dickens, N. J., et al. (2019). Genomic instability at the locus of sterol C24-methyltransferase promotes amphotericin B resistance in *Leishmania* parasites. *PLoS Negl. Trop. Dis.* 13:e0007052. doi: 10.1371/journal.pntd.0007052

Prieto Barja, P., Pescher, P., Bussotti, G., Dumetz, F., Imamura, H., Kedra, D., et al. (2017). Haplotype selection as an adaptive mechanism in the protozoan pathogen *Leishmania donovani*. *Nat. Ecol. Evol.* 1, 1961–1969. doi: 10.1038/s41559-017-0361-x

Purkait, B., Kumar, A., Nandi, N., Sardar, A. H., Das, S., Kumar, S., et al. (2012). Mechanism of amphotericin B resistance in clinical isolates of *Leishmania donovani*. *Antimicrob. Agents Chemother.* 56, 1031–1041. doi: 10.1128/AAC.00030-11

Rastrojo, A., García-Hernández, R., Vargas, P., Camacho, E., Corvo, L., Imamura, H., et al. (2018). Genomic and transcriptomic alterations in *Leishmania donovani* lines experimentally resistant to antileishmanial drugs. *Int. J. Parasitol. Drugs Drug Resist.* 8, 246–264. doi: 10.1016/j.ijpddr.2018.04.002

Santi, A. M. M., and Murta, S. M. F. (2022). Impact of genetic diversity and genome plasticity of *Leishmania* spp. in treatment and the search for novel chemotherapeutic targets. *Front. Cell. Infect. Microbiol.* 12:826287. doi: 10.3389/fcimb.2022.826287

Siriyasatien, P., Chusri, S., Kraivichian, K., Jariyapan, N., Hortiwakul, T., Silpapojakul, K., et al. (2016). Early detection of novel *Leishmania* species DNA in the saliva of two HIV-infected patients. *BMC Infect. Dis.* 16:89. doi: 10.1186/s12879-016-1433-2

Srivastava, P., Prajapati, V. K., Rai, M., and Sundar, S. (2011). Unusual case of resistance to amphotericin B in visceral leishmaniasis in a region in India where leishmaniasis is not endemic. *J. Clin. Microbiol.* 49, 3088–3091. doi: 10.1128/JCM.00173-11

Stauber, L. A., Franchino, E. M., and Grun, J. (1958). An eight-day method for screening compounds against *Leishmania donovani* in the Golden hamster. *J. Protozool.* 5, 269–273. doi: 10.1111/j.1550-7408.1958.tb02565.x

Turner, K. G., Vacchina, P., Robles-Murguia, M., Wadsworth, M., McDowell, M. A., and Morales, M. A. (2015). Fitness and phenotypic characterization of miltefosine-resistant *Leishmania major*. *PLoS Negl. Trop. Dis.* 9:e0003948. doi: 10.1371/journal.pntd.0003948

Van Bockstal, L., Bulté, D., Hendrickx, S., Sadlova, J., Volf, P., Maes, L., et al. (2020). Impact of clinically acquired miltefosine resistance by *Leishmania infantum* on mouse and sand fly infection. *Int. J. Parasitol. Drugs Drug Resist.* 13, 16–21. doi: 10.1016/j.ijpddr.2020.04.004

World Health Organization. (2022). Leishmaniasis. Available at: https://www.who.int/news-room/fact-sheets/detail/leishmaniasis (Accessed November 19, 2022).

Zhang, X., Goncalves, R., and Mosser, D. M. (2008). The isolation and characterization of murine macrophages. *Curr. Protoc. Immunol.* 83:14–1. doi: 10.1002/0471142735.im1401s83

frontiers | Frontiers in Microbiology

TYPE Review
PUBLISHED 05 May 2023
DOI 10.3389/fmicb.2023.1160984



OPEN ACCESS

EDITED BY
Laurent Rénia,
Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

REVIEWED BY
Vivek Podder,
Bangladesh Institute of Research and Rehabilitation for Diabetes Endocrine and Metabolic Disorders (BIRDEM), Bangladesh
Emmanuel Couacy-Hymann,
Laboratoire National d'Appui au Développement Agricole, Côte d'Ivoire

*CORRESPONDENCE
Zhidong Zhang
✉ zhangzhidong@swun.edu.cn
Yanmin Li
✉ Liyanmin@swun.edu.cn

RECEIVED 07 February 2023
ACCEPTED 27 March 2023
PUBLISHED 05 May 2023

CITATION
Ullah M, Li Y, Munib K and Zhang Z (2023) Epidemiology, host range, and associated risk factors of monkeypox: an emerging global public health threat.
*Front. Microbiol.* 14:1160984.
doi: 10.3389/fmicb.2023.1160984



# Epidemiology, host range, and associated risk factors of monkeypox: an emerging global public health threat

Munib Ullah[1,2], Yanmin Li[3]*, Kainat Munib[4] and Zhidong Zhang[3]*

[1]State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China, [2]Department of Clinical Studies, Faculty of Veterinary and Animal Sciences, PMAS-Arid Agriculture University Rawalpindi, Rawalpindi, Pakistan, [3]College of Animal Husbandry and Veterinary Medicine, Southwest Minzu University, Chengdu, China, [4]Department of Sociology, Allama Iqbal Open University Islamabad, Islamabad, Pakistan

Based on recent multiregional epidemiological investigations of Monkeypox (MPX), on 24 July 2022, the World Health Organization declared it a global public health threat. Retrospectively MPX was an ignored zoonotic endemic infection to tropical rainforest regions of Western and Central African rural communities until a worldwide epidemic in May 2022 verified the potential threat of monkeypox virus (MPXV) to be propagated across the contemporary world via transnational tourism and animal movements. During 2018–2022, different cases of MPX diagnosed in Nigerian travelers have been documented in Israel, the United Kingdom, Singapore, and the United States. More recently, on 27 September 2022, 66,000 MPX cases have been confirmed in more than 100 non-endemic countries, with fluctuating epidemiological footprinting from retrospective epidemics. Particular disease-associated risk factors fluctuate among different epidemics. The unpredicted appearance of MPX in non-endemic regions suggests some invisible transmission dynamic. Hence, broad-minded and vigilant epidemiological attention to the current MPX epidemic is mandatory. Therefore, this review was compiled to highlight the epidemiological dynamic, global host ranges, and associated risk factors of MPX, concentrating on its epidemic potential and global public health threat.

KEYWORDS

**epidemiology, monkeypox, host range, risk factors, global health, threat**

## 1. Introduction

The re-emergence of various transmissible infections, including Zika virus, swine flu (H1N1), Ebola virus, Nipah virus, avian influenza (H5N1), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Middle East respiratory syndrome coronaviruses (MERS-CoV), and recent regional outbreaks of monkeypox virus (MPXV) in the twentyfirst century, is alarming (Mourya et al., 2019). This spillover of viruses from animal origin to humans has predominantly been due to species barrier crossing (Bezerra-Santos et al., 2021). At a time when global health experts and world communities were awaiting the pandemic spread of coronavirus disease 2019 (COVID-19) to be diminished, contemporary global populations now face an unexpected MPX epidemic. In previous decades, irregular outbreaks, with thousands of MPX cases, have been predominantly limited to African countries.

MPX is enzootic in several sub-Saharan states and has co-occurred in African inhabitants for several years but has not received sufficient consideration from global technical experts. Excitingly, MPX, for the first time, acquired worldwide consideration when it appeared in the USA in 2003 (Reed et al., 2004). Such sporadic and confined occurrences of MPX in the non-enzootic world have been associated with international travel and the importation of infected animals (Reed et al., 2004). Although MPXV spread among humans has been soundly investigated, its extensive concurrent appearance in non-enzootic nations has hit the globe with another shock. Additionally, MPX epidemics have been poorly inspected, irregularly reported, and poorly epidemiologically defined in the past, and, ultimately, the picture of this infection is incomplete. This menace can intensify with temporal patterns in the case where there is a rise in virulence naturally or through genomic rearrangement, a spillover into extra extensively dispersed taxa, or entrance and cluster epizootics in non-enzootic states (Sklenovská and Van Ranst, 2018). All these risks are further worsened by enhanced desforestation, increasing population density, large-scale international travel, immigration, invasion and damage of natural animal habitations, and poor epidemiological approaches toward emerging and re-emerging disease investigations (Adler et al., 2022). Lately, MPX is making headlines due to the worldwide surge in the occurrence of the infection in many countries and continents. On 24 July 2022, the World Health Organization (WHO) declared MPX a global public health threat. As per the US Centers for Disease Control and Prevention report of 2 August 2022, ∼25,391 clinically confirmed MPX cases have been documented across 87 states globally (CDC, 2022b). This number has been ballooning prospectively in the USA, Brazil, Spain, the UK, and Germany (WHO, 2022d). More recently, on 27 September 2022, over 66,000 confirmed MPX cases have been documented in more than 100 non-endemic states, with fluctuating epidemiological footprinting from retrospective epidemics. In this scenario, when the global occurrence of MPX does not decline, the world might see another pandemic, which may be presently hanging over the head of the contemporary world and may easily become a global public health threat. Therefore, this review assembles updated literature on the different aspects of MPXV regarding disease epidemiology, host range, and associated risk factor, and also sheds light on its epizootic potential and global public health threat. Restoring public health setups and preparing for upcoming epidemics are required, particularly in underdeveloped countries with deprived healthcare delivery services.

## 2. MPX

Monkeypox (MPX) is a sporadic zoonotic viral infection caused by the MPXV, which belongs to the genus *Orthopoxvirus* of the family Poxviridae and is interrelated to the already eradicated smallpox virus. It is a large, enveloped virus comprising a dsDNA genome of 190 kbp and having a dumbbell-shaped core with horizontal figures (Kugelman et al., 2014). The MPXV has two distinct genomic groups, the West African clade and the Congo Basin clade. These genomic groups have been geologically isolated with diverse clinical and epizootological characteristics (WHO, 2022d). The Congo Basin clade is recognized to induce serious infection and can spread among humans with a fatality rate of ∼11%. However, the West African clade displays a fatality rate of <1% and has never been known to exhibit human-to-human spread (Jezek et al., 1987). The early signs and symptoms of MPX are frequent pyrexia, vigorous headache, myalgia, lymphadenopathy, and lethargy. After fever, the dermal wounds characteristically burst within 1 to 3 days. The rash tends to be more confined to the facial region and extremities as compared with the trunk region of the body. MPX is frequently a self-determining disease, and symptoms last from 2 to 4 weeks. The clinical appearance and indications of MPX are exactly like those of smallpox; however, it is a mild and rarely fatal infection (Soheili and Nasseri, 2022).

Monkeypox virus (MPXV) continues to present challenges to public health and healthcare providers in areas with endemic disease, owing to inadequate capacity to diagnose and clinically manage patients and to accurately identify exposures (McCollum, 2023). Mostly, MPX cases in the African subcontinent are mainly misdiagnosed with other zoonotic infections such as cutaneous anthrax, chickenpox (Varicella), staphylococcal-associated rash, or fungal diseases in cases with human immunodeficiency virus (HIV) infection (Formenty et al., 2010). In addition to the current outbreak, there have been multiple reports of initial misdiagnosis of patients who were later confirmed to have MPX (Heskin et al., 2022; Minhaj et al., 2022) due to an atypical clinical manifestation that does not resemble the MPX observed in African outbreaks. Laboratory evaluations for monkeypox cases include electron microscopy, immunohistochemistry, culture of material from rash specimens, serological testing for specific antibodies, and real-time or conventional polymerase chain reaction (PCR) assays. Confirmation of specimens from suspected MPX cases is performed using nucleic acid amplification testing, such as real-time or conventional polymerase chain reaction. Restriction fragment length polymorphism (RFLP) of PCR-amplified genes or gene fragments is also used to detect monkeypox DNA. However, this method is time-consuming and requires a virus culture. Whole-genome sequencing, using next-generation sequencing technologies, is the gold standard for the characterization of MPXV and other orthopoxviruses. However, the use of most of the above diagnostic tools is limited due to their high cost and advanced technology, especially in developing countries and regions with limited healthcare resources (MacNeil et al., 2011; Radonić et al., 2014; Brown and Leggat, 2016; Petersen et al., 2019b; Alakunle et al., 2020; Cohen-Gihon et al., 2020; Altindis et al., 2022).

Another issue that may cause the disease to re-emerge is a failure to offer vaccination to susceptible persons in places where human immunodeficiency virus (HIV) infection is widespread. Insufficient studies have been devoted to producing a specialized vaccine to prevent the infection (Heymann et al., 1998), given the recent return of infectious illnesses during an epidemic. Moreover, routine vaccination is currently not available in endemic countries having limited healthcare resources (Damon, 2011). The extent of protection against the MPXV outbreak offered by vaccines remains unclear. Similarly, there is currently no specific treatment approved for MPXV infection, though there are several antivirals that have been developed and are being tested to treat smallpox, including tecovirimat, brincidofovir, and cidofovir (Adler et al., 2022). The present regionalized spatial distribution of MPX-confirmed cases is shown in Figure 1 (Kaler et al., 2022).

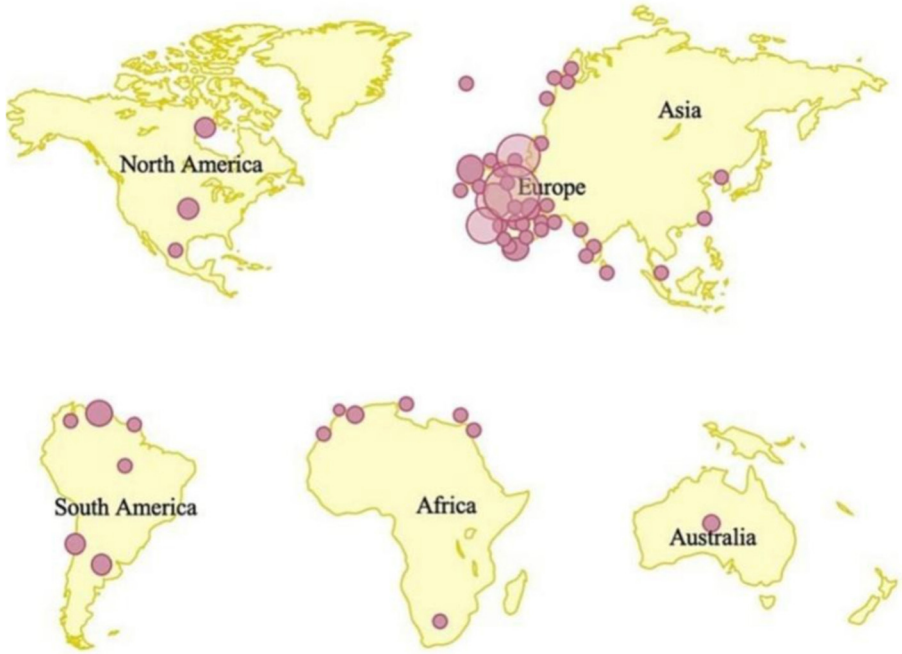


FIGURE 1
The present regionalized spatial distribution of MPX-confirmed cases.

## 3. Epidemiological dynamics of MPX

Monkeypox (MPX) is an infection of global public health significance as it not only affects states in central and western African regions, but also the contemporary globe is under threat (Reynolds et al., 2007). In the previous era, the frequency of human monkeypox (HMPX) infection was sporadic, and irregular cases were investigated in several African states. The first HMPX case was recognized in the 1970s in the Democratic Republic of the Congo (DRC), associated with a 9-month-old child (Foster et al., 1972; Arita et al., 1985). This investigation was expanded to further irregular cases recorded in 11 other states of Africa including Gabon, Cameroon, Benin, the Central African Republic, DRC, the Republic of the Congo, South Sudan, Nigeria, Côte d'Ivoire, Sierra Leone, and Liberia (Durski et al., 2018; World Health Organization, 2022a). From February 1996 to February 1997, a huge outbreak of MPX was recorded in the DRC, and ∼511 infected cases were investigated (Centers for Disease Control Prevention (CDC), 2022c). A systemic review and meta-analysis (Sham et al., 2022) explained details of suspected, confirmed, and fatal MPX cases by country and year-wise.

In 2003, an MPX epidemic occurred in the US, with 47 apparent or confirmed cases. Investigation showed that the affected individuals were exposed to the virus via pet prairie dogs retained with other mammals in a pet supply capacity, comprising the primary host and rodents from African Ghana (Bernard and Anderson, 2006). Petersen et al. (2019) investigated 116 clinically verified individuals with a death rate of 6.7%, and ∼280 suspicious cases appeared in Nigerian territory in 2018, with the large majority of cases in individuals under 40 years of age.

The frequency of the infection has affectedly amplified, and the DRC recorded 20 times more cases between 1981 and 1986 (7.2 cases per 100,000 people) and 2006 and 2007 (144.2 cases per 100,000 people), and a 5-fold rise from 2001 (0.64 cases per 100,000 people) to 2012 (3.11 cases per100, 000 people) (Hoff et al., 2017). Bunge et al. (2022) collected data from 28 available manuscripts and 15 gray database studies on HMPX infection point out that the incidence rate has been amplified since 1970s, with a rise in the intermediate age of infected individuals from 4 years old in the 1970s to 21 years old from 2010 to 2019. Table 1 adopted from Brown and Leggat (2016), Beer and Rao (2019), Adegboye et al. (2022) and Hatmal et al. (2022) shows the incidence of MPX and the number of deaths from 1970 to 2021.

In the retrospective outbreak investigations, MPX was recorded in youngsters and teenagers in the enzootic areas, with the same clinical picture and symptoms as observed in older individuals. The WHO has lately documented that serious cases of MPX more frequently occur among youngsters and are associated with the level of virus contact. Moreover, the severity of MPX cases may be associated with individual health conditions, the nature of complexities, and essential immune insufficiencies (WHO, 2022c). Individuals whose date of birth was after the 1980s are at greater risk because immunization for SPX stopped after its eradication, and this immunization can also defend people against MPX (Simpson et al., 2020). Additionally, it was thought that MPX infects women and men similarly, but, in the recent multi-country epidemic, several MPX cases have been reported in men who have sex with other men (MSM) (Bunge et al., 2022; Perez Duque et al., 2022; WHO, 2022c; Xiang and White, 2022). As per the CDC

10.3389/fmicb.2023.1160984

TABLE 1 Incidence of MPX and the number of deaths from 1970 to 2021.

| Country/Region | Timeframe | Total cases | Total deaths | References |
|---|---|---|---|---|
| Democratic Republic of the Congo (DRC) | 1970 | 1 | 1 | (Ladnyj et al., 1972) |
| | 1981–1986 | 338 | 33 | (Jezek and Fenner, 1988) |
| | 1996–1997 | 773 | 8 | (CDC, 1997) |
| | 2001–2013 | 19,646 | 335 | (Hoff et al., 2017) |
| | 2016 | 155 | 11 | (Laudisoit et al., 2016) |
| | 2019–2020 | 8388 | 244 | (WHO, 2022g) |
| Central African Republic | 2001 | 8 | 2 | (Berthet et al., 2011; Nakoune et al., 2017) |
| | 2010 | 2 | 0 | (Berthet et al., 2011) |
| | 2015 | 3 | 1 | (Nakoune et al., 2017) |
| | 2015–2016 | 62 | 5 | (Kalthan et al., 2018; WHO, 2022h) |
| | 2017–2018 | 41 | 1 | (Durski et al., 2018; WHO, 2022i) |
| Republic of the Congo | 2003 | 12 | 1 | (Learned et al., 2005) |
| | 2010 | 11 | 1 | (Reynolds et al., 2013) |
| | 2017 | 88 | 6 | (Durski et al., 2018) |
| Sudan Cameroon | 2005 | 37 | 0 | (Formenty et al., 2010) |
| | 1989 | 1 | 0 | (Tchokoteu et al., 1991) |
| Gabon | 1987 | 1 | 1 | (Müller et al., 1988) |
| | 1991 | 9 | 0 | (Durski et al., 2018) |
| Nigeria | 1971–1978 | 3 | 0 | (Breman et al., 1980) |
| | 2017–2018 | 228 | 6 | (Alakunle et al., 2020) |
| Sierra Leone | 1970–1971 | 1 | 0 | (Breman et al., 1980) |
| | 2014–2017 | 2 | 1 | (Durski et al., 2018) |
| Liberia | 1970–1971 | 4 | 0 | (Breman et al., 1980) |
| Côte d'Ivoire | 1971 | 1 | 0 | (Breman et al., 1977) |
| USA | 2003 | 47 | 0 | (Reed et al., 2004; Sejvar et al., 2004) |
| | 2021 | 2 | 0 | (World Health Organization, 2022e) |
| Singapore | 2019 | 1 | 0 | (Yong et al., 2020) |
| UK | 2018 | 4 | 0 | (Vaughan et al., 2020) |
| | 2019 | 1 | 0 | (UK, 2022) |
| | 2021 | 3 | 0 | (Yong et al., 2020) |

Source: Adopted from Brown and Leggat (2016), Beer and Rao (2019), Adegboye et al. (2022), and Hatmal et al. (2022).

report on the 2022 outbreak, the majority of MPX cases are due to MSM, which puts bisexual, transgender, and gay individuals at a greater threat of MPX (CDC, 2022b). Further investigations are mandatory for a better understanding of risk factors regarding sexual transmission dynamics of MPXV among MSM. The multi-state 2022 epidemic of MPX cases and deaths recorded by WHO (2022e) is shows in Table 2.

In some investigations, there is evidence of mixed infection of MPX with other blood-borne diseases and some sexually transmitted diseases (Liu et al., 2022), and people with HIV infection reflected a greater risk dynamic for MPX in the recent epidemic (Khaity et al., 2022; Bragazzi et al., 2023). In advanced cases of uncontrolled HIV infection, inappropriate immune response is significantly related to a weak prognosis, a longer period of disease signs, late curing of self-controlling MPX, and complex cures (Iñigo Martínez et al., 2022; Liu et al., 2022). Consequently, sorting MPX cases for HIV is extremely suggested in MSM (Liu et al., 2022). Recently, MPX has been accepted as a key factor that escalates the chance of contracting HIV (Davido et al., 2022; Patrocinio-Jesus and Peruzzu, 2022). A recent

TABLE 2 MPX cases and deaths recorded by the WHO during the multi-state 2022 epidemic (as of 8 June 2022) (World Health Organization, 2022e).

| WHO zone | State | Confirmed cases | Suspected cases | Deaths |
|---|---|---|---|---|
| AFRO | Liberia | 0 | 4 | 0 |
| | Sierra Leone | 0 | 2 | 0 |
| | Republic of Congo | 2 | 7 | 3 |
| | DRC | 10 | 1,356 | 64 |
| | Central African Republic | 8 | 17 | 2 |
| | Ghana | 5 | 12 | 0 |
| | Nigeria | 31 | 110 | 1 |
| | Cameroon | 3 | 28 | 2 |
| AMRO | Argentina | 2 | 0 | 0 |
| | Canada | 110 | 0 | 0 |
| | Mexico | 1 | 0 | 0 |
| | USA | 40 | 0 | 0 |
| EMRO | UAE | 13 | 0 | 0 |
| | Morocco | 1 | 0 | 0 |
| EURO | Austria | 1 | 0 | 0 |
| | Belgium | 24 | 0 | 0 |
| | Czech Republic | 6 | 0 | 0 |
| | Denmark | 3 | 0 | 0 |
| | Finland | 3 | 0 | 0 |
| | France | 66 | 0 | 0 |
| | Germany | 113 | 0 | 0 |
| | Hungary | 2 | 0 | 0 |
| | Ireland | 9 | 0 | 0 |
| | Italy | 29 | 0 | 0 |
| | Israel | 2 | 0 | 0 |
| | Latvia | 2 | 0 | 0 |
| | Malta | 1 | 0 | 0 |
| | Netherlands | 54 | 0 | 0 |
| | Norway | 2 | 0 | 0 |
| | Portugal | 191 | 0 | 0 |
| | Slovenia | 6 | 0 | 0 |
| | Spain | 259 | 0 | 0 |
| | Sweden | 6 | 0 | 0 |
| | Switzerland | 12 | 0 | 0 |
| | UK | 321 | 0 | 0 |
| WPRO | Australia | 6 | 1 | 0 |
| Cumulative | 36 countries | 1,344 | 1,537 | 72 |

epidemiological study from Madrid, Spain reported that 44.3% (225 cases out of 508 totals) of MPX-confirmed cases were linked to HIV infection (Iñigo Martínez et al., 2022). An additional report from London, UK indicated that 35.9% (70 cases out of 195 totals) of MPX-confirmed cases were linked to HIV infection (Patel et al., 2022). Similarly, mild infections of MPX among individuals with HIV and AIDS have been documented in Italy and Portugal (Antinori et al., 2022; Perez Duque et al., 2022), particularly among people with enhanced T-helper cell count, untraceable HIV viral genomic substance, and weak anti-retroviral treatment (Ortiz-Martínez et al., 2022). Infected individuals with immunological suppression initiated by HIV presented a clear-cut, wide scale of clinical appearances and characteristic MPX wounds. Fever, exanthema, inguinal lymphadenopathy, and genital ulcers were major clinical appearances in MPX-infected individuals during the epidemic in Portugal (Perez Duque et al., 2022). Pustules, papules, and a necrotic centralized wound in the perianal region, trunk, genitals, mouth, and facial region were recorded in a 24 year old bisexual man with HIV infection (De Sousa et al., 2022). Moreover, throughout the 2017–2018 MPX outbreaks in Nigerian regions, the majority of mortalities related to MPX were in individuals with unrestrained HIV, with AIDS appearances, who were not receiving proper medication (Yinka-Ogunleye et al., 2019). Another study on Nigeria showed that mixed HIV-infected MPX cases had a more prolonged disorder, greater wounds, and greater frequency of both genital ulcers and bacterial skin diseases, compared with HIV-negative MPX-infected individuals (Ogoina et al., 2020). Mixed infection with other sexually transmitted diseases (STDs) was also documented among MPX and HIV cases. An infected individual with unidentified progressive HIV and syphilis presented with a severed penis, oral mucosal infection, nasal necrotic wound, and MPX lesions spread over the entire body (Boesecke et al., 2022).

Active surveillance of MPX was carried out in nine regions of central DRC during 2005–2007, and ~760 MPX confirmed cases were recorded, with an annual occurrence of 55.3 per 100,000 people. Male gender, age <15, a history of vaccination against SPX, and inhabitants of afforested regions were the main associated risk factors of MPX (Rimoin et al., 2010). In 2017, a huge incidence of MPX was recorded in the Nigerian regions, with over 500 suspicious, over 200 confirmed cases, and a death rate of 3% (World Health Organization, 2022b). In an additional study, Beer and Rao (2019) investigated 71 reports relating to MPX cases and local epidemics during 1970–2018. The rates of documented occurrences were amplified since 1970, with an overall of 35 recorded epidemics outside the DRC, with 20 between 2010 and 2018.

The CDC, from 1 January 2022 to 5 August 2022, documented 28,220 confirmed cases of MPX in 88 states of the world (CDC, 2022e). The majority of these cases (27,875) were documented in 81 states that have not retrospectively documented MPX (CDC, 2022e). Additionally, a few months ago, the WHO investigated various human MPX outbreaks in different regions of Europe, the Americas, the Eastern Mediterranean, and the Western Pacific, with a total of 1,285 MPX confirmed cases, while 59 confirmed and 1,536 suspicious MPX cases were recorded, with 72 deaths occurring in African territories from January 2022 to June 2022 (World Health Organization, 2022e). The host range and susceptibility to MPXV infection was detected during

TABLE 3 Host range and animals susceptible to MPXV infection (Silva et al., 2020).

| Order/Family | Species | Tool of investigation* | Relationship to human infection** |
|---|---|---|---|
| Hominidae/Primates | *Homo sapiens* (Humans) | Virus isolation | Yes |
| | *Pongo pygmaeus* (Orangutans) | Virus isolation | Yes |
| | *Pan troglodytes* (Chimpanzees) | Virus isolation | No |
| Cercopithecidae/Primates | *Cercocebus atys* (Sooty mangabeys) | PCR/virus isolation | No |
| | *Macaca fascicularis* (Cynomolgus monkeys) | Virus isolation | Yes |
| Callithrichidae/Primates | *Callithrix jacchus* (White-tufted marmosets) | Lab. infection | No |
| Chinchillidae/Rodentia | *Oryctolagus cuniculus* (Rabbits) | Lab. infection | No |
| Muridae/Rodentia | *Mus musculus* (Inbred mouses) | Lab. infection | No |
| Cricetidae/Rodentia | Hamsters | Lab. infection | No |
| Nesomyidae/Rodentia | *Cricetomys sp.* (Giant-pouched rats) | PCR/virus isolation | No |
| Gliridae/Rodentia | *Graphiurus sp.* (African dormices) | PCR/virus isolation | No |
| Sciuridae/Rodentia | *Funisciurus sp.* (Rope squirrels) | PCR/virus isolation | Yes |
| | *Cynomys ludovicianus* (Black-tailed prairie dogs) | PCR | Yes |
| | *Marmota monax* (Woodchucks) | PCR/ virus isolation | No |
| Dipodidae/Rodentia | *Jaculus sp.* (Jerboas) | PCR/ virus isolation | No |
| Hystricidae/Rodentia | *Atherurus africanus* (Porcupines) | PCR/virus isolation | No |
| Macroscelididae/Pilosa | *Myrmecophaga tridactyla* (Ant-eaters) | Virus isolation | No |
| Didelphidae/Didelphimorphia | *Didelphis marsupialis* (Southern opossums) | PCR/ virus isolation | No |
| | *Monodelphis domestica* (Shot-tailed opossums) | PCR/virus isolation | No |
| Erinaceidae/Erinaceomorpha | *Atelerix sp.* (African hedgehogs) | PCR/virus isolation | No |

*Tool of investigation: virus isolation from naturally infected animals; laboratory infection; or molecular assay (PCR). Susceptibility to MPXV infection was detected during investigational research in the laboratory. **Transmission to humans previously described in the literature (Silva et al., 2020).

investigatory research in the laboratory by Silva et al. (2020) shown in Table 3. Several eco-bionomical, environmental, and geostrategic dynamics might have led to the regional and global appearance and re-appearance of MPX infection, including the misuse of rain timberlands, climate alteration, civil and military clashes in disease areas, highly mobile populations, declining herd immunity, and the ceasing of SPX immunization (Fauci, 2005; Liu et al., 2022). On the contrary, the reservoir host, natural history, and pathogenesis of MPXV are uncertain; hence, there are significant disputes in recognizing the epidemiological dynamics of MPX infection (Petersen et al., 2019).

## 3.1. Epidemiological dynamics of MPX retrospective to the global epidemic in 2022

Based on 50 years of retrospective analysis of MPX, the DRC has been the single state to constantly investigate HMPX patients, and, in the previous 30 years, the figure for documented infected individuals was over 1,000 per annum (Bunge et al., 2022; WHO, 2022f). During the year 2020, ~6,257 suspicious individuals of HMPX were investigated in the DRC (WHO, 2022f). In the initial 120 days of 2022, ~1,238 Central African clade-associated new MPX cases were documented in the DRC (Bunge et al., 2022; World Health Organization, 2022a).

Human monkeypox (HMPX) was only reported outside the African region when outbreaks linked to infected pet prairie dogs increased in the USA in 2003 (Brown and Leggat, 2016; Centers for Disease Control Prevention (CDC), 2020). None of the cases in this outbreak (a total of 81 recognized cases, 40% of which were confirmed cases) were attributed to secondary transmission, and the mortality rate was zero. The dogs acquired infections from infected exotic dormice and pouched rats, which were transported from Ghana.

Multiple factors are involved in the rise of HMPX since the 1970s. These include active, passive, and sentinel surveillance efforts, climatic dynamics, deforestation, and rapid demographic expansion of regions where the MPXV is retained in a huge population of host animals, with a surge in natural or incidental hosts. Furthermore, individuals aged 40–45 years or less lack immunity to the smallpox virus after the termination of immunization against smallpox in the 1980s. In summary, significantly associated dynamics also involve hominid behavior (for example, interaction with dead or live creatures, reservoir hosts, staying in tropically reforested or newly desforested ranges,

TABLE 4 Risk factors associated with MPX cases.

| Risk factor | References |
|---|---|
| Age | In Nigeria, the age of individuals affected by MPX was <40 years, with the absence of cross-protective resistance as they were born after the termination of the smallpox eradication campaign (Petersen et al., 2019). |
| Nosocomial infection | Healthcare-associated spread (Petersen et al., 2018). |
| Zoonotic infection | Interaction with infected prairie dogs (Kile et al., 2005) and wildlife, bites from peri-domestic animals, hunters (Meslin et al., 2000; Reynolds et al., 2007), household materials (Quiner et al., 2017; Yinka-Ogunleye et al., 2019; Guagliardo et al., 2020), and peridomestic rodents (Reynolds et al., 2010; Salzer et al., 2013). |
| Travelers | Immigrants to non-endemic monkeypox regions (Alakunle et al., 2020). |
| Human to human transmission | Inter-human transmission (Nolen et al., 2015). |
| Human-to-animal transmission | Human-to-dog transmission was reported in France and Brazil (Peters, 1966; Seang et al., 2022; Islam et al., 2023). |
| Men who have sex with men (MSM) | MPX was spread among MSM, those who have bisexual contact, and those who have sex with everyone, including male colleagues (Endo et al., 2022), young men who have sex with other men, engage in unsafe manners and actions comprising unsafe sex, HIV positivity, and retrospective records of sexually transmitted diseases (STDs), including syphilis (Bragazzi et al., 2023). |

hunting, close interaction with an infected individual, sharing a joint bedroom with an infected individual, sharing kitchenette kits with an infected individual, and preparation and intake of bush meat or monkeys), scarcity, military, and political conflicts, territorial movements, tourism, the trade of exotic animals, and public healthcare services (Hutin et al., 2001; Parker et al., 2007; Rimoin et al., 2010; Vaughan et al., 2020; Mauldin et al., 2022; Quarleri et al., 2022).

## 3.2. Epidemiological dynamics of MPX in the global epidemic in 2022

Since May 2022, many outbreaks of HMPX have been documented in European states for the first time, where the MPX infection is not prevalent (ECDC, 2022a; Sham et al., 2022; World Health Organization, 2022b,j). From 13 May 2022 to 16 May 2022, the UK documented six HMPX cases for the first time; these cases were investigated without any epidemiological associations with imported animals, travel to African countries, and with all cases self-distinguishing as men who have sex with other men, bisexual, or gay (WHO, 2022c). The majority of HMPX cases have a travel record to various states in Europe and America. Moreover, cases of HMPX in the enzootic world remain to be described.

Since early May and as of 19 September 2022, over 62,000 HMPX cases have been documented in the non-endemic world (Centers for Disease Control and Prevention (CDC), 2022d). As of 19 September 2022, ∼44 European republics have documented 24,017 cases, demonstrating 38.5% of all the globally documented cases in the recent epidemic. The highest figure (n = 6947) was documented in Spain, followed by France (n = 3898), Germany (n = 3563), and the UK (n = 3552); however, one case each was documented in Ukraine and Turkey. In this epidemic, the largest number of cases (n = 23,892) was documented in the USA, comprising 38.3% of the globally reported MPX cases. Variations in the incidence rate of HMPX by state might be relatively described by dissimilarities in demography and density population at threat, social and economic circumstances, under-diagnosis, and/or improper reporting.

The person-to-person transmission dynamic of HMPX has been documented in the European region for the first time (ECDC, 2022a; Vivancos et al., 2022). In the recent occurrence, clinical features that differ from retrospective documentations were investigated, including the lack of prodromal or very minor prodromic symptoms, a rash that appears earlier than the prodromic stage, a rash that exhibits only an ulcer or some abrasions, a skin rash restricted only to the perineal or anogenital region, and mainly inguinal site lymphadenopathy (Bunge et al., 2022; Iñigo Martínez et al., 2022; Thornhill et al., 2022). Based on the severity, MPX is categorized as mild and moderate, with ∼4 to 10% of patients admitted to hospitals (Centers for Disease Control and Prevention (CDC), 2022d; Girometti et al., 2022; WHO, 2022d). Due to encephalitis and comorbidities, ∼20 deaths due to MPX have been documented in the current multiregional epidemic, a figure that matches that in Africa as well as in non-endemic states (Centers for Disease Control and Prevention (CDC), 2022d; ECDC, 2022a; European Centre for Disease Prevention Control (ECDC), 2022c). Though several documentations specified a small number of cases without symptoms (Centers for Disease Control and Prevention (CDC), 2022d), a UK-based cohort study investigation showed interactions with an individual with confirmed MPX infection were recorded in ∼25% of cases (Patel et al., 2022). In this prospective epidemic, there has been no concrete evidence of animal-to-human or human-to-animal spread. In this occurrence, the investigated viruses were linked to the West African clade (Isidro et al., 2022; Kmiec and Kirchhoff, 2022).

An epidemiological study at 43 locations in 16 investigated states documented that ∼99% of men were affected by MPX, among whom 98% self-distinguished as bisexual men or gay, or men who have sex with other men (Thornhill et al., 2022). In the current study, the 18–50 years of age range was reported as having an average of 38 years of age. Among them, 41% closely interacted with HIV patients, and in most of the cases, HIV was considerably controlled. Pre-exposure prevention protocol was adopted by 57% of HIV-negative individuals or those patients who were not aware of their HIV status. In 29% of examined individuals, there was evidence of associated sexually communicated infections. In this study, confirmation of sexual transmission of infection was impossible, sex-related history was investigated in 95% of5

patients, 20% reported engaging in "chem sex" (sex-linked with the practice of medicines), and 32% reported attending on-site-sex events (Thornhill et al., 2022).

In the Spanish outbreak (Iñigo Martínez et al., 2022), ∼84.1% of MPX cases were documented as having a history of condomless sex or having sex with more than one sex partner within 3 weeks before the beginning of disease indications, 8.1% of infected persons confirmed having safe sexual activities, and 7.9% gave no response. Furthermore, in the present report, ∼80.3% of individuals were not aware of MPX or had no interaction with a recognized MPX case. One month before MPX diagnosis, numerous individuals had an international travel history to Italy, the UK, Germany, Belgium, Portugal, Peru, etc., with no recorded cases of travel to African countries. Furthermore, at a sauna region in Madrid and at the Gay Pride festival on a Spanish island, some cases of MPX were reported, with various secret gatherings also having a major role; dating via social networks was recorded by 56.9% of individuals as well as sexual activities in bars, touring zones, and secret studios. In this occurrence, the MPXV was investigated in seminal fluid samples of the patients, with sexual interaction acts a significantly associated factor in the disease occurrence. More investigation is required to explain the sexual transmission dynamics of MPX via genital fluids (Antinori et al., 2022; Iñigo Martínez et al., 2022; Thornhill et al., 2022; Noe et al., 2023).

Remarkably, various reports show that data were registered as having a lack of immunization status (Benites-Zapata et al., 2022). Among the US MPX cases for whom immunization status was accessible, 14% testified retrospectively to being vaccinated against smallpox (with 23% receiving single instead of double doses, 23% receiving pre-exposure prevention at an unidentified stage before the current occurrence, and 54% of individuals not providing an answer about vaccination status) (Philpott et al., 2022). To date, 344 MPX cases have been recorded among medical staff, and among them, some cases of spread via job-related exposure have been described in this occurrence (Centers for Disease Control and Prevention (CDC), 2022d). Worldwide, youngsters are most vulnerable to MPX because of the termination of smallpox immunization after the eradication of smallpox (Factsheet for health professionals on monkeypox: European Centre for Disease, 2022d). To avoid MPX, two vaccines (JYNNEOS and ACAM2000) are applied as follows: JYNNEOS vaccine is applied to safeguard against both smallpox and MPX, whereas the ACAM2000 vaccine is applied to protect against smallpox (Centers for Disease Control Prevention (CDC), 2022c; Factsheet for health professionals on monkeypox: European Centre for Disease, 2022d). The feedback of the immune system after vaccination is mainly based on cross-defense among the orthopoxviruses and vaccinia virus (McCollum and Damon, 2014; ECDC, 2022a). In the ongoing occurrence of MPX in the USA, men who have sex with other men, gender-diverse individuals, or transgender individuals who had sex with men in the previous 14 days might get the vaccination if they had sex with numerous individuals, or had sex at commercial sex clubs or bathhouses, or had sexual activities at an occasion, site, or in a zone where MPX spread is happening (Centers for Disease Control Prevention (CDC), 2022c). As per the recommendations of the WHO, several states in Europe, including the UK, Germany, France, and Spain, were providing immunization during the 2022 MPX epidemic (ECDC, 2022b; Factsheet for health professionals on monkeypox: European Centre for Disease, 2022d).

The WHO measures the MPX threat as sensible worldwide, with the exemption of the European and American regions, where the threat is evaluated as high (Factsheet for health professionals on monkeypox: European Centre for Disease, 2022d; Zachary and Shenoy, 2022). The recent global occurrence differs from previous epidemics in a few ways: the infrequent degree of incidence; unusual rapid expansion globally; spreading in non-endemic countries; mostly spreading among younger men (aged 18–44 years), with over 97% of them self-recognizing as men who have sexual intercourse with other men or unsafe sex with several individuals; the role of different super spreading occasions associated with transnational get-togethers; while asymptomatic infections and lack of or mild signs throughout the prodromal period make easier the transmission dynamics of the virus; and the occurrence of minor cases (Bunge et al., 2022; Centers for Disease Control and Prevention (CDC), 2022d; Delaney et al., 2022; WHO, 2022d). In summary, an advanced investigation is required to properly recognize and advance the supervision of HMPX.

## 4. MPX host range

Monkeypox virus (MPXV) isolates based on phenotypic and genetic deviations are divided into two different clades, specifically the Congo Basin and the West African clades (Likos et al., 2005). In contrast to the variola virus, which affects only humans, the MPXV is among those orthopoxviruses that can infect numerous animal hosts and can spread to humans (Parker et al., 2007; Parker and Buller, 2013; Patrono et al., 2020; Kmiec and Kirchhoff, 2022). The fixed reservoir host of the MPXV can even be unrecognized, but some small mammalians such as giant pouched rats (*Cricetomys spp.*), rope squirrels (*Funisciurus spp.*), sun squirrels (*Heliosciurus spp.*), and African dormice (*Graphiurus spp.*) are assumed to transmit the virus to human beings in Central and West Africa (Alakunle et al., 2020). MPXV is communicated from animals to human beings during hunting, trapping, treating infected animals, and dealing with their secretory and excretory fluids.

Based on experimental analyses and field investigations, MPXV has been documented in a wide range of rodents, including *Oryctolagus cuniculus* (rabbits), *Mus musculus* (mice), *Marmota monax* (woodchucks), hamsters, *Jaculus* sp. (jerboas), and *Atherurus africanus* (porcupines). Similarly, based on techniques such as molecular assay, virus separation, or *in vitro* contamination, vulnerability to MPXV was investigated in black-tailed prairie dogs (*Cynomys ludovicianus*), anteaters, short-tailed opossums (*Monodelphis domestica*), giant anteater (*Myrmecophaga tridactyla*), African hedgehogs (*Atelerix* sp.), southern opossums (*Didelphis marsupialis*), and various non-human primate species (Parker et al., 2007; Doty et al., 2017). The host range and susceptibility to MPXV infection is also shown in Table 3 (Silva et al., 2020).

In Africa, Asia, and Europe, non-human primates, chimpanzees (*Pan troglodytes*), orangutans (*Pongo pygmaeus*), cynomolgus monkeys (*Macaca fascicularis*), and sooty mangabeys (*Cercocebus atys*) can be infected with MPXV. In the USA and the UK,

non-human primates (Magnus et al., 1959; Wachtman and Mansfield, 2012; Alakunle et al., 2020) and common marmosets (*Callithrix jacchus*) were determined to be vulnerable to MPXV by intravenous injection (Mucker et al., 2015). Non-human primates may be affected by MPXV and show signs and symptoms, while small mammalians can be asymptomatic carriers of the virus (CDC, 2022a).

In 2003, HMPX infection in the USA was mainly linked with close interaction with ill pet prairie dogs introduced from the Ghana region of West Africa (Reed et al., 2004). This incident, as well as the rodent's infection, intensified alarms about the entry of MPX infection into the USA. In the meantime, the vulnerability of numerous African rodents to MPXV raised fear related to the spread of the virus to human beings, as these rodents are often maintained as pets (Centers for Disease Control Prevention (CDC), 2020; Sklenovská, 2020). Non-human primates, squirrels, and rodents have been observed to have MPXV based on sero-investigations in African territories. Wild animals are more susceptible to the disease. In 1985, MPXV was isolated from Thomas's rope squirrel (*Funisciurus anerythrus*) in the DRC and in 2012 from the sooty mangabey (*Cercocebus atys*) in Cote d Ivoire, signifying that these animal species might act as MPXV reservoirs hosts (Falendysz et al., 2017).

Human beings can also be accidental hosts (Parker et al., 2007) since the eradication of smallpox, based on MPXV morbidity and mortality, it is converted into the most significant infective zoonotic orthopoxvirus for humans. In 1970, the first human case of MPXV was documented in a 9-month-old child in the DRC, who presented with smallpox-like skin lesions (Arita and Henderson, 1968; Ladnyj et al., 1972). Numerous humanoid cases were investigated in subsequent years. During 1970–1999, the WHO documented almost 404 confirmed and 500 suspicious cases of human MPXV in various African states (Liberia, Gabon, Côte d'Ivoire, Central African Republic, and Cameroon, but predominantly in the DRC) (World Health Organization, 1997; Heymann et al., 1998; Sklenovská and Van Ranst, 2018). In May and June 2003, some MPX cases were reported to the Wisconsin Division of Public Health, with no mortality and no person-to-person spread observed (Centers for Disease Control Prevention (CDC), 2022c). The origin of this occurrence was traced back to the importation of exotic infected animals from Ghana (Khodakevich et al., 1986; Sklenovská and Van Ranst, 2018; CDC, 2022b). Luckily, the stage-wise episode of infected rodents in cages in the USA was temporary, and the pattern of spread in the country was destroyed (Petersen et al., 2019a). More recently, on 27 September 2022, 66,000 cases of MPX were confirmed in more than 100 non-endemic states, with fluctuating epidemiological footprinting from retrospective outbreaks (Li et al., 2022).

Human MPX cases have been snowballing globally with time, although they might have been miscalculated. Remarkably, diagnostic capacities in the affected states are mostly inadequate, while global healthcare personnel are mostly unaware of MPX disease. The emergence of the current MPX spread is linked with dynamics such as the growing invasion of hominids into wild habitations, the international and global travel of the public from enzootic regions to non-endemic areas, the introduction of pets and laboratory animals, lack of active disease surveillance, and improper prevention and control strategies (Essbauer et al., 2010). Furthermore, the termination of smallpox immunization and various reports of animals in captivity or experimental laboratories have made the global public susceptible to MPXV infection or other orthopoxvirus infection. As the MPX virus is an increasing global zoonotic threat with epidemic potential, and as most of its host range and life cycle in nature remains unclear, developments are immediately mandatory to recognize its biological cycle and host range for future prevention and control strategy.

## 5. Associated risk factors of MPX

Although the main associated risk factors fluctuate among different epidemics, the significance of obtaining the characteristics of particular individuals for calculating and predicting epidemic patterns cannot be ignored. Conventionally, MPX cases involving spread among human beings are more probable to be individuals who are women, non-vaccinated against smallpox, living in the same house, or providing cure to a primary case (JeŽek et al., 1988). Prominently, this information is based on clade 1-associated MPX in the DRC and did not represent other enzootic regions; outbreak investigations of different endemic states show that youngsters face the ample burden of the MPX infection. In an occurrence of clade 2B-associated MPX in Nigerian territory, mostly 21 to 40-year-old individuals were involved (Alakunle et al., 2020), although the index case was an 11-year-old teenager (Ogoina et al., 2019; Hobson et al., 2021). These associated risk factors specify the role of social and behavioral determining factors in helping the person-to-person spread of MPX infection. However, a systemic review and meta-analyses (Sham et al., 2022) explained the detailed associated risk factors for the primary introduction of MPX.

One of the serious associated risk factors for patients and healthcare workers is nosocomial MPXV infections (nosocomial infections, also referred to as healthcare-associated infections (HAI), are infection(s) acquired during the process of receiving healthcare that were not present at the time of admission) in both enzootic and non-enzootic areas. Smallpox was also mainly due to nosocomial occurrences (CDC, 1963), with the peak rate of spread occurring inside health centers (Kiang, 2003). Similarly, hospital-borne occurrences of MPX are mainly serious and long-term. These consistent multifactorial results include individuals who are susceptible to diseases, healthcare center sanitation patterns, and the usage of aerosol-producing measures (Judson, 2019). A total of six generations of MPXV spread were investigated in a public healthcare center in Impfondo, Republic of Congo, specifying MPXV's potential to spread if not rapidly handled in healthcare settings (Learned et al., 2005). On one occasion in the UK, a medical employee who had collected a blanket and dressing of an MPX-infected person was subsequently contracted MPXV (Vaughan et al., 2020).

Zoonotic transmission (transmission from animals to human beings) can arise from direct interaction with the blood, body fluids, or mucosal or cutaneous lesions of infected animals (Nigeria Centre for Disease (NCDC), 2022). In Africa, MPX has been reported

in various hosts including tree squirrels, rope squirrels, dormice, Gambian poached rats, several types of monkeys, and other animals (Kile et al., 2005; Yinka-Ogunleye et al., 2019). The reservoir of MPX has not cleared yet; however, rodents are the main expected but not clear yet (Kile et al., 2005). The intake of uncooked meat and other foodstuffs of infected animal origin is a probable risk factor (Petersen et al., 2018). Individuals living in or near forested regions could have an incidental or low-degree of exposure to infected animals.

Human-to-human spread can result from close contact with respiratory discharges, dermal abrasions of an infected individual, or a newly infected entity (Nolen et al., 2015). MPXV spread via respiratory particles typically requires lengthy and close interactions, which put community health staff, families, and other close contacts of active cases at greater risk (Petersen et al., 2018). The predictable sequence of spread in the public has grown in the current era from six to nine repeated human-to-human contaminations, and this could indicate decreasing protection in humans due to the end of smallpox immunization (Meslin et al., 2000).

Human-to-animal transmission of MPXV has not been reported yet and it is believed that the outbreak may not have been caused by infection from animals (Diaz-Cánova et al., 2022). European health administrators firmly recommend that rodent pets, e.g., guinea pigs and hamsters, that belong to patients with HMPX should be quarantined and watched or even euthanized to stop the spread of the virus (Heskin et al., 2022). However, the most recent identifications in August 2022 were two cases of human-to-dog transmission reported in France and Brazil (Peters, 1966; Islam et al., 2023). In Paris, a pet dog (a healthy 4-year-old male Italian Greyhound) of two individuals who were suffering from MPX was also diagnosed with MPXV. The virus was found in the individuals, and the dog showed homology on DNA sequencing (Seang et al., 2022). This dog tested positive for MPXV after showing symptoms such as abdominal abscesses. Based on the sequencing results and symptoms of the two patients as well as the dog, the researchers concluded that MPXV was indeed transmitted between humans and dogs (Seang et al., 2022).

As per disease investigations, the main concern is more for youngsters and immunocompromised adults, such as persons who have HIV infection (De Sousa et al., 2022). The recent global MPXV occurrence in human beings increases the probability that the virus might have mutated genetically and that human behavior may have altered or collected. These mutations might have occurred due to decreasing smallpox immunity, diminishing COVID-19 protective policies, sexual connections, and the restart of intercontinental movements (Zhu et al., 2022). An additional factor recognized in the current topographical distribution of MPX spread is sexual interaction, in particular among men who have sex with other men (ECDC, 2022a). Table 4 shows the updated risk factors associated with MPX cases worldwide.

## 6. MPXV as a potential bioweapon

Monkeypox (MPX) is no longer a rare, self-limiting disease limited to endemic countries. The MPXV is a high-danger pathogen that can spread to various regions and poses a significant threat to public health. Its ever-changing epidemiology and transmission dynamics have increased the possibility of it evolving into a much deadlier pathogen that can be used as a bioweapon due to its unanticipated development in places with no known epidemiological linkages, which permits undetected transmission for a long period and raises concerns about the virus's evolution (Ferdous et al., 2023). Despite the potential of MPXV to be used as a global bioweapon, the possibility of biological warfare and bioterrorism cannot be completely ruled out due to modern molecular biological advances and the spread of the virus to various regions due to rising globalization and cross-border animal mobility. As a result of these factors, MPXV, along with the variola virus and many other poxviruses, is on the NIH's highest danger list. The CDC has categorized it as a "select agent." Human travel is prevalent today, providing risk for the spread of MPX, and animals carried across borders represent an immediate danger of disease spread (Amir et al., 2023; Khattak et al., 2023).

## 7. Critical challenges associated with MPX research

To better understand the dynamics of MPX transmission and control, operational research is currently facing challenges, such as insufficient resources for detailed case investigations and contact follow-up in affected communities. A lack of adequate diagnostic facilities in laboratories is a serious problem. Owing to the lack of laboratory diagnosis capacity and access, as well as the difficulty of diagnosing MPX, it is difficult to discover any underlying etiology. A seroprevalence study would help to understand the epidemiology as well as subclinical infection among contacts in communities (Lederman et al., 2007). The currently available serological assays are generic orthopox tests; they do not specifically test for the MPXV. This is due to the fact that there is cross-reactivity between MPX and smallpox viruses, and therefore, we cannot distinguish between a MPXV infection and prior smallpox vaccinations or other orthopoxvirus infections. In addition, these assays are not currently available in the marketplace. It has been found that, according to data collected from Nigeria, ∼20% of 70 MPX-negative patients presenting rash illness with similar antigens also had orthopox antibodies. To identify the transmission of other orthopoxviruses in human and animal populations, further research, including using molecular and genomic approaches, is needed (Ihekweazu et al., 2020).

Precautions such as avoiding close interaction with reservoir hosts and infected persons, proper handwashing and disinfection, avoiding non-important travel, usage of suitable personal protective equipment, appropriate practices of waste management, and quarantine, treatment, and immunization of infected individuals must be applied to reduce the spread of MPXV. It is necessary to enhance continuous active investigation and monitoring of the MPXV in community health services and in the general population, particularly in livestock populations such as animal farmhouses, marketplaces, and slaughterhouses. Individuals traveling from regions of the world where the infection

is prevalent must be tested and declared free of disease before entry to another country. Infected persons must be supervised to stop the further spread of the virus to vulnerable populations. The public must be made aware of and educated on the threats of bushmeat intake, zoonotic spread, the significance of one's health, and the application of protective procedures and biosecurity against the MPXV. Finally, training public health facilitators on how to avoid the spread of the disease and how to protect themselves from the threat of infection is critical because they are at greater threat of being infected (Idris and Adesola, 2022).

## 8. Conclusion

The contemporary global public in the present era has already survived the COVID-19 pandemic and the extraordinary damages it produced. Due to globalization, communicable infections are becoming more widespread and pose a global public health threat. There is no method to determine subsequent emerging diseases, but one example, COVID-19, has re-taught the globe that what virus will arise as a major public health threat is somewhat unpredictable and that it is frequently too late to put in place counter-measures after the fact. The unpredicted appearance of MPX in the non-endemic world suggests some undetectable transmission dynamics. Hence, open-minded and vigilant epidemiological attention and global public awareness of the recent MPX epidemic are required, not only in developed economies but also in underdeveloped states that have been dominated by such viruses for several years. There is an urgent need for researchers and epidemiologists to participate more in this global public health threat, follow up on it, and conduct more molecular epidemiological research on the topic. Therefore, there is an urgent need for proper epidemiological approaches to be adopted to investigate the emergence of current MPX epidemics, as well as the true cause of the disease, transmission dynamics, identification of associated risk factors, and investigation of the global host range. Rapid documentation of new cases, active investigation, and syndromic observational surveillance approaches would provide insights into variations in epidemiological tendencies, particularly in situations where validating diagnostic techniques is challenging. Therefore, this review has been compiled to highlight the epidemiology, global host ranges, and associated risk factors of MPX, focusing on its epidemic potential and global public health threat.

## Author contributions

Conceptualization: MU and ZZ. Methodology, investigation, data curation, and writing-original review draft preparation: MU, YL, and ZZ. Writing-review and editing and visualization: MU and KM. Funding acquisition: ZZ and YL. All authors have read and agreed to the published version of the manuscript.

## Funding

This study was funded and supported by Southwest Mizu University Double World-Class Project (XM2023012), the Southwest Mizu University Research Startup Funds (16011211013), the Natural Science Foundation of Sichuan Province (2022NSFSC0073), and the Fundamental Research Funds for the Central Universities Southwest Minzu University (2021115).

## Acknowledgments

We would like to thank ZZ and YL of the College of Animal Husbandry and Veterinary Medicine, Southwest Minzu University, Chengdu, China, for their support during the compilation of the review and providing the main idea for the manuscript. We are sincerely grateful to Prof. Dr. Arfan Yousaf and Dr. Muhammad Arif Zafar of the Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi for their official support.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## References

Adegboye, O. A., Eugenia Castellanos, M., Alele, F. O., Pak, A., Ezechukwu, H. C., Hou, K., et al. (2022). Travel-related monkeypox outbreaks in the era of COVID-19 pandemic: are we prepared?. *Viruses* 14, 1283. doi: 10.3390/v14061283

Adler, H., Gould, S., Hine, P., Snell, L. B., Wong, W., Houlihan, C. F., et al. (2022). Clinical features and management of human monkeypox: a retrospective observational study in the UK. *Lancet Infect. Dis.* 22, 1153–1162. doi: 10.1016/S1473-3099(22)00228-6

Alakunle, E., Moens, U., Nchinda, G., and Okeke, M. I. (2020). Monkeypox virus in Nigeria: infection biology, epidemiology, and evolution. *Viruses* 12, 1257. doi: 10.3390/v12111257

Altindis, M., Puca, E., and Shapo, L. (2022). Diagnosis of monkeypox virus–An overview. *Travel Med. Infect. Dis.* 10, 2459. doi: 10.1016/j.tmaid.2022.102459

Amir, M., Vohra, M., Osoro, I., Sharma, A., and Kumar, R. (2023). Monkeypox (Mpox) re-emergence: prevalence, diagnostics, countermeasures, and its global effect. *J. Zoon. Dis.* 7, 199–206. doi: 10.22034/JZD.2023.16032

Antinori, A., Mazzotta, V., Vita, S., Carletti, F., Tacconi, D., Lapini, L. E., et al. (2022). Epidemiological, clinical and virological characteristics of four cases of monkeypox support transmission through sexual contact, Italy, May 2022. *Eurosurveillance* 27, 2200421. doi: 10.2807/1560-7917.ES.2022.27.22.2200421

Arita, I., and Henderson, D. A. (1968). Smallpox and monkeypox in non-human primates. *Bull. World Health Organiz.* 39, 277.

Arita, I., Jezek, Z., Khodakevich, L., and Ruti, K. (1985). Human monkeypox: a newly emerged orthopoxvirus zoonosis in the tropical rain forests of Africa. *Am. J. Tropic. Med. Hygiene* 34, 781–789. doi: 10.4269/ajtmh.1985.34.781

Beer, E. M., and Rao, V. B. (2019). A systematic review of the epidemiology of human monkeypox outbreaks and implications for outbreak strategy. *PLoS Neglect. Tropic. Dis.* 13, e0007791. doi: 10.1371/journal.pntd.0007791

Benites-Zapata, V. A., Ulloque-Badaracco, J. R., Alarcon-Braga, E. A., Hernandez-Bustamante, E. A., Mosquera-Rojas, M. D., Bonilla-Aldana, D. K., et al. (2022). Clinical features, hospitalisation and deaths associated with monkeypox: a systematic review and meta-analysis. *Ann. Clinic. Microbiol. Antimicrob.* 21, 36. doi: 10.1186/s12941-022-00527-1

Bernard, S. M., and Anderson, S. A. (2006). Qualitative assessment of risk for monkeypox associated with domestic trade in certain animal species, United States. *Emerg. Infect. Dis.* 12, 1827–1833. doi: 10.3201/eid1212.060454

Berthet, N., Nakoun,é, E., Whist, E., Selekon, B., Burguière, A. M., Manuguerra, J. C., et al. (2011). Maculopapular lesions in the Central African Republic. *The Lancet* 378, 1354. doi: 10.1016/S0140-6736(11)61142-2

Bezerra-Santos, M. A., Mendoza-Roldan, J. A., Thompson, R. A., Dantas-Torres, F., and Otranto, D. (2021). Illegal wildlife trade: a gateway to zoonotic infectious diseases. *Trends Parasitol.* 37, 181–184. doi: 10.1016/j.pt.2020.12.005

Boesecke, C., Monin, M. B., van Bremen, K., Schlabe, S., and Hoffmann, C. (2022). Severe monkeypox-virus infection in undiagnosed advanced HIV infection. *Infection* 2, 1-2. doi: 10.1007/s15010-022-01901-z

Bragazzi, N. L., Kong, J. D., Mahroum, N., Tsigalou, C., Khamisy-Farah, R., Converti, M., et al. (2023). Epidemiological trends and clinical features of the ongoing monkeypox epidemic: a preliminary pooled data analysis and literature review. *J. Med. Virol.* 95, e27931. doi: 10.1002/jmv.27931

Breman, J. G., Nakano, J. H., Coffi, E., Godfrey, H., and Gautun, J. C. (1977). Human poxvirus disease after smallpox eradication. *Am. J. Tropic. Med. Hygiene* 26, 273–281. doi: 10.4269/ajtmh.1977.26.273

Breman, J. G., Steniowski, M. V., Zanotto, E., Gromyko, A. I., and Arita, I. (1980). Human monkeypox, 1970–79. *Bull. World Health Organizat.* 58, 165–182.

Brown, K., and Leggat, P. A. (2016). Human monkeypox: current state of knowledge and implications for the future. *Tropic. Med. Infect. Dis.* 1, 8. doi: 10.3390/tropicalmed1010008

Bunge, E. M., Hoet, B., Chen, L., Lienert, F., Weidenthaler, H., Baer, L. R., et al. (2022). The changing epidemiology of human monkeypox—a potential threat? a systematic review. *PLoS Neglect. Tropic. Dis.* 16, e0010141. doi: 10.1371/journal.pntd.0010141

CDC. (1963). "Smallpox–Stockholm, Sweden," in *Morbidity and Mortality Weekly Report,* Centers for Disease Control and Prevention: Atlanta, GA.

CDC. (1997). Human monkeypox – Kasai oriental, Democratic Republic of Congo, February 1996-October 1997. *MMWR*. 46, 1168–1171.

CDC. (2022a). *Monkeypox in animals. Atlanta, Georgia.* Available online at: https://www.cdc.gov/poxvirus/monkeypox/veterinarian/monkeypox-in-animals.html (accessed August 9, 2022).

CDC. (2022b). *Monkeypox outbreak global map. Centers for Disease Control and Prevention*. Available online at: https://www.cdc.gov/poxvirus/monkeypox/response/2022/world-map.html (accessed August 2, 2022).

CDC. (2022e). *Monkeypox Outbreak Global Map 2022*. Available online at: https://www.cdc.gov/poxvirus/monkeypox/response/2022/world-map.html (accessed August 7, 2022).

Centers for Disease Control Prevention (CDC). (2020). Centers for Disease Control and Prevention. *Update: Multistate Outbreak of Monkeypox-Illinois, Indiana, Missouri, Ohio, and Wisconsin. 2003*. Available online at: https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5227a5.htm (accessed September 26, 2020).

Centers for Disease Control and Prevention (CDC). (2022d) Monkeypox; CDC: Atlanta, GE, USA, 2022. Available online: https://www.cdc.gov/poxvirus/monkeypox/index.html. (accessed September 20, 2022).

Centers for Disease Control and Prevention (CDC). (2022c). *Interim Clinical Considerations for Use of JYNNEOS and ACAM2000 Vaccines during the 2022 U.S. Monkeypox Outbreak*; CDC: Atlanta, GE, USA. Available online: https://www.cdc.gov/poxvirus/monkeypox/health-departments/vaccine-considerations.html (accessed September 20, 2022).

Cohen-Gihon, I., Israeli, O., Shifman, O., Erez, N., Melamed, S., Paran, N., et al. (2020). Identification and whole-genome sequencing of a monkeypox virus strain isolated in Israel. *Microbiol. Resour. Announc.* 9, e01524–e01519. doi: 10.1128/MRA.01524-19

Damon, I. K. (2011). Status of human monkeypox: clinical disease, epidemiology and research. *Vaccine* 29, D54–D59. doi: 10.1016/j.vaccine.2011.04.014

Davido, B., D'anglejan, E., Jourdan, J., Robinault, A., and Davido, G. (2022). Monkeypox 2022 outbreak: cases with exclusive genital lesions. *J. Travel Med.* 29, taac077. doi: 10.1093/jtm/taac077

De Sousa, D., Patrocínio, J., Frade, J., Correia, C., Borges-Costa, J., and Filipe, P. (2022). Human monkeypox coinfection with acute HIV: an exuberant presentation. *Int. J. STD AIDS* 33, 936–938. doi: 10.1177/09564624221114998

Delaney, K. P., Sanchez, T., Hannah, M., Edwards, O. W., Carpino, T., Agnew-Brune, C., et al. (2022). Strategies adopted by gay, bisexual, and other men who have sex with men to prevent monkeypox virus transmission - United States, August 2022. *MMWR Morb. Mortal Wkly. Rep.* 75, 1126–1130. doi: 10.15585/mmwr.mm7135e1

Diaz-Cánova, D., Moens, U. L., Brinkmann, A., Nitsche, A., and Okeke, M. I. (2022). Genomic sequencing and analysis of a novel human cowpox virus with mosaic sequences from North America and Old World Orthopoxvirus. *Front Microbiol* 13, 868887. doi: 10.3389/fmicb.2022.868887

Doty, J. B., Malekani, J. M., Kalemba, L. S. N., Stanley, W. T., Monroe, B. P., Nakazawa, Y. U., et al. (2017). Assessing monkeypox virus prevalence in small mammals at the human–animal interface in the Democratic Republic of the Congo. *Viruses* 9, 283. doi: 10.3390/v9100283

Durski, K. N., McCollum, A. M., Nakazawa, Y., Petersen, B. W., Reynolds, M. G., Briand, S., et al. (2018). Emergence of monkeypox—west and central Africa, 1970–2017. *Morbidity Mortal. Weekly Rep.* 67, 306–310. doi: 10.15585/mmwr.mm6710a5

ECDC. (2022a). *Communicable Disease Threats Report; Week 31, 31 July—6 August 2022*. ECDC: Stockholm, Sweden. Available online at: https://www.ecdc.europa.eu/sites/default/files/doc/Communicable-disease-threats-report-6-aug-2022-allusers.pdf (accessed August 31, 2022).

ECDC. (2022b). *Factsheet for Health Professionals on Monkeypox*. ECDC: Stockholm, Sweden. Available online at: https://www.ecdc.europa.eu/en/all-topics-z/monkeypox/factsheet-health-professionals. (accessed September 20, 2022)

Endo, A., Murayama, H., Abbott, S., Ratnayake, R., Pearson, C. A., Edmunds, W. J., et al. (2022). Heavy-tailed sexual contact networks and the epidemiology of monkeypox outbreak in non-endemic regions, May 2022. *MedRxiv*. 13, 6353. doi: 10.1101/2022.06.13.22276353

Essbauer, S., Pfeffer, M., and Meyer, H. (2010). Zoonotic poxviruses. *Vet. Microbiol.* 140, 229–236. doi: 10.1016/j.vetmic.2009.08.026

European Centre for Disease Prevention and Control (ECDC). (2022c). *Epidemiological update: Monkeypox outbreak [Internet]*. Solna, Sweden: ECDC. [updated 2022 May 20; cited 2022 Jun 6]. Available online at: https://www.ecdc.europa.eu/en/news-events/epidemiological-updatemonkeypox-outbreak

Factsheet for health professionals on monkeypox: European Centre for Disease, Prevention, and Control. (2022d). Available online at: https://www.ecdc.europa.eu/en/all-topics-z/monkeypox/factsheet-health-professionals.

Falendysz, E. A., Lopera, J. G., Doty, J. B., Nakazawa, Y., Crill, C., Lorenzsonn, F., et al. (2017). Characterization of Monkeypox virus infection in African rope squirrels (Funisciurus sp.). *PLoS Neglect. Tropic. Dis.* 11, e0005809. doi: 10.1371/journal.pntd.0005809

Fauci, A. S. (2005). Emerging and reemerging infectious diseases: the perpetual challenge. *Acad. Med.* 80, 1079–1085. doi: 10.1097/00001888-200512000-00002

Ferdous, J., Barek, M. A., Hossen, M. S., Bhowmik, K. K., and Islam, M. S. (2023). A review on monkeypox virus outbreak: new challenge for world. *Health Sci. Rep.* 6, e1007. doi: 10.1002/hsr2.1007

Formenty, P., Muntasir, M. O., Damon, I., Chowdhary, V., Opoka, M. L., Monimart, C., et al. (2010). Human monkeypox outbreak caused by novel virus belonging to Congo Basin clade, Sudan, 2005. *Emerg. Infect. Dis.* 16, 1539–1545. doi: 10.3201/eid1610.100713

Foster, S. O., Brink, E. W., Hutchins, D. L., et al. (1972). Human monkeypox. *Bull. World Health Organ.* 46, 569–576.

Girometti, N., Byrne, R., Bracchi, M., Heskin, J., McOwan, A., Tittle, V., et al. (2022). Demographic and clinical characteristics of confirmed human monkeypox virus cases in individuals attending a sexual health centre in London, UK: an observational analysis. *Lancet Infect. Dis.* 22, 1321–1328. doi: 10.1016/S1473-3099(22)00411-X

Guagliardo, S. A. J., Monroe, B., Moundjoa, C., Athanase, A., Okpu, G., Burgado, J., et al. (2020). Asymptomatic orthopoxvirus circulation in humans in the wake of a monkeypox outbreak among chimpanzees in Cameroon. *Am. J. Tropic. Med. Hygiene* 102, 206–212. doi: 10.4269/ajtmh.19-0467

Hatmal, M. M. M., Al-Hatamleh, M. A., Olaimat, A. N., Ahmad, S., Hasan, H., Ahmad Suhaimi, N. A., et al. (2022). Comprehensive literature review of monkeypox. *Emerg. Microb. Infect.* 11, 2600–2631. doi: 10.1080/22221751.2022.2132882

Heskin, J., Belfield, A., Milne, C., Brown, N., Walters, Y., Scott, C., et al. (2022). Transmission of monkeypox virus through sexual contact - A novel route of infection. *J. Infect.* 85, 334–363. doi: 10.1016/j.jinf.2022.05.028

Heymann, D. L., Szczeniowski, M., and Esteves, K. (1998). Re-emergence of monkeypox in Africa: a review of the past six years. *Br. Med. Bull.* 54, 693–702. doi: 10.1093/oxfordjournals.bmb.a011720

Hobson, G., Adamson, J., Adler, H., Firth, R., Gould, S., Houlihan, C., et al. (2021). Family cluster of three cases of monkeypox imported from Nigeria to the United Kingdom, May 2021. *Eurosurveillance* 26, 2100745. doi: 10.2807/1560-7917.ES.2021.26.32.2100745

Hoff, N. A., Doshi, R. H., Colwell, B., Kebela-Illunga, B., Mukadi, P., Mossoko, M., et al. (2017). Evolution of a disease surveillance system: an increase in reporting of human monkeypox disease in the Democratic Republic of the Congo, 2001–2013. *Int. J. Tropic. Dis. Health* 25, 35885. doi: 10.9734/IJTDH/2017/35885

Hutin, Y. J., Williams, R. J., Malfait, P., Pebody, R., Loparev, V. N., Ropp, S. L., et al. (2001). Outbreak of human monkeypox, Democratic Republic of Congo, 1996 to 1997. *Emerg. Infect. Dis.* 7, 434–438. doi: 10.3201/eid0703.017311

Idris, I., and Adesola, R. O. (2022). Current efforts and challenges facing responses to Monkeypox in United Kingdom. *Biomed. J.* 7, 7. doi: 10.1016/j.bj.2022.07.007

Ihekweazu, C., Yinka-Ogunleye, A., Lule, S., and Ibrahim, A. (2020). Importance of epidemiological research of monkeypox: is incidence increasing?. *Exp. Rev. Anti-infect. Therapy* 18, 389–392. doi: 10.1080/14787210.2020.1735361

Iñigo Martínez, J., Gil Montalbán, E., Jiménez Bueno, S., Martín Martínez, F., Nieto Juli,á, A., Sánchez Díaz, J., et al. (2022). Monkeypox outbreak predominantly affecting men who have sex with men, Madrid, Spain, 26 April to 16 June 2022. *Eurosurveillance* 27, 2200471. doi: 10.2807/1560-7917.ES.2022.27.27.2200471

Isidro, J., Borges, V., Pinto, M., Sobral, D., Santos, J. D., Nunes, A., et al. (2022). Phylogenomic characterization and signs of microevolution in the 2022 multi-country outbreak of monkeypox virus. *Nat. Med.* 28, 1569–1572. doi: 10.1038/s41591-022-01907-y

Islam, M. M., Dutta, P., Rashid, R., Jaffery, S. S., Islam, A., Farag, E., et al. (2023). Monkeypox at the human-animal-ecology interface: a comprehensive review of viral characteristics, transmission, pathobiology, prevention, and control strategies. *Virulence, (just-accepted)* 2, 2186357. doi: 10.1080/21505594.2023.2186357

Jezek, Z., and Fenner, F. (1988). *Human Monkeypox*. Basel, Switzerland: Karger Publishers.

JeŽek, Z., Grab, B., Szczeniowski, M., Paluku, K. M., and Mutombo, M. (1988). Clinico-epidemiological features of monkeypox patients with an animal or human source of infection. *Bull. World Health Organiz.* 66, 459.

Jezek, Z., Szczeniowski, M., Paluku, K. M., and Mutombo, M. (1987). Human monkeypox: clinical features of 282 patients. *J. Infect. Dis.* 156, 293–298. doi: 10.1093/infdis/156.2.293

Judson, S. M. V. (2019). Nosocomial transmission of emerging viruses via aerosol-generating medical procedures. *Viruses*. 11, 940. doi: 10.3390/v11100940

Kaler, J., Hussain, A., Flores, G., Kheiri, S., and Desrosiers, D. (2022). Monkeypox: a comprehensive review of transmission, pathogenesis, and manifestation. *Cureus.* 14.

Kalthan, E., Tenguere, J., Ndjapou, S. G., Koyazengbe, T. A., Mbomba, J., Marada, R. M., et al. (2018). Investigation of an outbreak of monkeypox in an area occupied by armed groups, Central African Republic. *Medecine et maladies infectieuses* 48, 263–268. doi: 10.1016/j.medmal.2018.02.010

Khaity, A., Hasan, H., Albakri, K., Elsayed, H., Abdelgawad, H. A. H., Islam, F., et al. (2022). Monkeypox from Congo 1970 to Europe 2022; is there a difference?. *Int. J. Surg.* 104, 106827. doi: 10.1016/j.ijsu.2022.106827

Khattak, S., Rauf, M. A., Ali, Y., Yousaf, M. T., Liu, Z., Wu, D. D., et al. (2023). The monkeypox diagnosis, treatments and prevention: a review. *Front. Cell. Infect. Microbiol.* 12, 2005. doi: 10.3389/fcimb.2022.1088471

Khodakevich, L., Jezek, Z., and Kinzanzka, K. (1986). Isolation of monkeypox virus from wild squirrel infected in nature. *Lancet (London, England)* 327, 98. doi: 10.1016/S0140-6736(86)90748-8

Kiang, K. K. M. (2003). Rates and risks of transmission of smallpox and mechanisms of prevention. *J. Lab. Clin. Med.* 142, 229–238. doi: 10.1016/S0022-2143(03)00147-1

Kile, J. C., Fleischauer, A. T., Beard, B., Kuehnert, M. J., Kanwal, R. S., Pontones, P., et al. (2005). Transmission of monkeypox among persons exposed to infected prairie dogs in Indiana in 2003. *Archiv. Pediatr. Adolesc. Med.* 159, 1022–1025. doi: 10.1001/archpedi.159.11.1022

Kmiec, D., and Kirchhoff, F. (2022). Monkeypox: a new threat?. *Int. J. Mol. Sci.* 23, 7866. doi: 10.3390/ijms23147866

Kugelman, J. R., Johnston, S. C., Mulembakani, P. M., Kisalu, N., Lee, M. S., Koroleva, G., et al. (2014). Genomic variability of monkeypox virus among humans, Democratic Republic of the Congo. *Emerg. Infect. Dis.* 20, 232–239. doi: 10.3201/eid2002.130118

Ladnyj, I. D., Ziegler, P., and Kima, E. (1972). A human infection caused by monkeypox virus in Basankusu Territory, Democratic Republic of the Congo. *Bull. World Health Organiz.* 46, 593–597.

Laudisoit, A., Komba, M., and Akonda, I. (2016). *Scientific report research bushmeat and monkeypox Yahuma health zone–Aketi health zone-Bombongolo health area. DRC.*

Learned, L. A., Reynolds, M. G., Wassa, D. W., Li, Y., Olson, V. A., Karem, K., et al. (2005). Extended interhuman transmission of monkeypox in a hospital community in the Republic of the Congo, 2003. *Am. J. Tropic. Med. Hyg.* 73, 428–434. doi: 10.4269/ajtmh.2005.73.428

Lederman, E. R., Reynolds, M. G., Karem, K., Braden, Z., Learned-Orozco, L. A., Wassa-Wassa, D., et al. (2007). Prevalence of antibodies against orthopoxviruses among residents of Likouala region, Republic of Congo: evidence for monkeypox virus exposure. *Am. J. Tropic. Med. Hygiene* 77, 1150–1156. doi: 10.4269/ajtmh.2007.77.1150

Li, H., Zhang, H., Ding, K., Wang, X. H., Sun, G. Y., Liu, Z. X., et al. (2022). The evolving epidemiology of monkeypox virus. *Cytokine Growth Fact. Rev.* 2, 102. doi: 10.1016/j.cytogfr.2022.10.002

Likos, A. M., Sammons, S. A., Olson, V. A., Frace, A. M., Li, Y., Olsen-Rasmussen, M., et al. (2005). A tale of two clades: monkeypox viruses. *Journal of General Virology* 86, 2661–2672. doi: 10.1099/vir.0.81215-0

Liu, X., Zhu, Z., He, Y., Lim, J. W., Lane, B., Wang, H., et al. (2022). Monkeypox claims new victims: the outbreak in men who have sex with men. *Infect. Dis. Pov.* 11, 91–93. doi: 10.1186/s40249-022-01007-6

MacNeil, A., Abel, J., Reynolds, M. G., Lash, R. R., Fonnie, R., Kanneh, L. D., et al. (2011). Serologic evidence of human orthopoxvirus infections in Sierra Leone. *BMC Res. Notes* 4, 1–5. doi: 10.1186/1756-0500-4-465

Magnus, P. V., Andersen, E. K., Petersen, K. B., and Birch-Andersen, A. (1959). A pox-like disease in cynomolgus monkeys. *Acta Pathologica Microbiologica Scandinavica* 46, 156–176. doi: 10.1111/j.1699-0463.1959.tb00328.x

Mauldin, M. R., McCollum, A. M., Nakazawa, Y. J., Mandra, A., Whitehouse, E. R., Davidson, W., et al. (2022). Exportation of monkeypox virus from the African continent. *J. Infect. Dis.* 225, 1367–1376. doi: 10.1093/infdis/jiaa559

McCollum, A. M. (2023). Epidemiology of Human Mpox—Worldwide, 2018–2021. *MMWR. Morbidity and Mortality Weekly Report* 72, 3. doi: 10.15585/mmwr.mm7203a4

McCollum, A. M., and Damon, I. K. (2014). Human monkeypox. *Clinic. Infect. Dis.* 58, 260–267. doi: 10.1093/cid/cit703

Meslin, F. X., Stöhr, K., and Heymann, D. (2000). Public health implications of emerging zoonoses. *Revue scientifique et technique (International Office of Epizootics)* 19, 310–317. doi: 10.20506/rst.19.1.1214

Minhaj, F. S., Ogale, Y. P., Whitehill, F., Schultz, J., Foote, M., Davidson, W., et al. (2022). Monkeypox outbreak—nine states, May 2022. *Morbid. Mortal. Weekly Rep.* 71, 764–769. doi: 10.15585/mmwr.mm7123e1

Mourya, D., Yadav, P., Ullas, P., Bhardwaj, S., Sahay, R., Chadha, M., et al. (2019). Emerging/re-emerging viral diseases and new viruses on the Indian horizon. *Ind. J. Med. Res.* 149, 447–467 doi: 10.4103/ijmr.IJMR_1239_18

Mucker, E. M., Chapman, J., Huzella, L. M., Huggins, J. W., Shamblin, J., Robinson, C. G., et al. (2015). Susceptibility of marmosets (Callithrix jacchus) to monkeypox virus: a low dose prospective model for monkeypox and smallpox disease. *PloS one* 10, e0131742. doi: 10.1371/journal.pone.0131742

Müller, G., Meyer, A., Gras, F., Emmerich, P., Kolakowski, T., and Esposito, J. J. (1988). Monkeypox virus in liver and spleen of child in Gabon. *The Lancet* 331, 769–770. doi: 10.1016/S0140-6736(88)91580-2

Nakoune, E., Lampaert, E., Ndjapou, S. G., Janssens, C., Zuniga, I., Van Herp, M., et al. (2017). "A nosocomial outbreak of human monkeypox in the Central African Republic," in *Open forum infectious di*seases (Vol. 4, No. 4, p. ofx168). US: Oxford University Press.

Nigeria Centre for Disease (NCDC). (2022). *Monkeypox: Nigeria recorded 558 cases,eight deaths in five years. Premium Times; May 10,2022.*

Noe, S., Zange, S., Seilmaier, M., Antwerpen, M. H., Fenzl, T., Schneider, J., et al. (2023). Clinical and virological features of first human monkeypox cases in Germany. *Infection* 51, 265–270. doi: 10.1007/s15010-022-01874-z

Nolen, L. D., Osadebe, L., Katomba, J., Likofata, J., Mukadi, D., Monroe, B., et al. (2015). Introduction of monkeypox into a community and household: risk factors and zoonotic reservoirs in the Democratic Republic of the Congo. *Am. J. Tropic. Med. Hyg.* 93, 410–415. doi: 10.4269/ajtmh.15-0168

Ogoina, D., Iroezindu, M., James, H. I., Oladokun, R., Yinka-Ogunleye, A., Wakama, P., et al. (2020). Clinical course and outcome of human monkeypox in Nigeria. *Clinic. Infect. Dis.* 71, e210–e214. doi: 10.1093/cid/ciaa143

Ogoina, D., Izibewule, J. H., Ogunleye, A., Ederiane, E., Anebonam, U., Neni, A., et al. (2019). The 2017 human monkeypox outbreak in Nigeria—report of outbreak experience and response in the Niger Delta University Teaching Hospital, Bayelsa State, Nigeria. *PloS one* 14, e0214229. doi: 10.1371/journal.pone.0214229

Ortiz-Martínez, Y., Zambrano-Sanchez, G., and Rodríguez-Morales, A. J. (2022). Monkeypox and HIV/AIDS: When the outbreak faces the epidemic. *Int. J. STD and AIDS* 33, 949–950. doi: 10.1177/09564624221114191

Parker, S., and Buller, R. M. (2013). A review of experimental and natural infections of animals with monkeypox virus between 1958 and 2012. *Future Virol.* 8, 129–157. doi: 10.2217/fvl.12.130

Parker, S., Nuara, A., Buller, R. M. L., and Schultz, D. A. (2007). Human monkeypox: an emerging zoonotic disease. *Fut. Microbiol.* 2, 17–34. doi: 10.2217/17460913.2.1.17

Patel, A., Bilinska, J., Tam, J. C., Fontoura, D. D. S., Mason, C. Y., Daunt, A., et al. (2022). Clinical features and novel presentations of human monkeypox in a central London centre during the 2022 outbreak: descriptive case series. *bmj* 378, e072410. doi: 10.1136/bmj-2022-072410

Patrocinio-Jesus, R., and Peruzzu, F. (2022). Monkeypox genital lesions. *N. Engl. J. Med.* 387, 66–66. doi: 10.1056/NEJMicm2206893

Patrono, L. V., Pléh, K., Samuni, L., Ulrich, M., Röthemeier, C., Sachse, A., et al. (2020). Monkeypox virus emergence in wild chimpanzees reveals distinct clinical outcomes and viral diversity. *Nat. Microbiol.* 5, 955–965. doi: 10.1038/s41564-020-0706-0

Perez Duque, M., Ribeiro, S., Martins, J. V., Casaca, P., Leite, P. P., Tavares, M., et al. (2022). Ongoing monkeypox virus outbreak, Portugal, 29 April to 23 May 2022. *Eurosurveillance* 27, 2200424. doi: 10.2807/1560-7917.ES.2022.27.22.2200424

Peters, J. C. (1966). An epizootic of Monkeypox at Rotterdam Zoo. *International Zoo Yearbook*. 6:274–275. doi: 10.1111/j.1748-1090.1966.tb01794.x

Petersen, B. W., Joelle Kabamba, J., McCollum, A. M., Lushima, R. S., Wemakoy, E. O., Tamfum, J. M., et al. (2018). Vaccinating against monkeypox in the Democratic Republic of the Congo. *Antiviral Res.* 162, 171–177.

Petersen, B. W., Kabamba, J., McCollum, A. M., Lushima, R. S., Wemakoy, E. O., Tamfum, J. J. M., et al. (2019). Vaccinating against monkeypox in the Democratic Republic of the Congo. *Antivir. Res.* 162, 171–177. doi: 10.1016/j.antiviral.2018.11.004

Petersen, E., Abubakar, I., Ihekweazu, C., Heymann, D., Ntoumi, F., Blumberg, L., et al. (2019a). Monkeypox-Enhancing public health preparedness for an emerging lethal human zoonotic epidemic threat in the wake of the smallpox post-eradication era. *Int. J. Infect. Dis.* 78, 78–84. doi: 10.1016/j.ijid.2018.11.008

Petersen, E., Kantele, A., Koopmans, M., Asogun, D., Yinka-Ogunleye, A., Ihekweazu, C., et al. (2019b). Human monkeypox: epidemiologic and clinical characteristics, diagnosis, and prevention. *Infect. Dis. Clinics* 33, 1027–1043. doi: 10.1016/j.idc.2019.03.001

Philpott, D., Hughes, C. M.;, Alroy, K. A., Kerins, J. L., Pavlick, J., Asbel, L., Crawley, A., et al. (2022). CDC multinational monkeypox response team. epidemiologic and clinical characteristics of monkeypox cases—United States, May 17–July 22, 2022. *MMWR Morb. Mortal. Wkly. Rep.* 71, 1018–1022. doi: 10.15585/mmwr.mm7132e3

Quarleri, J., Delpino, M. V., and Galvan, V. (2022). Monkeypox: considerations for the understanding and containment of the current outbreak in non-endemic countries. *Geroscience* 44, 2095–2103. doi: 10.1007/s11357-022-00611-6

Quiner, C. A., Moses, C., Monroe, B. P., Nakazawa, Y., Doty, J. B., Hughes, C. M., et al. (2017). Presumptive risk factors for monkeypox in rural communities in the Democratic Republic of the Congo. *PloS one* 12, e0168664. doi: 10.1371/journal.pone.0168664

Radonić, A., Metzger, S., Dabrowski, P. W., Couacy-Hymann, E., Schuenadel, L., Kurth, A., et al. (2014). Fatal monkeypox in wild-living sooty mangabey, Cote d'Ivoire, 2012. *Emerg. Infect. Dis.* 20, 1009–1329. doi: 10.3201/eid2006.131329

Reed, K. D., Melski, J. W., Graham, M. B., Regnery, R. L., Sotir, M. J., Wegner, M. V., et al. (2004). The detection of monkeypox in humans in the Western Hemisphere. *New Engl. J. Med.* 350, 342–350. doi: 10.1056/NEJMoa032299

Reynolds, M. G., Carroll, D. S., Olson, V. A., Hughes, C., Galley, J., Likos, A., et al. (2010). A silent enzootic of an orthopoxvirus in Ghana, West Africa: evidence for multi-species involvement in the absence of widespread human disease. *Am. J. tropic. Med. Hygiene* 82, 746–754. doi: 10.4269/ajtmh.2010.09-0716

Reynolds, M. G., Davidson, W. B., Curns, A. T., Conover, C. S., Huhn, G., Davis, J. P., et al. (2007). Spectrum of infection and risk factors for human monkeypox, United States, 2003. *Emerg. Infect. Dis.* 13, 1332–1339 doi: 10.3201/eid1309.070175

Reynolds, M. G., Emerson, G. L., Pukuta, E., Karhemere, S., Muyembe, J. J., Bikindou, A., et al. (2013). Detection of human monkeypox in the Republic of the Congo following intensive community education. *Am. J. Trop. Med. Hygiene* 88, 982–985. doi: 10.4269/ajtmh.12-0758

Rimoin, A. W., Mulembakani, P. M., Johnston, S. C., Lloyd Smith, J. O., Kisalu, N. K., Kinkela, T. L., et al. (2010). Major increase in human monkeypox incidence 30 years after smallpox vaccination campaigns cease in the Democratic Republic of Congo. *Proceed. Nat. Acad. Sci.* 107, 16262–16267. doi: 10.1073/pnas.1005769107

Salzer, J. S., Carroll, D. S., Rwego, I. B., Li, Y., Falendysz, E. A., Shisler, J. L., et al. (2013). Serologic evidence for circulating orthopoxviruses in peridomestic rodents from rural Uganda. *J. Wildlife Dis.* 49, 125–131. doi: 10.7589/2012-04-100

Seang, S., Burrel, S., Todesco, E., Leducq, V., Monsel, G., Le Pluart, D., et al. (2022). Evidence of human-to-dog transmission of monkeypox virus. *Lancet* 400, 658–659. doi: 10.1016/S0140-6736(22)01487-8

Sejvar, J. J., Chowdary, Y., Schomogyi, M., Stevens, J., Patel, J., Karem, K., et al. (2004). Human monkeypox infection: a family cluster in the midwestern United States. *J. Infect. Dis.* 190, 1833–1840. doi: 10.1086/425039

Sham, S., Sapna, F. N. U., Anjali, F. N. U., Kumar, S., Podder, V., Jaladi, S., et al. (2022). The changing global epidemiology of re-emerging human monkeypox virus infection: a systematic review. *medRxiv* 20, 22–12. doi: 10.1101/2022.12.09.22283261

Silva, N. I. O., de Oliveira, J. S., Kroon, E. G., Trindade, G. D. S., and Drumond, B. P. (2020). Here, there, and everywhere: the wide host range and geographic distribution of zoonotic orthopoxviruses. *Viruses* 13, 43. doi: 10.3390/v13010043

Simpson, K., Heymann, D., Brown, C. S., Edmunds, W. J., Elsgaard, J., Fine, P., et al. (2020). Human monkeypox–After 40 years, an unintended consequence of smallpox eradication. *Vaccine* 38, 5077–5081. doi: 10.1016/j.vaccine.2020.04.062

Sklenovská, N. (2020). "Monkeypox virus," in *Animal-origin viral zoonoses*. Springer: Singapore, pp. 39–68.

Sklenovská, N., and Van Ranst, M. (2018). Emergence of monkeypox as the most important orthopoxvirus infection in humans. *Front. Public Health* 6, 241. doi: 10.3389/fpubh.2018.00241

Soheili, M., and Nasseri, S. (2022). Monkeypox: virology, pathophysiology, clinical characteristics, epidemiology, vaccines, diagnosis, and treatments. *J. Pharm. Pharmaceutic. Sci.* 25, 322–297. doi: 10.18433/jpps33138

Tchokoteu, P. F., Kago, I., Tetanye, E., Ndoumbe, P., Pignon, D., and Mbede, J. (1991). "Variola or a severe case of varicella? a case of human variola due to monkeypox virus in a child from the Cameroon," in *Annales de la Societe Belge de Medecine Tropicale* (Vol. *71, No*. 2, pp. 123–128).

Thornhill, J. P., Barkati, S., Walmsley, S., Rockstroh, J., Antinori, A., Harrison, L. B., et al. (2022). Monkeypox virus infection in humans across 16 countries—April–June 2022. *New Engl. J. Med.* 387, 679–691. doi: 10.1056/NEJMoa2207323

UK, G. (2022). *Monkeypox Cases Confirmed in England-Latest Updates.*

Vaughan, A., Aarons, E., Astbury, J., Brooks, T., Chand, M., Flegg, P., et al. (2020). Human-to-human transmission of monkeypox virus, United Kingdom, October 2018. *Emerg. Infect. Dis.* 26, 782–785. doi: 10.3201/eid2604.191164

Vivancos, R., Anderson, C., Blomquist, P., Balasegaram, S., Bell, A., Bishop, L., et al. (2022). Community transmission of monkeypox in the United Kingdom, April to May 2022. *Eurosurveillance* 27, 2200422. doi: 10.2807/1560-7917.ES.2022.27.22.2200422

Wachtman, L., and Mansfield, K. (2012). Viral diseases of nonhuman primates. *Nonhum. Primates Biomed. Res.* 1, 2012.doi: 10.1016/B978-0-12-381366-4.00001-8

WHO (2022e). *Multi-country monkeypox outbreak: situation update 2022.* Available online at: https://www.who.int/emergencies/disease-outbreaknews/item/2022-DON392 (accessed July 5, 2022e).

WHO (2022f). *Regional Office for Africa. Weekly Bulletin on Outbreak and Other Emergencies: Week 4: 18–24 January 2021*. WHO: Geneva, Switzerland, 2021; Available online at: https://apps.who.int/iris/handle/10665/338891 (accessed August 31, 2022).

WHO. (2022c). *Disease Outbreak News; Multi-Country Monkeypox Outbreak in Non-Endemic Countries*. WHO: Geneva, Switzerland. Available online at: https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON385 (accessed August 31, 2022).

WHO. (2022d). *Monkeypox. World Health Organization*. Available online at: https://www.who.int/news-room/fact-sheets/detail/monkeypox (accessed August 2, 2022).

WHO. (2022g). *Monkeypox - Democratic Republic of the Congo*. Available online at: http://www.who.int/emergencies/disease-outbreak-news/item/monkeypox-democratic-republic-of-the-congo (accessed July 5, 2022).

WHO. (2022h). *Monkeypox—Central African Republic 2016*. Available online at: https://www.who.int/emergencies/disease-outbreak-news/item/13-october-2016-monkeypox-caf-en (accessed July 5, 2022).

WHO. (2022i). *Monkeypox-Cameroon 2018*. Available online at: https://www.who.int/emergencies/disease-outbreak-news/item/05-june-2018-monkeypoxcameroon-en#:text$=$From%2030%20April%20through%2030,and%20Pandemic%20diseases%20(DLMEP) (accessed July 5, 2022).

World Health Organization. (1997). Human monkeypox in Kasai Oriental, Democratic Republic of the Congo (former Zaire): Preliminary report of October, 1997 investigation. *Wkly Epidemiol Rec*. 72, 369–372.

World Health Organization. (2022a). Monkeypox - United States of America. *World Health Organization*. Available online at: https://www.who.int/emergencies/disease-outbreak-news/item/2021-DON344 (accessed June 20, 2022).

World Health Organization. (2022b). *Monkeypox – United States of America*. Available online at: who.int/emergencies/disease-outbreak-news/item/2021-DON344 (accessed June 20, 2022).

World Health Organization. (2022e). Multi-Country Monkeypox Outbreak: Situation Update. *World Health Organization*. Available online at: https://www.who.int/emergencies/disease-outbreaknews/item/2022-DON392 (accessed July 5, 2022).

World Health Organization. (2022j). Disease Outbreak News; Multi-country monkeypox outbreak in non-endemic countries: Update; WHO: Geneva, Switzerland, 2022. Available online: https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON396 (accessed September 20, 2022).

Xiang, Y., and White, A. (2022). Monkeypox virus emerges from the shadow of its more infamous cousin: family biology matters. *Emerg. Microbes Infect.* 11, 1768–1777. doi: 10.1080/22221751.2022.2095309

Yinka-Ogunleye, A., Aruna, O., Dalhat, M., Ogoina, D., McCollum, A., Disu, Y., et al. (2019). Outbreak of human monkeypox in Nigeria in 2017–18: a clinical and epidemiological report. *Lancet Infectious Dis.* 19, 872–879. doi: 10.1016/S1473-3099(19)30294-4

Yong, S. E. F., Ng, O. T., Ho, Z. J. M., Mak, T. M., Marimuthu, K., Vasoo, S., et al. (2020). Imported Monkeypox, Singapore. *Emerg. Infect. Dis.* 26, 1826–1830. doi: 10.3201/eid2608.191387

Zachary, K. C., and Shenoy, E. S. (2022). Monkeypox transmission following exposure in healthcare facilities in nonendemic settings: low risk but limited literature. *Infection Control Hosp. Epidemiol.* 43, 920–924. doi: 10.1017/ice.2022.152

Zhu, F., Li, L., and Che, D. (2022). Monkeypox virus under COVID-19: caution for sexual transmission—correspondence. *Int. J. Surg,* 104, 106768. doi: 10.1016/j.ijsu.2022.106768

frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 25 May 2023
DOI 10.3389/fmicb.2023.1181034



OPEN ACCESS

EDITED BY
Laurent Rénia,
Nanyang Technological University, Singapore

REVIEWED BY
Antonio Molinaro,
University of Naples Federico II, Italy
Jesús Campos-García,
Universidad Michoacana de San Nicolás de Hidalgo, Mexico

*CORRESPONDENCE
Helena Pětrošová
✉ hpetros@uvic.ca
David R. Goodlett
✉ goodlett@uvic.ca

RECEIVED 06 March 2023
ACCEPTED 02 May 2023
PUBLISHED 25 May 2023

CITATION
Pětrošová H, Mikhael A, Culos S, Giraud-Gatineau A, Gomez AM, Sherman ME, Ernst RK, Cameron CE, Picardeau M and Goodlett DR (2023) Lipid A structural diversity among members of the genus *Leptospira*. *Front. Microbiol.* 14:1181034. doi: 10.3389/fmicb.2023.1181034



# Lipid A structural diversity among members of the genus *Leptospira*

Helena Pětrošová[1,2]*, Abanoub Mikhael[2], Sophie Culos[1], Alexandre Giraud-Gatineau[3], Alloysius M. Gomez[1], Matthew E. Sherman[4], Robert K. Ernst[4], Caroline E. Cameron[1,5], Mathieu Picardeau[3] and David R. Goodlett[1,2]*

[1]Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, Canada, [2]University of Victoria Genome British Columbia Proteomics Center, University of Victoria, Victoria, BC, Canada, [3]Institut Pasteur, Université Paris Cité, CNRS UMR 6047, Biology of Spirochetes Unit, Paris, France, [4]Department of Microbial Pathogenesis, University of Maryland, Baltimore, MD, United States, [5]Department of Medicine, Division of Allergy and Infectious Diseases, University of Washington, Seattle, WA, United States

Lipid A is the hydrophobic component of bacterial lipopolysaccharide and an activator of the host immune system. Bacteria modify their lipid A structure to adapt to the surrounding environment and, in some cases, to evade recognition by host immune cells. In this study, lipid A structural diversity within the *Leptospira* genus was explored. The individual *Leptospira* species have dramatically different pathogenic potential that ranges from non-infectious to life-threatening disease (leptospirosis). Ten distinct lipid A profiles, denoted L1-L10, were discovered across 31 *Leptospira* reference species, laying a foundation for lipid A-based molecular typing. Tandem MS analysis revealed structural features of *Leptospira* membrane lipids that might alter recognition of its lipid A by the host innate immune receptors. Results of this study will aid development of strategies to improve diagnosis and surveillance of leptospirosis, as well as guide functional studies on *Leptospira* lipid A activity.

KEYWORDS

**lipid A, *Leptospira*, mass spectrometry, structure-activity relationship, molecular typing, lipopolysaccharide (LPS), fast lipid analysis technique, pathogenicity**

## 1. Introduction

Lipopolysaccharide (LPS) is one of the hallmark virulence factors of Gram-negative pathogens. It consists of three parts: O-antigen, core oligosaccharide antigen, and lipid A. The O-antigen is a polysaccharide exposed to the extracellular milieu, and its size and structural complexity delays the recognition of LPS by the host immune system and limits binding to host antibodies (Duerr et al., 2009; Domínguez-Medina et al., 2020). Core oligosaccharide consists of several different monosaccharide units, and connects O-antigen with lipid A. It contributes to stability of the outer membrane and it has antigenic properties (Silipo and Molinaro, 2010). Finally, lipid A, also known as endotoxin, anchors LPS to the outer leaflet of the outer membrane. Lipid A is the membrane anchor of LPS which attaches it to the outer leaflet of the outer membrane. It comprises two glucosamine sugars decorated with fatty acyl chains and terminal phosphate groups that can be further adorned with other functional moieties (Simpson and Trent, 2019).

The biological function of lipid A is dependent on its chemical structure. Bacteria modify their lipid A to adapt to changes in their surrounding environment (Needham and Trent, 2013;

Simpson and Trent, 2019; Kawahara, 2021). These structural adaptations include, for example, modifications to the length and saturation of fatty acyl chains to overcome temperature shifts (Gunn and Ernst, 2007; Hassan et al., 2020) or addition of functional groups to gain resistance to antimicrobial peptides (Trent et al., 2001; Zhou et al., 2001), and are covered in great detail in recent reviews (Simpson and Trent, 2019; Kawahara, 2021). Lipid A is also a pathogen-associated molecular pattern. It interacts with the Toll-Like Receptor 4/Myeloid Differentiation protein 2 (TLR4/MD2) complex in a structure-dependent manner (Park et al., 2009; Ohto et al., 2012; Scott et al., 2017). The canonical hexa-acylated lipid A from *Escherichia coli* strongly activates TLR4/MD2, and is therefore highly endotoxic (Park et al., 2009). In contrast, tetra-acylated lipid A molecules are TLR4/MD2 antagonists (Baldridge and Crane, 1999; Deguchi et al., 2016). Similarly, lipid A molecules with two terminal phosphates are stronger TLR4/MD2 ligands than their monophosphorylated counterparts (Baldridge and Crane, 1999; Kong et al., 2012). Some pathogens, such as *Yersinia* and *Salmonella*, modify their lipid A structures accordingly to evade host inflammatory responses when establishing infection (Kawahara et al., 2002; Rebeil et al., 2004; Kong et al., 2011; Chandler et al., 2020).

*Leptospira* is a diverse group of bacteria comprising non-infectious free-living spirochetes, as well as pathogens that cause leptospirosis in a wide variety of hosts (Coburn et al., 2021). Unlike other spirochetes, all *Leptospira* possess LPS in their envelopes, and this molecule is central to the host immune responses to infection (Werts et al., 2001; Nahori et al., 2005; Viriyakosol et al., 2006; Murray et al., 2010; Srikram et al., 2011; Marcsisin et al., 2013). The lipid A structure has been established in serovars of the pathogenic *Leptospira* (Que-Gewirth et al., 2004; Eshghi et al., 2015; Novak et al., 2022). In contrast to the canonical di-glucosamine backbone of lipid A with amide- and ester-linked primary fatty acids (Simpson and Trent, 2019), the backbone of *Leptospira* lipid A comprises di-aminoglucose sugars, which results in linkage of all primary fatty acids through amide bonds (Que-Gewirth et al., 2004; Eshghi et al., 2015; Novak et al., 2022). In addition, the lipid A has only one terminal phosphate that is methylated; a structural feature that has not been described in any other bacterial species to date (Que-Gewirth et al., 2004; Simpson and Trent, 2019). These unique lipid A features are likely involved in the inability of *Leptospira* lipid A to bind to human TLR4/MD2 (Werts et al., 2001). Similar to other bacterial pathogens, *L. interrogans* modify their lipid A structure to adapt to temperature shifts (Gunn and Ernst, 2007; Eshghi et al., 2015).

Given the enormous diversity of the *Leptospira* genus (Vincent et al., 2019), the structural diversity of its lipid A is curiously understudied (Patra et al., 2015; Vanithamani et al., 2021; Novak et al., 2022). *Leptospira* are fastidious bacteria that grow slowly in rich and complex culturing media supplemented with host factors (Zuerner, 2005). The traditional protocols for lipid A extraction that require large volumes of bacterial culture are therefore likely the cause of this knowledge gap. To circumvent these limitations, we employed a rapid protocol for lipid A structural characterization, FLAT$^n$ (Leung et al., 2017; Sorensen et al., 2020; Yang et al., 2022a), that allowed us to utilize an estimated equivalent of $10^7$ *Leptospira* cells in 1 ml volume per assay. We examined lipid A mass spectral profiles, from which representative structures were proposed, in 31 *Leptospira* species from different phylogenetic groups. This work therefore represents the first comprehensive comparison of lipid A structure in virulent versus nonvirulent *Leptospira* species.

## 2. Materials and methods

### 2.1. *Leptospira* species

*Leptospira* species used in this study are listed in Table 1. *Leptospira* were grown in the Ellinghausen–McCullough–Johnson–Harris (EMJH) medium, as modified by Ellis and Thierman (EMJH T80/T40/LH); medium was prepared without the addition of rabbit serum and superoxide dismutase (Ellis and Thiermann, 1986; Zuerner, 2005). Cultures were kept at 30°C and shaking at 100 rpm. For all experiments, *Leptospira* species were grown in biological triplicates to mid-logarithmic phase (approximately 5× 10^8 cells/ml), as assessed by density and motility under a dark-field microscope (Zuerner, 2005).

### 2.2. Fast lipid analysis technique (FLAT)

Lipid A structural analyses were performed using FLAT (Sorensen et al., 2020) and its tandem-MS version FLAT$^n$ (Yang et al., 2022a). Five milliliter of logarithmic *Leptospira* culture was centrifuged at 4,000x g for 15 min. Resulting pellets were washed twice with 1 mL of phosphate buffered saline (Sigma Aldrich, St. Luis, MO, USA), and resuspended in 200 µL of MS-grade water (Fisher Chemical, Hampton, NH, USA). One microliter of the sample was spotted on a MALDI plate (MFX µFocus plate 12×8 c 2,400 µm 0.7 T; Hudson Surface Technology, Inc., South Korea) and air dried. One microliter of the FLAT extraction buffer (0.2 M citric acid, 0.1 M sodium citrate in MS-grade water; both from Fisher Chemical) was added on the top of each sample. MALDI plate was placed in an in-house made humidifier chamber and incubated at 110°C for 30 min. Plate was gently washed with MS-grade water for approximately 30s and let air dry. Finally, 1 µL of norharmane matrix (Sigma Aldrich) was spotted on the top of each sample and let dry. Norharmane matrix was prepared at 10 mg/mL in 2:1 v/v MS-grade chloroform and methanol (both from Fisher Chemical).

### 2.3. MALDI MS analysis of lipid A

Mass spectra were obtained on a timsTOF *flex* MALDI-2 instrument (Bruker, Bremen, Germany) in the negative ion mode. Instrument was calibrated before each experiment in an electrospray mode by a direct infusion of the Agilent Calibration mix (Agilent Technologies, Santa Clara, CA, USA). Tandem MS analyses were performed with the following settings: 3,000 shots/spot on average, collision energy: 110–120 eV, isolation width: *m/z* 4, collision RF: 1,000 Vpp, transfer time: 110 µs and prepulse storage: 11 µs. To detect product ions in the low range *m/z*, the collision RF and transfer time were changed to 300 Vpp and 30 µs, respectively. Data were analyzed using mMass v5.5.0 (Strohalm et al., 2010) and Compass Data Analysis v 6.0 (Bruker). Fragmentation patterns of predicted lipid A structures were confirmed in ChemDraw v18.0 (PerkinElmer Informatics, Waltham, MA, USA). Theoretical isotopic distributions

were predicted using Peak-by-Peak Metabolomics software v 2022.8.0 (Spectroswiss, Lausanne, Switzerland).

## 3. Results and discussion

Recent advances in the field of *Leptospira* genomics led to identification of 68 reference *Leptospira* species, and their reclassification into four distinct phylogenetic subclades (Vincent et al., 2019; Korba et al., 2021). The P1 subclade encompasses species formerly known as "pathogens," the P2 subclade comprises species formerly known as "intermediates," and finally, the S1 and S2 subclades encompass non-infectious saprophytic species. The P1 subclade is further divided into P1 high virulence (P1*hv*) and P1 low virulence (P1*lv*) groups. *Leptospira* species most frequently involved in human disease, such as *L. interrogans* and *L. noguchii*, belong to the P1*hv* group, whereas species with no/unknown pathogenic potential cluster to the P1*lv* group (Vincent et al., 2019). Although the differences between the individual subclades are clear on the genome level, additional knowledge on phenotypic differences is warranted to fully understand the pathogenesis of leptospirosis. Here we examined clade-specific differences in the structure of lipid A, the hallmark virulence factor of bacterial pathogens.

### 3.1. Ten unique lipid A profiles were detected between the individual *Leptospira* subclades

The negative ion mass spectra of lipid A from 31 *Leptospira* species were examined by FLAT (Sorensen et al., 2020). This included five P1*hv*, five P1*lv*, nine P2, nine S1, and three S2 species (Table 1). In total, 10 different lipid A profiles denoted L1-L10 were detected across the examined species (Figure 1; Table 2). In the P1*hv* group, *L. interrogans*, *L. noguchii,* and *L. weilii* shared the L1 profile (Figure 1A), and L2 and L3 profiles were detected in *L. mayottensis* and *L. santarosai*, respectively (Figures 1B,C). The P1*lv* group was homogenous; all P1*lv* species shared the L4 profile (Figure 1D). In the P2 subclade, 7 out of the 9 species shared the L5 lipid A profile with *L. licerasiae* (Figure 1E). The L6 profile was detected in *L. fluminis* (Figure 1F) and the L7 profile in *L. perolatii* (Figure 1G). The lipid A profiles of S1 and S2 species were very similar to each other. Seven out of nine S1 species shared the L8 profile with the model saprophytic species *L. biflexa* (Figure 1H). *L. noumeaensis* and *L. kanakyensis* displayed the L9 phenotype (Figure 1I). Finally, the L10 profile was detected in all S2 species (Figure 1J). The individual lipid A profiles of all examined *Leptospira* species can be found in Supplementary material (Supplementary Figures S1–S3).

There was no obvious association between the origin of the examined *Leptospira* species and their lipid A profiles. For example, all but one examined P1*lv* species and all S1 species were isolated from water and soil environments (Table 1), yet their lipid A profiles were different (Figure 1). Presence of lipid A modifications that could aid survival of *Leptospira* in water and soil environments cannot be excluded. However, environment-induced lipid A modifications are often transient (Rebeil et al., 2004; Li et al., 2012) and unlikely to be carried over to bacteria grown under conditions where these modifications are not required. At the growth conditions used in this study (modified EMJH T80/T40/LH, 30°C, and shaking), the strongest association was observed between the lipid A profiles of the individual *Leptospira* species and their phylogenetic classification (Figure 1; Table 2).

### 3.2. *Leptospira* lipid A profiles were complex, displaying high intraspecies heterogeneity

The structures of the representative lipid A ions of each profile (L1–L10) were proposed based on tandem mass spectrometry analysis (FLAT$^n$) (Yang et al., 2022a). The lipid A structure of *L. interrogans* (L1) corresponded to the previously reported structure for this species (Que-Gewirth et al., 2004; Eshghi et al., 2015), validating our methodology (Figures 2A,B). Interpretation of lipid A profiles can be challenging. However, one main lipid A ion is usually surrounded by satellite molecules resulting from substantial modifications to this lipid A molecule (such as addition of a sugar moiety or a terminal phosphate group) (Leung et al., 2017; Liang et al., 2019). In contrast, all *Leptospira* lipid A profiles were complex with several clusters of lipid A ions separated by 26 or 28 Da (Figures 1, 2E). These mass differences corresponded to an addition of two carbons connected by a double bond or a single bond, respectively, and were previously described in *L. interrogans* and *L. kirschneri* (Novak et al., 2022) (Figure 2E). Each of these clusters was further predicted to consist of five individual lipid A ions separated by 2 Da (a double bond), revealing an unusual lipid A heterogeneity within a single bacterial species (Figure 2E). Briefly, if only a single lipid A ion was present, the isotopic distribution would look as depicted in Figure 2C. Instead, the measured isotopic distribution in each lipid A cluster (Figure 2E) closely corresponded to a mixed isotopic distribution consisting of five lipid A ions differentiated by a presence of a double bond (Figure 2D). Mass spectrometry-based strategies to locate positions of double bonds in unsaturated lipid molecules exist. They include chemical derivatization prior mass spectrometry analysis, and are yet to be tested on complex mixtures of lipid A molecules detected in *Leptospira* species (Figure 1E; Novak et al., 2022). Alternatively, proposed lipid A structures can be supported with other analytical techniques, such as nuclear magnetic resonance (NMR). However, dissolving lipid A in NMR-compatible solvents is challenging due to its amphipathic nature (Ribeiro et al., 1999; Zähringer et al., 2001; Silipo et al., 2002). The NMR approach is therefore more appropriate for characterization of the water-soluble components of LPS (core oligosaccharide and O-antigen). Both above-mentioned strategies require pure lipid A extracts from large volume of *Leptospira* culture, chemical derivatization reagents and rigorous method optimization for complex lipid A samples. Localization of double bonds was therefore not possible within the scope of this study. Like others (Eshghi et al., 2015; Novak et al., 2022) we therefore proposed structures of the representative lipid A for each of the lipid A profiles (L1–L10), and concluded that additional degrees of unsaturation were present (Figure 3). It is important to note that our approach allowed us to obtain valid structural information on *Leptospira* lipid A from an equivalent of $10^7$ cells (approximately 100 µL of exponential culture). Experiments were therefore performed in a controlled manner, using biological triplicates on two independent experimental days. The low amount of starting material does not affect the results. Lipid A structures of *Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Klebsiella pneumoniae* proposed by FLAT$^n$

TABLE 1 Reference *Leptospira* species used in this study.

| Species | Strain | Group | Origin | Virulence (hamster) | Reference |
|---|---|---|---|---|---|
| *L. interrogans* | L495 | P1*hv* | Human; Manila, Philippines | Yes | Koizumi and Watanabe (2003) |
| *L. mayottensis* | 200901116 | P1*hv* | Human; Mayotte | Yes | Bourhy et al. (2014) |
| *L. noguchii* | 201102933 | P1*hv* | Human; Guadeloupe | Yes | Vincent et al. (2019) |
| *L. santarosai* | LT821 | P1*hv* | *Proechimys semispinosus* (spiny rat); Panama Canal Zone | Yes | Yasuda et al. (1987) |
| *L. weilii* | 14535 | P1*hv* | Human; Laos | Yes | Vincent et al. (2019) |
| *L. adleri* | M7A | P1*lv* | Water; Mayotte | ND | Vincent et al. (2019) |
| *L. ainazelensis* | 201903074 10/E/19 | P1*lv* | Water through (cow breeding); Aïn Azel, Algeria | ND | Korba et al. (2021) |
| *L. dzianensis* | M12A | P1*lv* | Water; Dziani, Mayotte | ND | Vincent et al. (2019) |
| *L. gomenensis* | KG8-B22 | P1*lv* | Soil; Kaala-Gomen, New Caledonia | ND | Vincent et al. (2019) |
| *L. tipperaryensis* | GWTS1 | P1*lv* | *Crocidura russula* (greater white-toothed shrew); Tipperary, Ireland | ND | Nally et al. (2016) |
| *L. fluminis* | SCS5 | P2 | Soil; Sungai Congkak, Malaysia | ND | Vincent et al. (2019) |
| *L. haakeii* | ATI7-C-A2 | P2 | River bank; Unia, New Caledonia | ND | Thibeaux et al. (2018) |
| *L. hartskeerlii* | MCA1-C-A1 | P2 | Soil; Ponerihouen, New Caledonia | ND | Thibeaux et al. (2018) |
| *L. langatensis* | SSW18 | P2 | Water; Sungai Congkak, Malaysia | ND | Vincent et al. (2019) |
| *L. licerasiae* | VAR010 | P2 | Human; Iquitos, Peru | No | Ricaldi et al. (2012) |
| *L. neocaledonica* | ES4-C-A1 | P2 | River bank; Koné, New Caledonia | No | Thibeaux et al. (2018) |
| *L. perolatii* | FH1-B-B1 | P2 | River bank; Touho, New Caledonia | No | Thibeaux et al. (2018) |
| *L. selangorensis* | SCW17 | P2 | Water; Sungai Congkak, Malaysia | ND | Vincent et al. (2019) |
| *L. venezuelensis* | CLM-U50 | P2 | *Rattus norvegicus* (rat); Venezuela | ND | Puche et al. (2018) |
| *L. bandrabouensis* | 201601111 M10A | S1 | Water; Bandraboua, Mayotte | ND | Vincent et al. (2019) |
| *L. biflexa* | Patoc 1 | S1 | Water; Italy, France | No | Picardeau et al. (2008) |
| *L. bourretii* | 201800280 PZF7-6 | S1 | Soil; Nouméa, New Caledonia | ND | Vincent et al. (2019) |
| *L. bouyouniensis* | 201601297 M1A | S1 | Water; Bouyouni, Mayotte | ND | Vincent et al. (2019) |
| *L. harrisiae* | 201602189 FH2-B A1 | S1 | River bank; Touho, New Caledonia | ND | Thibeaux et al. (2018) |
| *L. kanakyensis* | 201800292 TK5-11 | S1 | Soil; Koné, New Caledonia | ND | Vincent et al. (2019) |
| *L. montravelensis* | 201800279 PZF5-3 | S1 | Water; Nouméa, New Caledonia | ND | Vincent et al. (2019) |
| *L. mtsangambouensis* | 201601298 M2A | S1 | Water; Mtsangamboua, Mayotte | ND | Vincent et al. (2019) |
| *L. noumeaensis* | 201800287 PZF14-4 | S1 | Water; Nouméa, New Caledonia | ND | Vincent et al. (2019) |
| *L. idonii* | 201300427 DSM26084; Eri-1 | S2 | Water; Fukuoka, Japan | No | Saito et al. (2013) |
| *L. kobayashii* | E30 | S2 | Soil; Gifu, Japan | ND | Masuzawa et al. (2019b) |
| *L. ryugenii* | YH101 | S2 | Water; Shizuoka, Japan | ND | Masuzawa et al. (2019a) |

Virulence column refers to the golden Syrian hamster model of leptospirosis. ND, not experimentally determined.

corresponded to those determined by other methodologies (Yang et al., 2022a). Here we also validated FLAT$^n$ on lipid A of *L. interrogans* serovar Manilae strain L495 (Figure 1A; Eshghi et al., 2015).

## 3.3. The interspecies variability of *Leptospira* lipid A: 2 and 2′primary acyl chains

Tandem MS analysis revealed that some previously described *Leptospira* lipid A features (Que-Gewirth et al., 2004; Eshghi et al., 2015) were conserved in all 31 examined species. Specifically, representative lipid A molecules were hexa-acylated and monophosphorylated, with all primary fatty acyl residues linked *via* amide bonds and a methylated terminal phosphate group present at 1 position of the di-aminoglucose backbone (Figure 3). A newly discovered conserved feature was the presence of C12:0 (OH) residues at the 3 and 3′ primary positions (Figure 3).

The structural variability of lipid A between the *Leptospira* species was determined by the length and saturation of 2 and 2′ primary acyl and 2′ and 3′ secondary acyl chains (Figure 3). The identity of 2 primary acyl chains could be deducted from the main

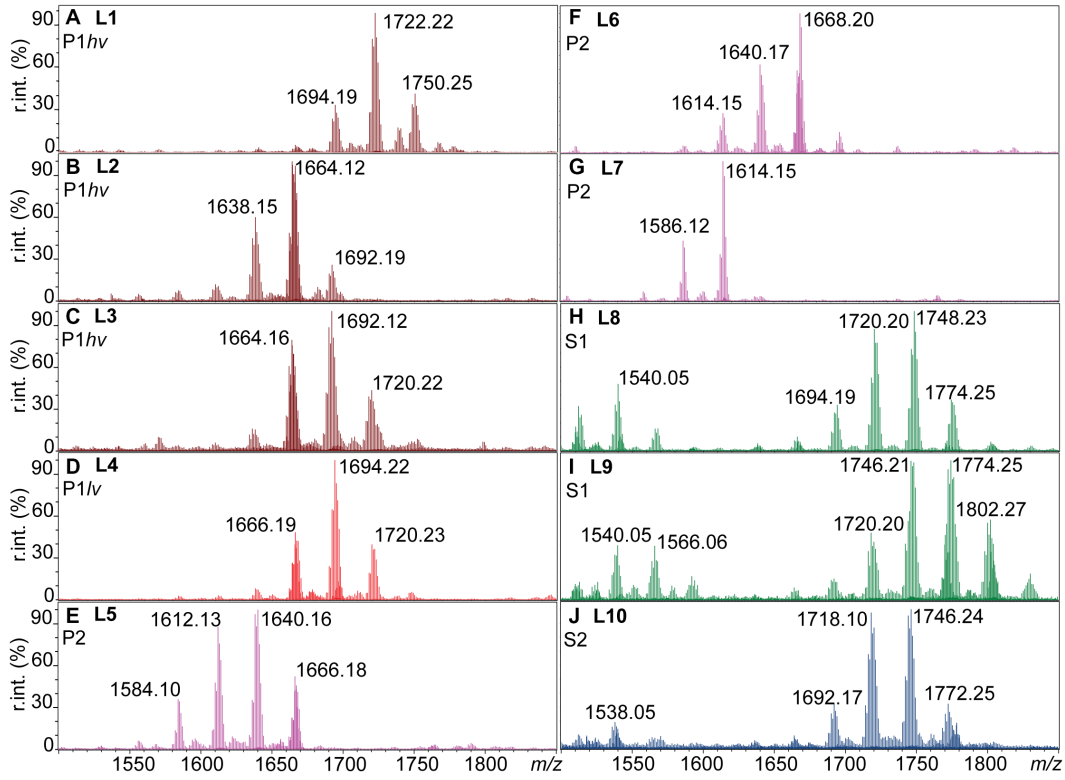


FIGURE 1
Ten lipid A profiles identified within the individual *Leptospira* subclades (L1-L10). One microliter of logarithmic cell suspensions in MS-grade water was spotted on a MALDI plate and subjected to FLAT (Sorensen et al., 2020). MS1 scans were acquired in the negative ion mode, relative intensities (r. int.) are shown. **(A–C)** P1*hv* group (dark red). **(D)** P1*lv* group (light red). **(E–G)** P2 subclade (purple). **(H,I)** S1 subclade (green). **(J)** S2 subclade (blue). Lipid A profiles of the individual *Leptospira* species can be found in Supplementary Figures S1–S3, and the information is summarized in Table 2.

product $B_1$ ion that results from fragmentation of the bond connecting the glucosamine backbone (Figures 2A,B). The three main $B_1$ ions identified in this study were *m/z* 695, *m/z* 721 and *m/z* 723 indicating acylation of the phosphorylated sugar unit (Glc*N* I) at position 2 with C14:0 (OH), C16:1 (OH) and C16:0 (OH), respectively (Supplementary Figures S4–S6). The 2 and 2′ primary acyl chains are usually identical, which stems from the mechanism of the lipid A biosynthesis (Raetz et al., 2009; Simpson and Trent, 2019). Lipid A is synthesized in a series of conserved reactions mediated by the family of Lpx enzymes; homologs of most Lpx enzymes were identified across *Leptospira* species (Hinckley et al., 2005; Eshghi et al., 2015; Nieves et al., 2023). In the early steps, LpxA, LpxC, and LpxD produce a molecule of uridine phosphate (UDP)-2,3-diacylglucosamine from UDP-N-acetylglucosamine and fatty acids bound to acyl carrier proteins. While LpxA is responsible for the addition of a fatty acyl to the 3 primary position, LpxD adds a fatty acyl to the 2 primary position of the glucosamine backbone. Both LpxA and LpxD have affinity toward specific fatty acyl chains, and this affinity differs across bacterial species (Simpson and Trent, 2019). Subsequently, a molecule of "lipid X" (2,3-diacylglucosamine-1-phoshate) is produced from some UDP-2,3-diacylglucosamine precursors *via* activity of LpxH or its homologs LpxI or LpxG. One UDP-2,3-diacylglucosamine and one "lipid X" molecule are then condensed together *via* the activity of LpxB, resulting in identical acyl chains in the 2 and 2′ and in the 3 and 3′ primary positions. As follows, each of the individual *Leptospira* subclades had a predominant primary acyl chain at the 2 and 2′ primary positions: C16:0 (OH) acyls were detected exclusively in P1*hv* species (Figures 3A,C), C14:0 (OH) in all P1*lv* and P2 species (Figures 3D–G) and C16:1 (OH) in all S1 and S2 species (Figures 3H,I). This was consistent with a previous study where C16 (OH) were detected exclusively in the pathogenic *L. interrogans* (Patra et al., 2015). Lipid A of *L. mayottensis* incorporated two C14:0 (OH) as the 2 and 2′ primary residues and its lipid A therefore resembled those of the P1*lv* and P2 species (Figure 3B, L2 profile).

Interestingly, fragmentation of the L3 and L9 representative lipid A ions resulted in two $B_1$ ions instead of one (Supplementary Figures S4D, S6F). In L3 (*L. santarosai*), the two $B_1$ ions *m/z* 695 and *m/z* 723 were detected, suggesting that the 2 and 2′ primary acyl chains were interchangeable, creating two possible isomers. A combination of C14:0 (OH)/C16:0 (OH) at the 2/2′ positions resulted in the *m/z* 695 $B_1$ product ion, while the opposite configuration, C16:0 (OH)/C14:0 (OH) at the 2/2′ positions, resulted in the *m/z* 723 $B_1$ ion (Figure 3C). The lipid A profile of this strain was also the most complex one with two extra double bonds in the base lipid A ion that could not be localized using the MS data alone (Figure 3C). In L9 (*L. kanakyensis* and *L. noumeaensis*), *m/z* 721 and *m/z* 747 were detected, likely resulting from combinations of C16:1 (OH)/C18:2 (OH) and C18:2 (OH)/C16:1 (OH) at the 2/2′ primary positions, respectively

TABLE 2 Lipid A profiles identified in the individual subclades.

| Subclade | Profile | Incidence | Lipid A ions (*m/z*) | *Leptospira* species |
|---|---|---|---|---|
| **P1*hv*** | **L1** | (3/5) | 1,694, **1,722** and 1,750 | *L. interrogans*, *L. noguchii* and *L. weilii* |
| | **L2** | (1/5) | 1,638, **1,664** and 1,692 | *L. mayottensis* |
| | **L3** | (1/5) | 1,664, **1,692** and 1,720 | *L. santarosai* |
| **P1*lv*** | **L4** | (5/5) | 1,666, **1,694** and 1,720 | *L. adleri*, *L. ainazelensis*, *L. dzianensis*, *L. gomenensis*, and *L. tipperaryensis* |
| **P2** | **L5** | (7/9) | 1,584, 1,612, **1,640** and 1,666 | *L. haakeii*, *L. hartskeerli*, *L. langatensis*, *L. licerasiae*, *L. neocaledonica*, *L. selangorensis*, and *L. venezuelensis* |
| | **L6** | (1/9) | 1,614, 1,640 and **1,668** | *L. fluminis* |
| | **L7** | (1/9) | 1,586 and 1,614 | *L. perolatii* |
| **S1** | **L8** | (7/9) | 1,540, 1,694, 1,720, **1,748** and 1,774 | *L. bandrabouensis*, *L. biflexa*, *L. bouyouniensis*, *L. bourrettii*, *L. harrisiae*, *L. mtsangambouensis*, and *L. montravelensis* |
| | **L9** | (2/9) | 1,540, 1,566, 1720, 1746, **1774** and 1802 | *L. noumeaensis* and *L. kanakyensis* |
| **S2** | **L10** | (3/3) | 1,538, 1,692, 1720, **1746** and 1772 | *L. idonii*, *L. kobayashii*, and *L. ryugenii* |

Numbers in brackets correspond to the number of interrogated *Leptospira* species with the corresponding lipid A profile. The most common lipid A profile is listed first for each subclade; base peak ions are highlighted in bold.

(Figure 3H). These unusual lipid A structures could be a result of simultaneous activity of two LpxD enzymes, as two copies of *lpxD* genes have been annotated in all *Leptospira* genomes except for those belonging to the P2 subclade (Supplementary Figure S7). To date, the function of two separate LpxD enzymes was studied only in two bacterial species (Simpson and Trent, 2019). In *Francisella*, the expression of LpxD1 and LpxD2 is temperature dependent. LpxD1 adds two C18:0 (OH) and LpxD2 adds two C16:0 (OH) to the 3 and 3′ primary positions of the lipid A when grown at 37°C and 25°C, respectively, aiding adaptation to temperature shifts (Gunn and Ernst, 2007; Scott et al., 2016). In *L. interrogans*, LpxD1 contributes to pathogenicity, adaptation to temperature changes and presence of toxic compounds (Eshghi et al., 2015). However, the conditions warranting expression of LpxD1/LpxD2 in *L. interrogans* remain elusive (Eshghi et al., 2015; Simpson and Trent, 2019). The representative structures of L3 and L9 phenotypes might provide a first hint to function of LpxD1 and LpxD2 in other *Leptospira* species. It occurs that *Leptospira* species with the L3 and the L9 phenotypes, co-expressed LpxD1 and LpxD2 enzymes might compete to add acyl residues to the 2 primary position of the early UDP-2,3-diacyl glucosamine product in lipid A biosynthesis. A similar phenomenon was described for the late acetyltransferases LpxL1 and LpxL2 in *Klebsiella pneumoniae* that compete to add either C12:0 or C14:0 at the 2′ secondary position (Li et al., 2016; Mills et al., 2017; Simpson and Trent, 2019). Annotated tandem mass spectra for all representative lipid A ions can be found in the supplementary material (Supplementary Figures S4–S6).

## 3.4. The interspecies variability of *Leptospira* lipid A: 2′ And 3′ secondary acyl chains

All *Leptospira* species incorporated short fatty acyl chains at the 2′ and 3′ secondary positions of their lipid A. In the representative lipid A molecules, these residues consisted of a combination of C12:1/C14:1 (L1, L3 and L6; Figures 3A,C,F), two C12:1 (L5; Figure 3E) or two C14:1 (L4, L8-10; Figures 3D,H,I). Representative lipid A molecules of *L. mayottensis* (L2; Figure 3B) and *L. perolatii* (L7; Figure 3G) contained a combination of C14:2/C12:1 and C14:1/C8:0, respectively (Figure 3). Secondary acyl residues are added to the lipid A by late acyltransferases (homologs of LpxL and LpxM from *Escherichia coli*) (Raetz et al., 2009). Each of these enzymes often adds an acyl chain of a specific length and degree of saturation (Simpson and Trent, 2019). To date, only one bi-functional acyltransferase capable of adding two different acyl chains to the 2′ and 3′ secondary positions was reported in *Acinetobacter baumannii* (Boll et al., 2015). Given the great variability of secondary acyl chains across *Leptospira*, its LpxL homolog is likely a multifunctional acyltransferase.

## 3.5. Penta-acylated lipid A molecules were detected in S1 and S2 *Leptospira* species

A novel structural feature of *Leptospira* lipid A was found in S1 and S2 subclades. In these species, additional clusters with lower *m/z* were identified (Figure 1; Supplementary Figure S3). Upon tandem mass spectrometry analysis, it was determined that these were penta-acylated lipid A molecules (Figure 3; Supplementary Figure S6). The mechanisms of synthesis of these penta-acylated lipid A species are unclear. In other bacteria, fatty acyl chains can be removed *via* activity of LpxR (Simpson and Trent, 2019). Although homologs of LpxR were identified in *Leptospira*, the LpxR usually removes two acyl chains, not one. PagL and PagP enzymes can remove a single acyl chain from the lipid A molecule (Ernst et al., 2006; Thaipisuttikul et al., 2014), however, homologs of these enzymes were not found in saprophytic *Leptospira* (Picardeau et al., 2008). Finally, in bacteria harboring two LpxL enzymes, such as *Neisseria meningitidis*, loss of one copy

10.3389/fmicb.2023.1181034

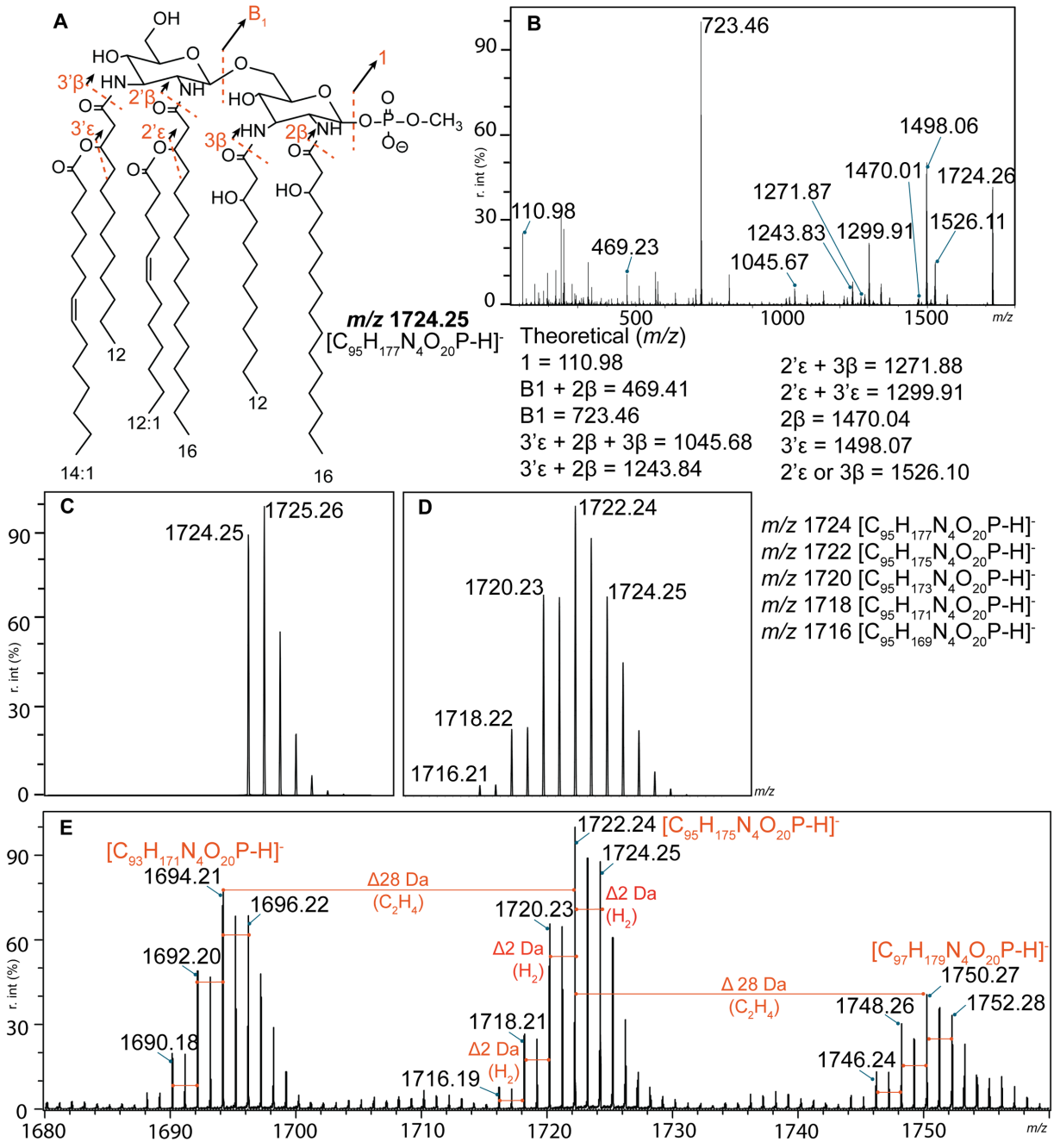


FIGURE 2
Structural determination of *L. interrogans* lipid A (L1) by tandem mass spectrometry. **(A)** Proposed structure of the *m/z* 1724.25 ion corresponds to the previously published structure for this species (Que-Gewirth et al., 2004; Eshghi et al., 2015). Fragmentation patterns are depicted as dashed red lines. **(B)** Product ion scan of the precursor ion *m/z* 1724.25. Calculated *m/z* of the product ions are listed at the bottom of the panel. **(C,D)** Theoretical isotopic distributions. **(C)** Isotopic distribution of a single lipid A ion (*m/z* 1724.25 corresponding to the structure in panel **A**). **(D)** Mixed isotopic distribution of five lipid A ions that differ from each other by a presence of a single double bond (2 Da). Please note that the abundances of each ion were not equal; ratio used for the simulation was 9:21:17:6:1 (*m/z* 1724:1722:1720:1718:1716). **(E)** Annotated mass spectrum of *L. interrogans* lipid A profile (L1). Three main clusters of lipid A ions separated by 28 Da were identified. Each individual cluster likely consisted of five lipid A ions that differ by a presence of a double bond (red lines). r. int. – relative intensity.

leads to synthesis of penta-acylated lipid A species (Fransen et al., 2009). This cannot be the case in *Leptospira* where only one LpxL homolog was annotated (Picardeau et al., 2008). Nonetheless, penta-acylated lipid A molecules are known to elicit reduced immune responses in the host (Fransen et al., 2010; Scott et al., 2017), and their presence in saprophytic species is intriguing.

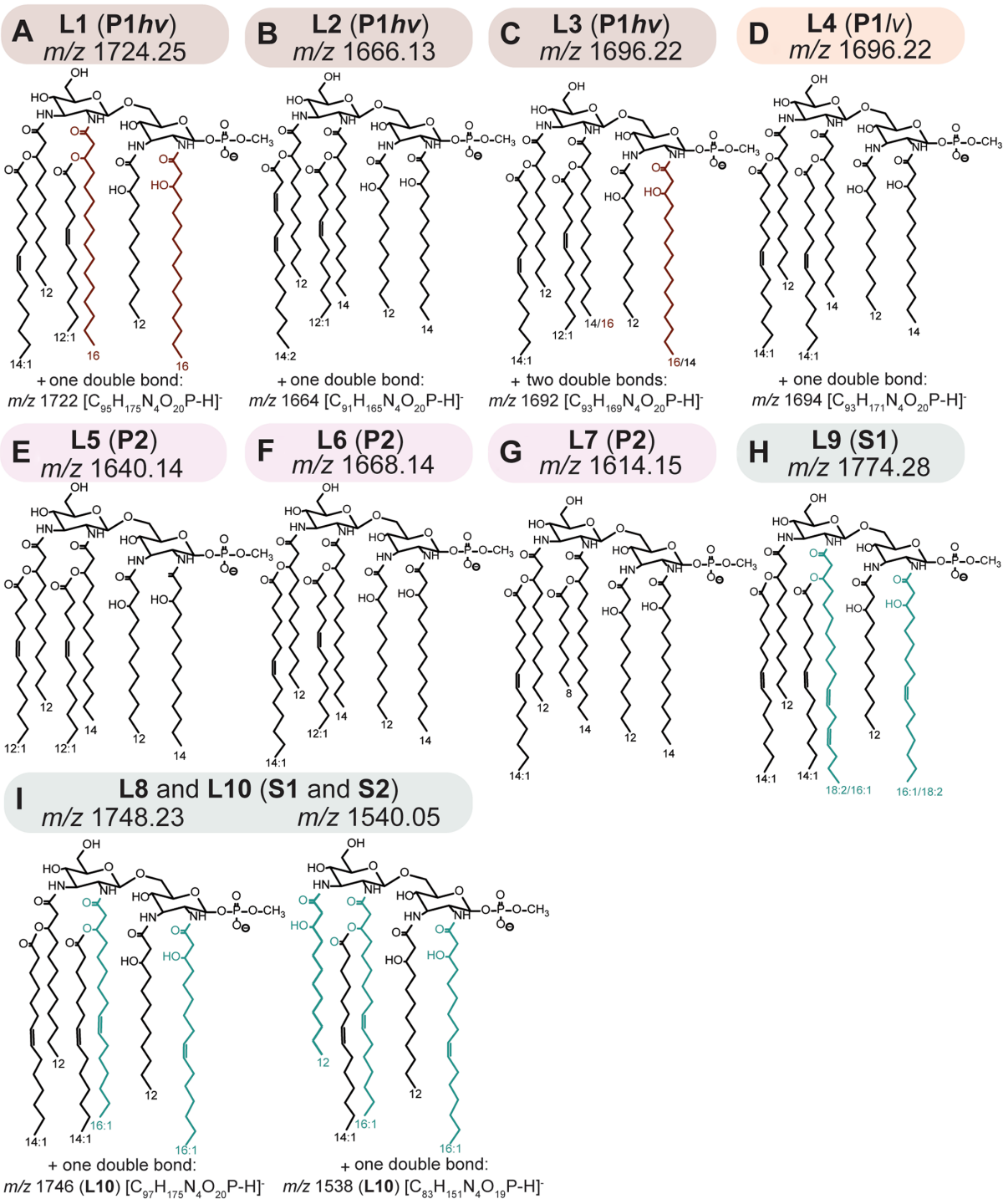


FIGURE 3
**(A–C)** L1-L3; P1hv group. **(D)** L4; P1lv group. **(E–G)** L5-L7; P2 subclade. **(H)** L9; S1 subclade. **(I)** L8 and L10; S1 and S2 subclades. Putative structures of lipid A ions representative of the L1–L10 profiles. Structures were proposed based on fragmentation patterns of the representative lipid A ions, as detected by FLAT$^n$ (Yang et al., 2022a; Supplementary Figures S4–S6). Sugar and anomeric configurations were assigned in homology with previous data (Que-Gewirth et al., 2004; Eshghi et al., 2015; Novak et al., 2022) and the placement of double bonds is putative. Additional approaches discussed in Section 3.2 are needed to fully validate the proposed structures (out of the scope of the current study). Please refer to the Table 2 for information on distribution of the individual phenotype across *Leptospira* species.

## 3.6. Which structural features of *Leptospira* lipid A might contribute to pathogenicity?

Lipid A of pathogenic *Leptospira* has been previously shown to evade recognition by the human TLR4/MD2 (Werts et al., 2001). It has been speculated that this might be due to monophosphorylation of *Leptospira* lipid A that is associated with a reduced endotoxic activity in other bacteria (Baldridge and Crane, 1999; Wang et al., 2007). However, this is complicated by the unusual presence of a methyl group on the single terminal phosphate (Que-Gewirth et al.,

2004). Here, we revealed other structural features that might contribute to this phenomenon.

Degree of TLR4/MD2 activation is also dependent on the length of fatty acyl residues. While C12 or C14 are optimal for TLR4/MD2 binding, C16 is not favorable (Rietschel et al., 1994; Park and Lee, 2013; Facchini et al., 2018). In deep-sea *Moritella* species, the lipid A either activates TLR4/MD2 or is "immune-silent," not eliciting responses *via* TLR4/MD2 or other related host receptors (Gauthier et al., 2021). While the basic structural features between *Moritella* lipid As are conserved (hexa-acylated bis-phosphorylated molecules), the immune-silent *Moritella* lipid A has higher C16 content (Gauthier et al., 2021). In this study, C16 (OH) residues were found exclusively in pathogenic P1*hv* species (Figures 3A,C), which was consistent with previous findings (Patra et al., 2015). We therefore hypothesize that lipid A of P1*lv* and P2 species might be better binding partners of the innate immune receptors, contributing to faster clearance of these species and their lower pathogenic potential in humans. Future studies including assessing endotoxin activity of P1*lv* and P2 lipid A extracts using reporter assays are warranted to explore this hypothesis.

Finally, while the discussion to this point has centered around lipid A, other lipid molecules are known to confer immune evasion. Cardiolipins have been shown to suppress stimulatory activity of LPS (Khan et al., 2018). Cardiolipin species have been identified in pathogenic as well as non-pathogenic *Leptospira* species (Supplementary Figure S8). Since our lipid preparations for FLAT and FLAT$^n$ consisted of whole cells, it is not possible to determine if the cardiolipins were located to the inner or the outer membrane and if they can attenuate LPS-mediated immune activation. However, their presence in the *Leptospira* membrane is intriguing and warrants further investigation.

### 3.7. Lipid A-based molecular typing as a complementary strategy for *Leptospira* identification and classification

Novel *Leptospira* species are isolated from various hosts or the environment on regular basis (Thibeaux et al., 2018; Masuzawa et al., 2019b; Korba et al., 2021). Extensive phenotype profiling including serotyping, assessing growth at 37°C, growth in presence of purine analog 8-azaguanine, and ultimately animal infection studies are needed to distinguish pathogens from saprophytes during characterization of novel species (Vincent et al., 2019). Here, we propose the use of L1-L10 lipid A profiles combined with FLAT for rapid classification of *Leptospira* isolates into the individual subclades (L1-L3 for P1*hv*, L4 for P1*lv*, L5-L7 for P2, L8-9 for S1 and L10 for S2 subclades). Lipid A-based MALDI-TOF assays allow for rapid (within an hour) identification of bacteria directly from a specimen using minimal input and hands-on-time (Leung et al., 2017; Liang et al., 2019; Sorensen et al., 2020). Lipid A-based assays allow for simultaneous identification and screening for antibiotic resistance markers and can be used directly from urine (Smith et al., 2021, 2022; Yang et al., 2022b). Thanks to minimal background in the *m/z* area where lipid A is detected, individual species can also be identified from multi-bacterial samples (Fondrie et al., 2018; Ryu et al., 2020). Protein-based profiling *via* MALDI-TOF is routinely used to characterize *Leptospira* species (Thibeaux et al., 2018; Sonthayanon et al., 2019; Girault et al., 2020; Korba et al., 2021), and the addition of lipid A phenotyping would provide valuable information while utilizing the existing infrastructure.

## 4. Conclusion

This is the first study focused on structural analysis of lipid A across the whole *Leptospira* genus. Ten distinct lipid A profiles were revealed that can be used for rapid molecular typing of novel clinical and environmental *Leptospira* isolates, aiding the leptospirosis surveillance. In addition, revealed structural differences between lipid A of individual species can lead to novel hypotheses on *Leptospira* pathogenicity.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

## Author contributions

HP conceived and designed the experiments. HP, AM, SC, AG-G, and MS performed the experiments. HP, AM, and DG analyzed the data. AG, RE, CC, MP, and DG contributed reagents, materials, and analysis tools. HP and DG prepared the original draft. HP, AM, SC, AG-G, AG, MS, RE, CC, MP, and DG reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

## Funding

Funding for this study was provided to DRG from Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant (RGPIN-2022-04433). DG and RE received funding from National Institutes of Health (NIH) and National Institute of Allergy and Infectious Diseases (NIAID) #R01AI147314. DG and CC received support from the Canada Institutes of Health Research (CIHR), Catalyst Grant Sexually Transmitted and Blood Borne Infections Research in Canada: Beyond HIV/AIDS and Hepatitis (SC1–178732). DG and the work performed at the University of Victoria-Genome BC Proteomics Centre was also supported by funding to the Metabolomics Innovation Centre (TMIC) from Genome Canada and Genome British Columbia, through the Genomics Technology Platform (GTP) program for operations and technology development (265MET), as well operations support from the Canadian Foundation for Innovation Major Sciences Initiative (CFI-MSI) program (35456). Infrastructure and operations funding to support his project was provided to DRG from PacifiCan (Project No: 22591). MP was supported by Institut Pasteur.

## Acknowledgments

We thank Darryl Hardie and Jun Han of the UVic Genome BC Proteomic Centre for their assistance with the LC–MS/MS assays. We thank Angela Jackson of the UVic Genome BC Proteomic Centre for her support and managing the laboratory's day-to-day operations. We also thank Dr. Yuri O. Tsybin of Spectroswiss for assistance with isotopic distribution modeling.

## Conflict of interest

RE and DG are co-founders and vice presidents of Patagain, a company that develops mass spectrometry-based microbiology tests to identify disease pathogens and determine antimicrobial resistance.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1181034/full#supplementary-material

## References

Baldridge, J. R., and Crane, R. T. (1999). Monophosphoryl lipid A (MPL) formulations for the next generation of vaccines. *Methods* 19, 103–107. doi: 10.1006/meth.1999.0834

Boll, J. M., Tucker, A. T., Klein, D. R., Beltran, A. M., Brodbelt, J. S., Davies, B. W., et al. (2015). Reinforcing lipid A acylation on the cell surface of *Acinetobacter baumannii* promotes cationic antimicrobial peptide resistance and desiccation. *Survival* 6, e00478–e00415. doi: 10.1128/mBio.00478-15

Bourhy, P., Collet, L., Brisse, S., and Picardeau, M. (2014). *Leptospira mayottensis* sp. nov., a pathogenic species of the genus *Leptospira* isolated from humans. *Int. J. Syst. Evol. Microbiol.* 64, 4061–4067. doi: 10.1099/ijs.0.066597-0

Chandler, C. E., Harberts, E. M., Pelletier, M. R., Thaipisuttikul, I., Jones, J. W., Hajjar, A. M., et al. (2020). Early evolutionary loss of the lipid A modifying enzyme PagP resulting in innate immune evasion in *Yersinia pestis*. *Proc. Natl. Acad. Sci. U. S. A.* 117, 22984–22991. doi: 10.1073/pnas.1917504117

Coburn, J., Picardeau, M., Woods, C. W., Veldman, T., and Haake, D. A. (2021). Pathogenesis insights from an ancient and ubiquitous spirochete. *PLoS Pathog.* 17:e1009836. doi: 10.1371/journal.ppat.1009836

Deguchi, A., Tomita, T., Ohto, U., Takemura, K., Kitao, A., Akashi-Takamura, S., et al. (2016). Eritoran inhibits S100A8-mediated TLR4/MD-2 activation and tumor growth by changing the immune microenvironment. *Oncogene* 35, 1445–1456. doi: 10.1038/onc.2015.211

Domínguez-Medina, C. C., Pérez-Toledo, M., Schager, A. E., Marshall, J. L., Cook, C. N., Bobat, S., et al. (2020). Outer membrane protein size and LPS O-antigen define protective antibody targeting to the *Salmonella* surface. *Nat. Commun.* 11:851. doi: 10.1038/s41467-020-14655-9

Duerr, C. U., Zenk, S. F., Chassin, C., Pott, J., Gütle, D., Hensel, M., et al. (2009). O-antigen delays lipopolysaccharide recognition and impairs antibacterial host defense in murine intestinal epithelial cells. *PLoS Pathog.* 5:e1000567. doi: 10.1371/journal.ppat.1000567

Ellis, W. A., and Thiermann, A. B. (1986). Isolation of *Leptospira interrogans* serovar Bratislava from sows in Iowa. *Am. J. Vet. Res.* 47, 1458–1460.

Ernst, R. K., Adams, K. N., Moskowitz, S. M., Kraig, G. M., Kawasaki, K., Stead, C. M., et al. (2006). The *Pseudomonas aeruginosa* lipid A deacylase: selection for expression and loss within the cystic fibrosis airway. *J. Bacteriol.* 188, 191–201. doi: 10.1128/JB.188.1.191-201.2006

Eshghi, A., Henderson, J., Trent, M. S., and Picardeau, M. (2015). *Leptospira interrogans* lpxD homologue is required for thermal acclimatization and virulence. *Infect. Immun.* 83, 4314–4321. doi: 10.1128/IAI.00897-15

Facchini, F. A., Zaffaroni, L., Minotti, A., Rapisarda, S., Calabrese, V., Forcella, M., et al. (2018). Structure-activity relationship in monosaccharide-based toll-like receptor 4 (TLR4) antagonists. *J. Med. Chem.* 61, 2895–2909. doi: 10.1021/acs.jmedchem.7b01803

Fondrie, W. E., Liang, T., Oyler, B. L., Leung, L. M., Ernst, R. K., Strickland, D. K., et al. (2018). Pathogen identification direct from Polymicrobial specimens using membrane glycolipids. *Sci. Rep.* 8:15857. doi: 10.1038/s41598-018-33681-8

Fransen, F., Hamstra, H. J., Boog, C. J., van Putten, J. P., van den Dobbelsteen, G. P. J. M., and van der Ley, P. (2010). The structure of *Neisseria meningitidis* lipid A determines outcome in experimental meningococcal disease. *Infect. Immun.* 78, 3177–3186. doi: 10.1128/IAI.01311-09

Fransen, F., Heckenberg, S. G. B., Hamstra, H. J., Feller, M., Boog, C. J. P., van Putten, J. P. M., et al. (2009). Naturally occurring lipid A mutants in *Neisseria meningitidis* from patients with invasive meningococcal disease are associated with reduced coagulopathy. *PLoS Pathog.* 5:e1000396. doi: 10.1371/journal.ppat.1000396

Gauthier, A. E., Chandler, C. E., Poli, V., Gardner, F. M., Tekiau, A., Smith, R., et al. (2021). Deep-sea microbes as tools to refine the rules of innate immune pattern recognition. *Sci. Immunol.* 6:eabe0531. doi: 10.1126/sciimmunol.abe0531

Girault, D., Kainiu, M., Barsac, E., Thibeaux, R., and Goarant, C. (2020). Use of MALDI-ToF mass spectrometry for identification of Leptospira. *Methods Mol. Biol.* 2134, 23–29. doi: 10.1007/978-1-0716-0459-5_3

Gunn, J. S., and Ernst, R. K. (2007). The structure and function of *Francisella* lipopolysaccharide. *Ann. N. Y. Acad. Sci.* 1105, 202–218. doi: 10.1196/annals.1409.006

Hassan, N., Anesio, A. M., Rafiq, M., Holtvoeth, J., Bull, I., Haleem, A., et al. (2020). Temperature driven membrane lipid adaptation in glacial psychrophilic Bacteria. *Front. Microbiol.* 11:824. doi: 10.3389/fmicb.2020.00824

Hinckley, M. B., Reynolds, C. M., Ribeiro, A. A., McGrath, S. C., Cotter, R. J., Lauw, F. N., et al. (2005). A *Leptospira interrogans* enzyme with similarity to yeast Ste14p that methylates the 1-phosphate group of lipid A. *J. Biol. Chem.* 280, 30214–30224. doi: 10.1074/jbc.M506103200

Kawahara, K. (2021). Variation, modification and engineering of lipid A in endotoxin of gram-negative Bacteria. *Int. J. Mol. Sci.* 22:2281. doi: 10.3390/ijms22052281

Kawahara, K., Tsukano, H., Watanabe, H., Lindner, B., and Matsuura, M. (2002). Modification of the structure and activity of lipid A in *Yersinia pestis* lipopolysaccharide by growth temperature. *Infect. Immun.* 70, 4092–4098. doi: 10.1128/IAI.70.8.4092-4098.2002

Khan, M. M., Ernst, O., Sun, J., Fraser, I. D. C., Ernst, R. K., Goodlett, D. R., et al. (2018). Mass spectrometry-based structural analysis and systems Immunoproteomics strategies for deciphering the host response to endotoxin. *J. Mol. Biol.* 430, 2641–2660. doi: 10.1016/j.jmb.2018.06.032

Koizumi, N., and Watanabe, H. (2003). Identification of a novel antigen of pathogenic *Leptospira* spp. that reacted with convalescent mice sera. *J. Med. Microbiol.* 52, 585–589. doi: 10.1099/jmm.0.05148-0

Kong, Q., Six, D. A., Liu, Q., Gu, L., Roland, K. L., Raetz, C. R. H., et al. (2011). Palmitoylation state impacts induction of innate and acquired immunity by the *Salmonella enterica* serovar typhimurium *msbB* mutant. *Infect. Immun.* 79, 5027–5038. doi: 10.1128/IAI.05524-11

Kong, Q., Six, D. A., Liu, Q., Gu, L., Wang, S., Alamuri, P., et al. (2012). Phosphate groups of lipid A are essential for *Salmonella enterica* serovar Typhimurium virulence and affect innate and adaptive immunity. *Infect. Immun.* 80, 3215–3224. doi: 10.1128/IAI.00123-12

Korba, A. A., Lounici, H., Kainiu, M., Vincent, A. T., Mariet, J.-F., Veyrier, F. J., et al. (2021). *Leptospira ainlahdjerensis* sp. nov., *Leptospira ainazelensis* sp. nov., *Leptospira abararensis* sp. nov. and *Leptospira chreensis* sp. nov., four new species isolated from water sources in Algeria. *Int. J. Syst. Evol. Microbiol.* 71:005148. doi: 10.1099/ijsem.0.005148

Leung, L. M., Fondrie, W. E., Doi, Y., Johnson, J. K., Strickland, D. K., Ernst, R. K., et al. (2017). Identification of the ESKAPE pathogens by mass spectrometric analysis of microbial membrane glycolipids. *Sci. Rep.* 7:6403. doi: 10.1038/s41598-017-04793-4

Li, Y., Powell, D. A., Shaffer, S. A., Rasko, D. A., Pelletier, M. R., Leszyk, J. D., et al. (2012). LPS remodeling is an evolved survival strategy for bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 109, 8716–8721. doi: 10.1073/pnas.1202908109

Li, Y., Yun, J., Liu, L., Li, Y., and Wang, X. (2016). Identification of two genes encoding for the late acyltransferases of lipid A in *Klebsiella pneumoniae*. *Curr. Microbiol.* 73, 732–738. doi: 10.1007/s00284-016-1117-6

Liang, T., Leung, L. M., Opene, B., Fondrie, W. E., Lee, Y. I., Chandler, C. E., et al. (2019). Rapid microbial identification and antibiotic resistance detection by mass spectrometric analysis of membrane lipids. *Anal. Chem.* 91, 1286–1294. doi: 10.1021/acs.analchem.8b02611

Marcsisin, R. A., Bartpho, T., Bulach, D. M., Srikram, A., Sermswan, R. W., Adler, B., et al. (2013). Use of a high-throughput screen to identify Leptospira mutants unable to colonize the carrier host or cause disease in the acute model of infection. *J. Med. Microbiol.* 62, 1601–1608. doi: 10.1099/jmm.0.058586-0

Masuzawa, T., Saito, M., Nakao, R., Nikaido, Y., Matsumoto, M., Ogawa, M., et al. (2019a). Molecular and phenotypic characterization of *Leptospira johnsonii* sp. nov., *Leptospira ellinghausenii* sp. nov. and *Leptospira ryugenii* sp. nov. isolated from soil and water in Japan. *Microbiol. Immunol.* 63, 89–99. doi: 10.1111/1348-0421.12671

Masuzawa, T., Uno, R., Matsuhashi, N., Yamaguchi, M., Xu, J., and Nakamura, S. (2019b). Comparison of *Leptospira* species isolated from environmental water and soil in Japan. *Microbiol. Immunol.* 63, 469–473. doi: 10.1111/1348-0421.12741

Mills, G., Dumigan, A., Kidd, T., Hobley, L., and Bengoechea, J. A. (2017). Identification and characterization of two *Klebsiella pneumoniae lpxL* lipid A late Acyltransferases and their role in virulence. *Infect. Immun.* 85, e00068–e00017. doi: 10.1128/IAI.00068-17

Murray, G. L., Srikram, A., Henry, R., Hartskeerl, R. A., Sermswan, R. W., and Adler, B. (2010). Mutations affecting *Leptospira interrogans* lipopolysaccharide attenuate virulence. *Mol. Microbiol.* 78, 701–709. doi: 10.1111/j.1365-2958.2010.07360.x

Nahori, M.-A., Fournié-Amazouz, E., Que-Gewirth, N. S., Balloy, V., Chignard, M., Raetz, C. R. H., et al. (2005). Differential TLR recognition of leptospiral lipid A and lipopolysaccharide in murine and human cells. *J. Immunol.* 175, 6022–6031. doi: 10.4049/jimmunol.175.9.6022

Nally, J. E., Arent, Z., Bayles, D. O., Hornsby, R. L., Gilmore, C., Regan, S., et al. (2016). Emerging infectious disease implications of invasive mammalian species: the greater white-toothed shrew (*Crocidura russula*) is associated with a novel Serovar of pathogenic *Leptospira* in Ireland. *PLoS Negl. Trop. Dis.* 10:e0005174. doi: 10.1371/journal.pntd.0005174

Needham, B. D., and Trent, M. S. (2013). Fortifying the barrier: the impact of lipid A remodelling on bacterial pathogenesis. *Nat. Rev. Microbiol.* 11, 467–481. doi: 10.1038/nrmicro3047

Nieves, C., Vincent, A. T., Zarantonelli, L., Picardeau, M., Veyrier, F. J., and Buschiazzo, A. (2023). Horizontal transfer of the *rfb* cluster in *Leptospira* is a genetic determinant of serovar identity. *Life Sci. Alliance* 6:e202201480. doi: 10.26508/lsa.202201480

Novak, A., Pupo, E., Van't Veld, E., VPMG, R., Broere, F., and Sloots, A. (2022). Activation of canine, mouse and human TLR2 and TLR4 by inactivated *Leptospira* vaccine strains. *Front. Immunol.* 13:823058. doi: 10.3389/fimmu.2022.823058

Ohto, U., Fukase, K., Miyake, K., and Shimizu, T. (2012). Structural basis of species-specific endotoxin sensing by innate immune receptor TLR4/MD-2. *Proc. Natl. Acad. Sci. U. S. A.* 109, 7421–7426. doi: 10.1073/pnas.1201193109

Park, B. S., and Lee, J.-O. (2013). Recognition of lipopolysaccharide pattern by TLR4 complexes. *Exp. Mol. Med.* 45:e66. doi: 10.1038/emm.2013.97

Park, B. S., Song, D. H., Kim, H. M., Choi, B.-S., Lee, H., and Lee, J.-O. (2009). The structural basis of lipopolysaccharide recognition by the TLR4–MD-2 complex. *Nature* 458, 1191–1195. doi: 10.1038/nature07830

Patra, K. P., Choudhury, B., Matthias, M. M., Baga, S., Bandyopadhya, K., and Vinetz, J. M. (2015). Comparative analysis of lipopolysaccharides of pathogenic and intermediately pathogenic *Leptospira* species. *BMC Microbiol.* 15:244. doi: 10.1186/s12866-015-0581-7

Picardeau, M., Bulach, D. M., Bouchier, C., Zuerner, R. L., Zidane, N., Wilson, P. J., et al. (2008). Genome sequence of the saprophyte *Leptospira biflexa* provides insights into the evolution of *Leptospira* and the pathogenesis of leptospirosis. *PLoS One* 3:e1607. doi: 10.1371/journal.pone.0001607

Puche, R., Ferrés, I., Caraballo, L., Rangel, Y., Picardeau, M., Takiff, H., et al. (2018). *Leptospira venezuelensis* sp. nov., a new member of the intermediate group isolated from rodents, cattle and humans. *Int. J. Syst. Evol. Microbiol.* 68, 513–517. doi: 10.1099/ijsem.0.002528

Que-Gewirth, N. L. S., Ribeiro, A. A., Kalb, S. R., Cotter, R. J., Bulach, D. M., Adler, B., et al. (2004). A methylated phosphate group and four amide-linked acyl chains in *Leptospira interrogans* lipid A. the membrane anchor of an unusual lipopolysaccharide that activates TLR2. *J. Biol. Chem.* 279, 25420–25429. doi: 10.1074/jbc.M400598200

Raetz, C. R. H., Guan, Z., Ingram, B. O., Six, D. A., Song, F., Wang, X., et al. (2009). Discovery of new biosynthetic pathways: the lipid A story. *J. Lipid Res.* 50, S103–S108. doi: 10.1194/jlr.R800060-JLR200

Rebeil, R., Ernst, R. K., Gowen, B. B., Miller, S. I., and Hinnebusch, B. J. (2004). Variation in lipid A structure in the pathogenic *yersiniae*. *Mol. Microbiol.* 52, 1363–1373. doi: 10.1111/j.1365-2958.2004.04059.x

Ribeiro, A. A., Zhou, Z., and Raetz, C. R. H. (1999). Multi-dimensional NMR structural analyses of purified lipid X and lipid A (endotoxin). *Magn. Reson. Chem.* 37, 620–630. doi: 10.1002/(SICI)1097-458X(199909)37:9<620::AID-MRC517>3.0.CO;2-Q

Ricaldi, J. N., Fouts, D. E., Selengut, J. D., Harkins, D. M., Patra, K. P., Moreno, A., et al. (2012). Whole genome analysis of *Leptospira licerasiae* provides insight into leptospiral evolution and pathogenicity. *PLoS Negl. Trop. Dis.* 6:e1853. doi: 10.1371/journal.pntd.0001853

Rietschel, E. T., Kirikae, T., Schade, F. U., Mamat, U., Schmidt, G., Loppnow, H., et al. (1994). Bacterial endotoxin: molecular relationships of structure to activity and function. *FASEB J.* 8, 217–225. doi: 10.1096/fasebj.8.2.8119492

Ryu, S. Y., Wendt, G. A., Ernst, R. K., and Goodlett, D. R. (2020). MGMS2: membrane glycolipid mass spectrum simulator for polymicrobial samples. *Rapid Commun. Mass Spectrom.* 34:e8824. doi: 10.1002/rcm.8824

Saito, M., Villanueva, S. Y. A. M., Kawamura, Y., Iida, K.-I., Tomida, J., Kanemaru, T., et al. (2013). *Leptospira idonii* sp. nov., isolated from environmental water. *Int. J. Syst. Evol. Microbiol.* 63, 2457–2462. doi: 10.1099/ijs.0.047233-0

Scott, A. J., Flinders, B., Cappell, J., Liang, T., Pelc, R. S., Tran, B., et al. (2016). Norharmane matrix enhances detection of endotoxin by MALDI-MS for simultaneous profiling of pathogen, host, and vector systems. *Pathog Dis.* 74:ftw097. doi: 10.1093/femspd/ftw097

Scott, A. J., Oyler, B. L., Goodlett, D. R., and Ernst, R. K. (2017). Lipid A structural modifications in extreme conditions and identification of unique modifying enzymes to define the toll-like receptor 4 structure-activity relationship. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1862, 1439–1450. doi: 10.1016/j.bbalip.2017.01.004

Silipo, A., Lanzetta, R., Garozzo, D., Lo Cantore, P., Iacobellis, N. S., Molinaro, A., et al. (2002). Structural determination of lipid A of the lipopolysaccharide from *Pseudomonas reactans*. A pathogen of cultivated mushrooms. *Eur. J. Biochem.* 269, 2498–2505. doi: 10.1046/j.1432-1033.2002.02914.x

Silipo, A., and Molinaro, A. (2010). "The diversity of the Core oligosaccharide in lipopolysaccharides" in *Endotoxins: Structure, function and recognition subcellular biochemistry*. eds. X. Wang and P. J. Quinn (Dordrecht: Springer Netherlands), 69–99.

Simpson, B. W., and Trent, M. S. (2019). Pushing the envelope: LPS modifications and their consequences. *Nat. Rev. Microbiol.* 17, 403–416. doi: 10.1038/s41579-019-0201-x

Smith, R. D., Izac, J. R., Ha, M., Yang, H., Johnson, J. K., and Ernst, R. K. (2021). Rapid identification of mcr-1-positive *Escherichia coli* from patient urine using a novel lipid-based MALDI-TOF-MS assay. *Access Microbiol.* 3:000309. doi: 10.1099/acmi.0.000309

Smith, R. D., McElheny, C. L., Izac, J. R., Gardner, F. M., Chandler, C. E., Goodlett, D. R., et al. (2022). A novel lipid-based MALDI-TOF assay for the rapid detection of Colistin-resistant *Enterobacter* species. *Microbiol. Spectr.* 10:e0144521. doi: 10.1128/spectrum.01445-21

Sonthayanon, P., Jaresitthikunchai, J., Mangmee, S., Thiangtrongjit, T., Wuthiekanun, V., Amornchai, P., et al. (2019). Whole cell matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for identification of *Leptospira* spp. in Thailand and Lao PDR. *PLoS Negl. Trop. Dis.* 13:e0007232. doi: 10.1371/journal.pntd.0007232

Sorensen, M., Chandler, C. E., Gardner, F. M., Ramadan, S., Khot, P. D., Leung, L. M., et al. (2020). Rapid microbial identification and colistin resistance detection via MALDI-TOF MS using a novel on-target extraction of membrane lipids. *Sci. Rep.* 10:21536. doi: 10.1038/s41598-020-78401-3

Srikram, A., Zhang, K., Bartpho, T., Lo, M., Hoke, D. E., Sermswan, R. W., et al. (2011). Cross-protective immunity against leptospirosis elicited by a live, attenuated lipopolysaccharide mutant. *J. Infect. Dis.* 203, 870–879. doi: 10.1093/infdis/jiq127

Strohalm, M., Kavan, D., Novák, P., Volný, M., and Havlícek, V. (2010). mMass 3: a cross-platform software environment for precise analysis of mass spectrometric data. *Anal. Chem.* 82, 4648–4651. doi: 10.1021/ac100818g

Thaipisuttikul, I., Hittle, L. E., Chandra, R., Zangari, D., Dixon, C. L., Garrett, T. A., et al. (2014). A divergent *Pseudomonas aeruginosa* palmitoyltransferase essential for cystic fibrosis-specific lipid A. *Mol. Microbiol.* 91, 158–174. doi: 10.1111/mmi.12451

Thibeaux, R., Iraola, G., Ferrés, I., Bierque, E., Girault, D., Soupé-Gilbert, M.-E., et al. (2018). Deciphering the unexplored *Leptospira* diversity from soils uncovers genomic evolution to virulence. *Microb. Genom.* 4:e000144. doi: 10.1099/mgen.0.000144

Trent, M. S., Ribeiro, A. A., Lin, S., Cotter, R. J., and Raetz, C. R. (2001). An inner membrane enzyme in Salmonella and *Escherichia coli* that transfers 4-amino-4-deoxy-L-arabinose to lipid A: induction on polymyxin-resistant mutants and role of a novel lipid-linked donor. *J. Biol. Chem.* 276, 43122–43131. doi: 10.1074/jbc.M106961200

Vanithamani, S., Akino Mercy, C. S., Kanagavel, M., Sumaiya, K., Bothammal, P., Saranya, P., et al. (2021). Biochemical analysis of leptospiral LPS explained the difference between pathogenic and non-pathogenic serogroups. *Microb. Pathog.* 152:104738. doi: 10.1016/j.micpath.2021.104738

Vincent, A. T., Schiettekatte, O., Goarant, C., Neela, V. K., Bernet, E., Thibeaux, R., et al. (2019). Revisiting the taxonomy and evolution of pathogenicity of the genus *Leptospira* through the prism of genomics. *PLoS Negl. Trop. Dis.* 13:e0007270. doi: 10.1371/journal.pntd.0007270

Viriyakosol, S., Matthias, M. A., Swancutt, M. A., Kirkland, T. N., and Vinetz, J. M. (2006). Toll-like receptor 4 protects against lethal *Leptospira interrogans* Serovar

Icterohaemorrhagiae infection and contributes to in vivo control of Leptospiral burden. *Infect. Immun.* 74, 887–895. doi: 10.1128/IAI.74.2.887-895.2006

Wang, X., Ribeiro, A. A., Guan, Z., Abraham, S. N., and Raetz, C. R. H. (2007). Attenuated virulence of a *Francisella* mutant lacking the lipid A 4′-phosphatase. *Proc. Natl. Acad. Sci. U. S. A.* 104, 4136–4141. doi: 10.1073/pnas.0611606104

Werts, C., Tapping, R. I., Mathison, J. C., Chuang, T. H., Kravchenko, V., Saint Girons, I., et al. (2001). Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. *Nat. Immunol.* 2, 346–352. doi: 10.1038/86354

Yang, H., Smith, R. D., Chandler, C. E., Johnson, J. K., Jackson, S. N., Woods, A. S., et al. (2022a). Lipid A structural determination from a single Colony. *Anal. Chem.* 94, 7460–7465. doi: 10.1021/acs.analchem.1c05394

Yang, H., Smith, R. D., Sumner, K. P., Goodlett, D. R., Johnson, J. K., and Ernst, R. K. (2022b). A matrix-assisted laser desorption ionization-time of flight mass spectrometry direct-from-urine-specimen diagnostic for gram-negative pathogens. *Microbiol. Spectr.* 10:e0373022. doi: 10.1128/spectrum.03730-22

Yasuda, P. H., Steigerwalt, A. G., Sulzer, K. R., Kaufmann, A. F., Rogers, F., and Brenner, D. J. (1987). Deoxyribonucleic acid relatedness between Serogroups and Serovars in the family *Leptospiraceae* with proposals for seven new *Leptospira* species. *Int. J. Syst. Evol. Microbiol.* 37, 407–415. doi: 10.1099/00207713-37-4-407

Zähringer, U., Salvetzki, R., Wagner, F., Lindner, B., and Ulmer, A. J. (2001). Structural and biological characterisation of a novel tetra-acyl lipid A from *Escherichia coli* F515 lipopolysaccharide acting as endotoxin antagonist in human monocytes. *J. Endotoxin Res.* 7, 133–146. doi: 10.1177/09680519010070020801

Zhou, Z., Ribeiro, A. A., Lin, S., Cotter, R. J., Miller, S. I., and Raetz, C. R. (2001). Lipid A modifications in polymyxin-resistant *Salmonella typhimurium*: PMRA-dependent 4-amino-4-deoxy-L-arabinose, and phosphoethanolamine incorporation. *J. Biol. Chem.* 276, 43111–43121. doi: 10.1074/jbc.M106960200

Zuerner, R. L. (2005). Laboratory maintenance of pathogenic *Leptospira*. *Curr. Protoc. Microbiol.* Chapter 12:Unit 12E.1. doi: 10.1002/9780471729259.mc12e01s00

frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 05 June 2023
DOI 10.3389/fmicb.2023.1196044



OPEN ACCESS

EDITED BY
Laurent Rénia,
Nanyang Technological University, Singapore

REVIEWED BY
Marco Antonio Natal Vigilato,
Pan American Health Organization,
United States
Ana Cláudia Coelho,
University of Trás-os-Montes and Alto Douro,
Portugal

*CORRESPONDENCE
Jolly Musoke
✉ Musokej@ufs.ac.za

RECEIVED 29 March 2023
ACCEPTED 16 May 2023
PUBLISHED 05 June 2023

CITATION
van der Westhuizen CG, Burt FJ, van Heerden N, van Zyl W, Anthonissen T and Musoke J (2023) Prevalence and occupational exposure to zoonotic diseases in high-risk populations in the Free State Province, South Africa.
*Front. Microbiol.* 14:1196044.
doi: 10.3389/fmicb.2023.1196044



# Prevalence and occupational exposure to zoonotic diseases in high-risk populations in the Free State Province, South Africa

Cornelius G. van der Westhuizen[1], Felicity J. Burt[2,3], Nina van Heerden[1], Willie van Zyl[4], Tonia Anthonissen[4] and Jolly Musoke[1,5]*

[1]Department of Medical Microbiology, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa, [2]Division of Virology, National Health Laboratory Service, Universitas, Bloemfontein, South Africa, [3]Division of Virology, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa, [4]Department of Agriculture, Land Reform and Rural Development, Division of Veterinary Services, Kroonstad, South Africa, [5]Department of Medical Microbiology, National Health Laboratory Service, Universitas, Bloemfontein, South Africa

**Introduction:** Zoonotic diseases are responsible for 2.5 billion human cases globally and approximately 2.7 million deaths annually. Surveillance of animal handlers and livestock for zoonotic pathogens contributes to understanding the true disease burden and risk factors within a community. This study investigated the prevalence of selected zoonoses in cattle, farm workers and occupational exposure to endemic zoonotic diseases and their associated risk factors.

**Methods:** Sputum samples from farmworkers were screened for *Mycobacterium bovis*. Blood specimens from farmworkers and archived sera were tested for serological evidence of *Brucella* sp., hantaviruses, and *Leptospira* sp. Communal and commercial cattle herds were tested for bovine tuberculosis and brucellosis.

**Results:** *Mycobacterium bovis* was not isolated from human samples. A total of 327 human sera were screened, and 35/327 (10.7%) were *Brucella* sp. IgG positive, 17/327 (5.2%) *Leptospira* sp. IgM positive, and 38/327 (11.6%) hantavirus IgG positive (95% CI). A higher proportion of *Brucella* sp. IgG-positive samples were detected among veterinarians (value of $p=0.0006$). Additionally, two cattle from a commercial dairy farm were bovine tuberculosis (bTB) positive using the bTB skin test and confirmatory interferon-gamma assay. A higher percentage of confirmed brucellosis-positive animals were from communal herds (8.7%) compared to commercial herds (1.1%).

**Discussion:** These findings highlight the brucellosis and *M. bovis* prevalence in commercial and communal herds, the zoonotic disease risk in commercial and subsistence farming in developing countries, and the occupational and rural exposure risk to zoonotic pathogens.

KEYWORDS

**bovine TB transmission, hantavirus, *Leptospira*, zoonotic, risk factors, brucellosis, seroprevalence, tuberculosis**

# 1. Introduction

Zoonoses are transmitted from vertebrate animals to humans and are accountable for more than 60% of all recognized human diseases and 75% of emerging infectious diseases (EID; Jones et al., 2008). In developing countries, including South Africa (SA), the mortality rate associated with EID is 47.3% (Wang et al., 2016). The majority (71.8%) of EID originate from wildlife (Jones et al., 2008). The pandemic potential of EID, as seen in the recent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak, justifies increased preparedness and research to understand these pathogens (LeDuc and Barry, 2004). Low- and middle-income countries are potentially more at risk of zoonotic pathogenic outbreaks due to limited resources. Hence, surveillance studies are vital to determine disease burden with potential public and veterinary health implications.

*Mycobacterium bovis* is the etiological agent for bovine tuberculosis (bTB) in cattle and zoonotic tuberculosis (TB) in humans. Evidence suggests an increase in intra- and inter-species transmission of *M. bovis* has previously been reported in South Africa, with bTB infection confirmed in cattle and 16 different animal species, including one domestic porcine and 15 wildlife species (Hlokwe et al., 2014). However, the prevalence of human TB due to *M. bovis* remains relatively unknown in South Africa and globally, primarily due to routine diagnostics being unable to differentiate between *M. bovis* and *Mycobacterium tuberculosis*. Though the two *Mycobacterium* species are genetically closely related, *M. bovis* does require a different treatment as it is inherently resistant to pyrazinamide, an important first-line medication used in a TB drug regimen (World Health Organization, 2017). A crude estimated rate of overall median proportions of zoonotic TB incidents was 2.8% for African countries (Müller et al., 2013). In South Africa, bTB and bovine brucellosis (BB) infection in cattle are controlled diseases associated with extensive morbidity that consequently lead to livestock production losses. Furthermore, human diseases caused by these bacteria are a notifiable condition due to their associated mortality and morbidity and, therefore, a considerable public health concern with substantial economic impact.

*Brucella* spp. are the etiological agents for brucellosis, commonly referred to as undulant fever or Malta fever. *Brucella abortus*, responsible for BB, is considered one of the predominant zoonotic pathogens in animals and humans (Rahman et al., 2017). Brucellosis is annually responsible for approximately 500 000 new human cases worldwide, with most cases reported in regions where the disease has reached levels of endemicity (Lai et al., 2017). The last formally published study focusing on the incidence rate of *Brucella* sp. in the South Africa human population reported a rate of >0.2 per 100 000 population based on a survey from 1956 to 1959 (Schrire, 1962). Sporadic cases have been reported subsequently, but surveillance remains limited (Govindasamy, 2020).

Leptospirosis, also known as Weil's disease, is a widespread and potentially fatal zoonotic bacterial disease transmitted to humans from contact with infected animals' urine (Haake and Levett, 2015). Globally, between 300,000 and 500,000 cases of leptospirosis are reported each year, with case fatality rates of up to 30% (Tilahun et al., 2013). In South Africa, an annual incidence of between 0.15 and 0.66/100,000 population has been documented, with sporadic outbreaks reported (Naidoo et al., 2020; Gizamba et al., 2022).

Hantaviruses are transmitted to humans from contact with excreta from infected rodents and have been identified as the cause of mild to severe diseases with fatalities in most parts of the world except Africa. Serological evidence of hantaviruses has been detected in sub-Saharan, East-, and West Africa, and molecular evidence in rodents has been described. However, there are no reports on human disease except occasionally imported cases (Moolla et al., 2022). The limited studies on potential rodent and insectivore hosts have not, to date, shown evidence of hantavirus infection in hosts in South Africa, although limited surveillance studies in humans have suggested a low level of serological evidence. The moderately low seroprevalence rate from patients in South Africa does not exclude the possibility of hantavirus disease occurring in the country and certainly justifies further investigation.

Zoonotic pathogen surveillance studies at the animal-human interface and among populations at occupational risk with direct animal exposure or exposure due to residing in rural conditions are crucial for identifying circulating pathogens with public health implications. These zoonoses clinically present with symptoms generally shared with a range of other common infectious diseases (i.e., malaria, typhoid fever), leading to difficulties in diagnosis and underestimating the true burden of these diseases (Salyer et al., 2017).

This study aimed to investigate the prevalence and associated risk factors of *M. bovis* and *Brucella* sp. in cattle and farmworkers from two farming communities: communal or backyard (subsistence) farming and large-scale commercial farming. Furthermore, this study aimed to document occupational and environmental exposure to *Brucella* sp., *Leptospira* sp., and hantaviruses across the Free State province, South Africa.

# 2. Materials and methods

## 2.1. Study design

A prospective, cross-sectional study was conducted using two populations which included workers with occupational exposure to animals and cattle. The study was conducted between November 2019 and March 2020. All farms selected for this study had cattle scheduled for routine bTB and BB screening as part of the Department of Agriculture Land Reform and Rural Development (DALRRD) Free State province surveillance program. Subsequently, farmworkers on the chosen farms were screened for *Mycobacterium tuberculosis* complex (MTBC) and *Brucella* IgG antibodies and asked to complete a questionnaire.

Furthermore, a retrospective analysis was performed on archived serum samples from workers with occupational exposure to animals and residing in rural areas to document zoonotic exposure to *Brucella* sp., *Leptospira* sp., and hantaviruses amongst high-risk occupational groups.

## 2.2. Study area

This bTB and BB study was conducted on two distinct farming populations, one communal farm and four commercial farms consisting of two beef and two dairy farms. The informal communal farm was located in Maokeng, Kroonstad rural, Free State province, South Africa, and the commercial farms were located within the


FIGURE 1
Study sites in the different municipal regions within the Fezile Dabi district, Free State province, South Africa. Available at https://municipalities.co.za/map/107.

Moqhaka and Ngwathe municipal regions in the Free State province (Figure 1).

## 2.3. Study populations

### 2.3.1. Animals

Two commercial dairy farms, designated as farms A and B and two commercial beef farms, designated as farms C and D, were selected by the Kroonstad State Veterinary Services as per the routine screening schedule. Convenience sampling was done for bTB and brucellosis based on the cattle availability on the farm on the day of testing and DALRRD testing history. The screening was conducted on all animals on each farm above the ages of 6 months for bTB and 18 months for BB.

### 2.3.2. High-risk human occupational workers

The bTB and BB study comprised two sample groups, A and B. In group A, 26 on-site/prospective sputum and serum samples were collected from farmworkers in the Maokeng community kraal ($n = 13$), commercial dairy farm B ($n = 7$), and commercial beef farm C ($n = 6$). A convenience sampling method was used. All participants above 18 years old were approached and enrolled if they agreed to participate in the current study. Specimen collection could not be achieved on farms A and D due to COVID-19 travel restrictions.

In group B, a total of 301 archived serum samples collected from healthy individuals between April 2016 and February 2017 as part of a previous study (HSREC34/2016 and ETOVS152/06) were included. The individuals lived in rural areas and had exposure to animals due to their occupation.

All 327 samples (from groups A and B) used in this study were collected within the Free State province and included the following high-risk populations: farmworkers ($n = 28$), abattoir workers ($n = 207$), veterinarians ($n = 12$), stable grooms ($n = 32$), recreational hunters ($n = 46$), and laboratory workers ($n = 2$).

## 2.4. Tuberculosis test in animals

A total of 321 cattle were tested for bTB, including 33, 126, and 91 cattle from farms A, B, and C, respectively. Farm D was only scheduled for BB screening and not bTB (previously tested negative), and 71 cattle were screened for bTB from the Maokeng community kraal.

### 2.4.1. Single intradermal skin test

All cattle herds from the Maokeng community kraal, farms A and C were initially screened for bTB using a single intradermal skin test (SIST), as described in the Bovine Tuberculosis Manual, approved by DALRRD (Department of Agriculture, Forestry and Fisheries of SA (DAFF), 2016). Briefly, the skin thickness of the animal was measured pre-injection using a Hauptner pistol grip. The animal was then injected intradermal with 0.1 ml of 5,000 International Units (IU) of bovine tuberculin purified protein derivative (PPD) (Onderstepoort Biological Products (OBP), Pretoria) using a McClintock syringe. After 72 h, reaction sites were observed for evidence of swelling or a color change and examined for reaction consistency (hard or soft swelling), presence of edema, and heat. The measurements were recorded, and the difference in skin thickness, pre-and post-injection, was

determined for each animal by subtracting the measurement obtained after 72 h from the initial skin thickness measurement to determine reaction type.

Herds were regarded as negative when animals had a change in skin thickness of <6 mm, including non–specific reactions. Suspect herds were defined as having a single animal with an increase in skin thickness of >6 mm, combined with evidence of positive skin reactions. Herds with animals showing large typical inflammatory reactions with an increase of skin thickness of ≥20 mm were regarded as positive.

### 2.4.2. Comparative intradermal skin test

The comparative intradermal skin test (CIST) was performed only on farms A and B due to COVID-19 restrictions. On farm A, all cattle were tested, whereas, on farm B, all SIST-suspect and -positive herds were re-tested after 3 months using a CIST. The CIST followed the same procedure as the SIST. However, instead of solely injecting 0.1 ml of 5,000 IU bovine tuberculin PPD, 0.1 ml of 2,500 IU avian tuberculin PPD (OBP, Pretoria) was injected approximately 20 cm apart. The bovine reaction increase was determined by subtracting the 72 h post-injection skin measurement (bovine PPD) from the pre-injection normal skin measurement. The avian reaction increase was determined by subtracting the 72 h post-injection skin measurement (avian PPD) from the pre-injection normal skin measurement. A positive difference between bovine and avian of <2 mm was regarded as a negative reactor; 3–4 mm suspect; an increase of >5 mm was regarded as a positive bovine reactor.

### 2.4.3. Confirmatory interferon-gamma release assay

The interferon-gamma (IFN-γ) release assay was performed on heparinized blood samples collected from CIST-suspect and -positive reactors as per the Bovigam™ (Thermo Fisher Scientific, United States) standard operating procedure at the Tuberculosis Laboratory at Onderstepoort Veterinary Institute (OVI), within 6 h after collection. Briefly, blood samples were stimulated with bovine PPD, avian PPD, fortuitum PPD (OBP, Pretoria), and pokeweed mitogen (OBP, Pretoria) positive control and incubated at ±37°C for 24 h. Plasma was harvested, and interferon-gamma release was detected per the manufacturer's instructions (Bovigam™).

The release of IFN-γ from stimulated blood was detected using a BovigamTM test kit per the manufacturer's instructions. Steps requiring plate washing were done using a 96-well plate washer (BioTek ELx50, United States), and the optical density (O.D.) of the samples was measured at 450 nm using a plate reader (BioTek Elx800, United States). Whole blood stimulated with pokeweed mitogen was used as a positive control, and unstimulated blood was used as a negative control. The O.D. values for the plasma stimulated with bovine PPD, avian PPD, fortuitum PPD, and pokeweed mitogen were recorded as O.D.bov, O.D.av., O.D.fort, and O.D.pwh, respectively. Unstimulated blood was recorded as O.D.neg. Animals were considered bTB positive when (O.D.bov - O.D.av. >2 and O.D.fort - O.D.neg ≤0.15). Animals were classified as avian reactors when O.D.av. > (O.D.bov + 0.1 × O.D.bov). Animals demonstrating an immune response to bovine PPD and fortuitum PPD were classified as multiple reactors if (O.D.bov – O.D.av. <0.2 and O.D.fort – O.D.neg >0.15). Animals demonstrating an equivalent immune response to both bovine PPD and avian PPD were classified as equal reactors (O.D.bov + 0.1 × O.D.bov) > O.D.av. > (O.D.bov – 0.1 × O.D.bov). The test was considered valid if the O.D. value of the blood stimulated with pokeweed mitogen (O.D.pwh) was >0.5.

All samples stimulated with bovine PPD were initially screened to determine any positive reactors. Any sample with an O.D. of ≥0.38 was regarded as positive, as Michel (2008) described. All positive reactors were subject to re-testing with the inclusion of avian PPD, fortuitum PPD, and controls.

### 2.4.4. Molecular characterization

From DALRRD's biobank, previous samples were obtained from bTB-positive cattle from the same study farms and were characterized using Mycobacterial interspersed repetitive-unit-variable number tandem repeat (MIRU-VNTR). The typing was performed using the 24 MIRU-VNTR typing kit supplied by Quadruplex versions (GenoScreen, France), according to the manufacturer's guidelines by the Tuberculosis Laboratory at OVI, Pretoria, South Africa. The MIRU-VNTR profiles were reported as numbers corresponding to the number of alleles at each locus and were entered in an excel sheet. These numerical patterns were then analyzed using the MIRU-VNTRplus database.[1]

## 2.5. Tuberculosis test in animal products

### 2.5.1. Milk culture

Milk was collected from all CIST-suspected and positive female animals. All samples were transported and processed for culture at the Tuberculosis Laboratory at OVI, Pretoria, South Africa. Milk samples were decontaminated using 1% cetylpyridinium chloride to achieve a final volume of 150 mL and incubated at 20°C ± 2°C for 1 week in the dark. After that, samples were centrifuged at 3500 ×*g* for 30 min, the supernatant discarded, and the remaining pellet was inoculated onto 4X Lowenstein Jensen (LJ) - pyruvate and 2X LJ- glycerol media. Incubation followed at 37°C ± 2°C for 10 weeks.

## 2.6. Tuberculosis test in humans

### 2.6.1. Sputum decontamination and culture

The 26 sputum samples collected were decontaminated using a BD BBL™ MycoPrep™ kit per the manufacturer's instructions. Following this, a BD BACTEC™ MGIT™ 960 Supplement Kit was required for selective growth, containing both a growth supplement and an antibiotic mixture. Samples were cultured within a BD BACTEC™ MGIT™ 960 Mycobacteria Culture System at ±37°C for up to 42 days. Positive cultures were initially screened using a modified Ziehl–Neelsen staining technique to determine phenotypic characteristics.

### 2.6.2. DNA extraction and line probe assay

DNA was extracted from positive cultures using a GenoLyse® DNA Extraction kit from Hain Lifescience according to instructions.

Per the manufacturer's instructions, *M. tuberculosis* was amplified and genotyped using a GenoType Mycobacterium CM kit. Amplicon hybridization was performed using a GenoType

1 www.miru-vntrplus.org

Mycobacterium CM VER 2.0 kit (Hain Lifescience) in an automated GT–Blot 48 (Hain Lifescience) hybridization apparatus. Visible hybridization bands on the DNA strips were compared to a reference key to differentiate and speciate between the *M. tuberculosis* complex and 27 clinically relevant Nontuberculous Mycobacteria (NTM).

## 2.7. Brucellosis test in animals

A total of 1862 whole blood samples from the communal farm and commercial farms were collected from the tail vein of cattle. This included 33, 117, 449, and 1194 cattle from commercial farms A, B, C, and D, respectively. In the Maokeng community, kraal 69 cattle were screened for BB. These samples were sent to OVI or Grahamstown Veterinary Laboratory for brucellosis screening. An initial rose Bengal test (RBT) was performed on all samples, and confirmatory testing was performed on positive reactors using a complement fixation test (CFT).

## 2.8. Serological testing of human samples for antibodies against *Brucella* sp., *Leptospira* sp., and hantaviruses

All serum samples were screened for brucella IgG-specific antibodies using a commercially available indirect ELISA (Vircell; Granada, Spain), and steps were carried out per the manufacturer's instructions. Optical density was measured at a wavelength of 450 nm (with a reference read at 630 nm) using a BioTek® 800TS™ Absorbance Reader (Winooski, United States). The mean O.D. value was calculated for the cut-off serum provided, and the antibody index was calculated as follows: (sample O.D./cut-off serum mean O.D.) × 10. An index of <9 was considered negative, 9–11 equivocal, and >11 positive. All results that returned as equivocal were re-tested.

*Leptospira*-specific antibodies were detected using a commercially available Panbio IgM ELISA (Windsor, Australia), according to the manufacturer's instructions. Optical density values were measured at a 450 nm wavelength with a reference filter at 630 nm. The cut-off value was determined by calculating the average absorbance of the calibrators tested in triplicate, multiplied by the calibrator factor (batch specific). Results were calculated as "Panbio units": sample absorbance/cut-off value. A result of <0.9, 0.9 to 1.1, and >1.1 was defined as negative, equivocal, or positive, respectively. All samples with an equivocal result were re-tested.

A commercially available EUROIMMUN Anti-Hanta Virus Pool 1 "Eurasia" ELISA (Lübeck, Germany) was used to detect hantavirus-specific IgG antibodies. This *in vitro* assay can detect human IgG antibodies against old-world hantavirus strains (Hantaan, Dobrava, and Puumala), and the procedure was carried out according to the manufacturer's instructions. Results were determined semi-quantitatively. The ratio of the test sample to the provided calibrator was determined as follows: absorbance of the serum sample/absorbance of calibrator two (20 RU/ml). A ratio of <0.8 was considered negative, ≥0.8 to <1.1 equivocal, and ≥1.1 positive. Equivocal samples were re-tested.

## 2.9. Occupational and environmental zoonotic risk factors

Demographical, occupational information, food preparation practices, and risk factors (e.g., livestock exposure, source of livestock food products, and any reports of illness after a participant was directly exposed to animal tissue/fluids) were obtained through a questionnaire previously used (Vawda et al., 2018).

## 2.10. Statistical analysis

Database establishment and the necessary manipulation of data were done in Excel® 2016. Due to skewed distributions, descriptive statistics were calculated, namely frequencies, percentages for categorical variables, medians, and quartiles for numerical variables. Associations between categorical variables and laboratory outcomes were assessed using chi-squared or Fisher's exact test in the case of sparse data. Differences between laboratory outcome groups regarding numerical variables were assessed using Mann–Whitney tests. All statistical analyses were performed by the University of the Free State Department of Biostatistics using SAS Version 9.4.

## 2.11. Ethical considerations

Ethical approval was obtained from the University of the Free Health Sciences Research Ethics Committee (UFS-HSD2019/1075/270801), Animal Research Ethics Committee (UFS-AED2019/0111), and Environmental & Biosafety Research Ethics Committee (UFS-ESD2019/0086). Furthermore, permission was obtained from the Department of Agriculture, Land Reform and Rural Development (DALRRD) before any animal testing was conducted. Verbal consent from farm owners was obtained for the collection of animal samples. Signed informed consent was obtained from volunteers before the samples were collected.

# 3. Results

## 3.1. Tuberculosis test in animals

### 3.1.1. Single and comparative intradermal skin test

A total of 321 cattle were screened for bTB using SIST and CIST, including 71/321 (22.1%) from the Maokeng community kraal, 33/321 (10.3%) from farm A, 126/321 (39.3%) from farm B and 91/321 (28.3%) from farm C. bTB results were read 72 h after inoculation. bTB results were available for 301/321 (93.8%). The Maokeng community kraal had the least number of animals that returned for bTB results ($n = 51/71$). Based on the SIST results, 3/51 (5.9%) cattle were suspect reactors in the Maokeng community, and 4/33 (12.1%) in farm A (Table 1). No positive bTB results with ≥20 were detected.

Based on the bTB confirmatory test, CIST, the four SIST-suspected positive animals from farm A were negative using CIST. In contrast, CIST results indicated that 8/126 (6.3%) cattle were positive reactors and 8/126 (6.3%) were suspect reactors on farm B. No CIST was

TABLE 1 Results of the single intradermal skin test and comparative intradermal skin test in cattle from the Moqhaka and Ngwathe municipality regions.

| | | Community | Commercial dairy | | Commercial beef |
|---|---|---|---|---|---|
| | | Maokeng | Farm A | Farm B | Farm C |
| No. of cattle screened with SIST | | 71 | 33 | N/A | 91 |
| No. of cattle returned after 72h for result readings | | 51 | 33 | N/A | 91 |
| SIST screening results | Positive | 0 | 0 | N/A | 0 |
| | Suspect | 3 | 4 | N/A | 0 |
| | Negative | 48 | 29 | N/A | 91 |
| No. of cattle screened with CIST | | N/A | 33 | 126 | N/A |
| No. of cattle returned after 72h for result readings | | N/A | 33 | 126 | N/A |
| CIST screening results | Positive | N/A | 0 | 8 | N/A |
| | Negative | N/A | 33 | 110 | N/A |
| | Suspect | N/A | 0 | 8 | N/A |

*Farm D was not scheduled for bTB screening. SIST, single intradermal skin test; CIST, comparative intradermal skin test. N/A represents not applicable as testing was not done.

TABLE 2 Interferon-gamma release assay and milk culture result from cattle with positive and suspect CIST reactions.

| Animal status based on CIST result | CIST skin reaction increase (mm) | External characteristics of injection site | IFN-γ results | Milk culture results |
|---|---|---|---|---|
| Positive | 10.4 | A, skin condition | Positive | Negative |
| Positive | 6.7 | C | Negative | Negative |
| Positive | 5.6 | C | Negative | Negative |
| Positive | 5.4 | C | Negative | Negative |
| Positive | 4.8 | C, mild D | Positive | Negative |
| Positive | 4.7 | C | Negative | Negative |
| Positive | 4.6 | C | Negative | Negative |
| Suspect | 3.9 | C | Negative | Negative |
| Suspect | 3.9 | C | AV | Negative |
| Suspect | 3.8 | C | Negative | Negative |
| Suspect | 3.7 | F | Negative | Negative |
| Suspect | 3.6 | C | Negative | Negative |
| Suspect | 3.3 | C | Negative | Negative |

C, circumscribed; A, adhesions, D, diffuse; F, flat; AV, avian reactor.

performed on samples from animals in the Maokeng community due to the animals not being available for the three-month follow-up due to covid restrictions.

The IFN-γ release assay was scheduled to be performed on all 16 CIST-suspect and -positive animals from farm B. Of the 16 positive/suspect animals, whole blood was collected from 13/16 (81%). Two animals died during the three-month waiting period, and the third had hypocalcemia when sampling was conducted (no sample was available). Therefore, the IFN-γ assay was performed on 7/8 CIST-positive and 6/8 -suspect animals. Three of the 13 cattle had a positive IFN-γ result, two bovine reactors, and one avian reactor. The remaining ten samples were negative (Table 2). Subsequently, all milk samples collected were culture-negative (Figure 2).

### 3.1.2. Confirmatory gamma-interferon assay

#### 3.1.2.1. Molecular characterization

**3.1.2.1.1. Tuberculosis test in humans**

*Mycobacterium bovis* or MTBC species were not detected in the 26 human sputum samples that were tested. Seven of the 26 samples (27%) were flagged as culture positive. *Nocardia* sp. was detected in 2/7 (28.6%) samples, *Mycobacterium intracellulare* in 2/7 (28.6%) samples, and 3/7 (42.8%) samples were identified as other *Mycobacterium* sp. excluding MTBC and the 27 clinically significant NTMs (Table 3).

## 3.2. *Brucella* test in animals

A total of 1862 cattle were available for *Brucella* sp. using the RBT (Table 4). However, three samples were hemolysed and not included. A total of 52/1859 (2.8%) cattle had a positive RBT result and were subjected to confirmatory testing using the CFT. From these results, 19/52 (36.5%), 6/52 (11.5%), and 27/52 (51.9%) were confirmed CFT positive, suspect, and negative, respectively.

## 3.3. Serological testing of human samples for antibodies against *Brucella* sp., *Leptospira* sp., and hantaviruses

A total of 327 human serum samples were screened for IgG antibodies against *Brucella* sp. and hantaviruses and IgM antibodies against *Leptospira* sp. For each assay, the results were normalized by calculating the ratio for each sample to that of the cut-off control (according to manufacturers' instructions). Ratio values calculated for each sample in all three assays (*Brucella* sp., *Leptospira* sp., and hantavirus) were plotted (Figures 3A–C).

10.3389/fmicb.2023.1196044

| Sample Id | MIRU 04 580 | MIRU 26 2996 | MIRU 40 0802 | MIRU 16 1644 | MIRU 24 2387 | MIRU 10 960 | MIRU 31 3192/ETRE | VNTR 42 424 | VNTR 43 577/ETRC | ETRA 2165 | VNTR 47 2401 | VNTR 52 3690 | VNTR 53 4156 | QUB 11b 2163b | VNTR 1955 | QUB 26 4052 | MIRU 02 0154 | MIRU 23 2531 | MIRU 39 4348 | MIRU 20 2059 | MIRU27 3007 | VNTR 46 2347 | VNTR 48ETR B - 2461 | VNTRR 49 3171 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3.5 | 4 | 2 | 2 | 3 | 6 | 6 | 3 | 5 | - | 4 | 9 | 1 | - | 3 | 3 | - | 4 | 2 | 2 | 1 | 3 | 5 | 3 |

FIGURE 2
Mycobacterial interspersed repetitive-unit-variable number tandem repeat (MIRU-VNTR) typing of *Mycobacterium tuberculosis* isolated from a previously bTB CIST-positive cattle from farm B.

**TABLE 3** Sputum culture-positive results collected from farm workers and herd status.

| Sample no. | Sex | Age | Herd status | Species |
|---|---|---|---|---|
| **Maokeng community kraal** | | | | |
| 1 | Male | 52 | Suspect | *Nocardia* sp. |
| 2 | Male | 23 | Suspect | *Mycobacterium intracellulare* |
| 3 | Male | 74 | Suspect | *Mycobacterium intracellulare* |
| 5 | Female | 57 | Suspect | *Mycobacterium* sp. |
| **Commercial dairy farm B** | | | | |
| 23 | Female | 30 | Positive | *Mycobacterium* sp. |
| **Commercial beef farm C** | | | | |
| 21 | Male | 27 | Negative | *Mycobacterium* sp. |
| 24 | Male | 25 | Negative | *Nocardia* sp. |

Results obtained from the *Brucella* sp. IgG, *Leptospira* sp. IgM and hantavirus IgG ELISA for each occupational group are shown in Table 5. *Brucella* IgG-positive reactors were detected in all occupations. Specific to group A (the 26 farm workers), *Brucella* IgG antibodies were detected in three Maokeng community farmers and one farm B worker (Table 3). *Leptospira* sp. IgM and Hantavirus IgG antibodies were mainly detected in abattoir workers and stable groomsmen.

## 3.4. Occupational and environmental zoonotic risk factors

*P*-value analysis of the *Brucella* IgG positive results showed that age (value of $p = 0.0008$), veterinary work (value of $p = 0.0006$), and laboratory work (value of $p = 0.031$) were all significant risk factors. Based on the participant's response to the questionnaire, illness post-exposure to animal tissue/blood (value of $p = 0.029$) was statistical significance to *Brucella* IgG seropositivity.

The maximum, minimum, and mean age for all Hantavirus IgG-positive participants was 65, 19, and 39, respectively. Statistical analysis of Hantavirus IgG showed no significance (value of $p < 0.05$) to any risk factor variables.

Analysis conducted on the *Leptospira* seropositive IgM results depicted abattoir work or informal slaughtering (value of $p = 0.024$) as the only significant risk factors.

# 4. Discussion

This study aimed to investigate the prevalence of *M. bovis* and *Brucella* sp. in cattle and farm workers in two farming communities (communal and commercial) and their associated risk factors. In addition, this study documents occupational and environmental exposure to *Brucella* sp., *Leptospira* sp., and hantaviruses across the Free State province, South Africa.

Based on the available data and confirmatory IFN-γ assay and skin tests, the cattle bTB prevalence detected in this study was 0.7% (two animals), all originating from a commercial dairy farm B (Table 1). These findings are lower than reports from other sub-Saharan countries, 6.2% in Algeria, 7.4% in Sudan, and ± 27% in Ethiopia (Ameni et al., 2011). Our results demonstrate the potentially effective control schemes in lowering bTB transmission in the study site. In South Africa, the bTB eradication and control scheme was implemented in 1969, following the 'test and slaughter' approach, due to the economic importance of the disease (Michel et al., 2006). The approach has been met with great success and led to a substantial decrease in bTB in cattle within the commercial sector, from a prevalence of 11.8% in 1971 to 0.39% in 1995 (Arnot and Michel, 2020). Another explanation for the lower incidence rate could be an absent reservoir host, such as buffalo or other wildlife species neighboring the study area. Spill-over of bTB from wildlife to neighboring livestock does reportedly occur at the wildlife-livestock interface in South Africa (Musoke et al., 2015).

Unfortunately, neither positive animals were slaughtered to inspect for visible lesions and culture. Therefore, no differentiation could be made between *M. bovis* and *M. tuberculosis* or any other member of the MTBC. However, on farm B, in 2018, an animal had a positive CIST result and was subsequently slaughtered. No visible lesions were detected (personal communication from the veterinarian). Nonetheless, lymph node tissue was sent for culture, and *M. tuberculosis* was confirmed (Figure 2). Therefore, the possibility arises that both animals may be infected with *M. tuberculosis* based on the farms' history. Both animals were first-time reactors, having tested negative with the CIST 8 months before the positive result.

Conversations with the farm owner and workers revealed that in 2018 (when *M. tuberculosis* was cultured from an animal on the farm), a farm worker diagnosed with TB was present. The worker passed away at the end of 2018. Further investigations into the possibility of reverse zoonotic TB are required on farm B. Additionally, throughout this study, no cattle were introduced into the herd. Therefore, another possible explanation could be latent TB reactivation. Previous reports

TABLE 4 Livestock rose bengal test, complement fixation test, and bovine brucellosis results of cattle from Maokeng community kraal and four commercial farms linked to farmworkers IgG results.

| | No. of conclusive results | No. of RBT positive reactions (%) | No. of CFT positive reactions (%) | No. of CFT suspect reactions (%) | No. of CFT negative reactions (%) | No. of farmworkers with a positive IgG detected/No. tested (%) |
|---|---|---|---|---|---|---|
| **Community** | | | | | | |
| Maokeng Com Kraals | 69 | 8 (11.6) | 6 (8.7) | 0 | 2 (2.9) | 3/13 (23.07) |
| **Commercial (dairy)** | | | | | | |
| Farm A | 33 | 0 | N/A | N/A | N/A | N/A |
| Farm B | 117 | 0 | N/A | N/A | N/A | 1/13 (7.69) |
| **Commercial (beef)** | | | | | | |
| Farm C | 448 | 9 (2) | 0 | 4 (0.9) | 5 (1.1) | N/A |
| Farm D | 1192 | 35 (2.9) | 13 (1.1) | 2 (0.2) | 20 (1.7) | N/A |
| Total | **1 859** | **52 (2.8)** | **19 (1)** | **6 (0.3)** | **27 (1.5)** | |

N/A represents not applicable as testing was not done.

have shown that cavitation of caseous lesions can occur in cattle herds infected with bTB and is required for the bacteria to go into a state of dormancy (Van Rhijn et al., 2008). This phenomenon of reactivation is documented more frequently in humans than in cattle. However, the Australian TB eradication program reports evidence of latent bTB reactivation in cattle whereby several infected animals were detected, culled, and then years after, more infected animals were detected with no external infection source (Cassidy, 2006).

A possible zoonotic transmission source to humans was not found in commercial or community-based settings, as all sputum culture results were MTBC negative. Rather symbiotically similar *Nocardia* sp. was isolated from two farm workers (2/7), who responded in the questionnaire that they regularly consume unpasteurized milk. Based on the SIST results, one of the *Nocardia* sp. positive cases was from a farmer in the Maokeng community with a bTB suspect herd. Previous studies have confirmed the transmission of *Nocardia* sp. from cattle to humans by consuming dairy products from cattle infected with the bacteria (Wahba et al., 2011).

Limited studies have reported BB's prevalence or incidence rate in South Africa cattle populations. Previous findings have reported on the seroprevalence of BB in Gauteng, Mpumalanga (Mnisi area), and KwaZulu Natal and determined seroprevalence of 2.33, 0.88, and 1.3%, respectively, with the latter two focusing on communal cattle in municipal dip tanks (Matekwe, 2011; Chisi et al., 2014; Govindasamy et al., 2021). Moreover, introducing compulsory calf vaccinations in South Africa has considerably decreased the overall BB prevalence from approximately 10.5% in 1976 to 1.4% in 1988 (Godfroid et al., 2004). These results agree with the findings from this study, where an incidence rate of 1% was determined. Based on the confirmatory BB test and CFT assay, 1% of all cattle screened were positive. Of this, 6/69 (8.7%) cattle were CFT-positive from the Maokeng community, whereas commercial beef farm D had 13/1192 (1.1%) BB-positive animals. The BB higher seroprevalence in the Maokeng farm was expected as literature reports that in subsistence farming communities, BB almost always exceeds 5% in sub-Saharan Africa (Chisi et al., 2014). Bovine brucellosis' higher incidence rate in communal settings is likely attributed to how animals are managed and a lack of disease awareness among farmers (Cloete et al., 2019). In commercial settings, animals are raised on enclosed land where movement is restricted and controlled. In addition, in these settings, BB control measures such as mass herd vaccinations and annual testing are implemented more stringently to adhere to specific standards. However, in communal farming, grazing land is shared amongst farmers where cattle herds interact with other herds, increasing the risk of transmission (Madzingira et al., 2020).

*Brucella* sp. IgG antibody was detected in 4/26 (15.4%) farm workers, including 3/13 (23%) Maokeng community farmers and one farm B worker. The positive reactors from the Maokeng community kraal were from a farm on which BB-positive herds were identified. Two of the three farmers reported consuming unpasteurized milk regularly from their herd, and the third confirmed to have assisted his animals in parturition several times over the past years. The positive reactor from the commercial beef farm was from a BB suspect herd, as determined by the CFT results. The incidence rate of *Brucella* sp. seropositivity in the community could result from limited knowledge regarding disease prevention and transmission and the higher incidence among the herd, increasing the risk of potential exposure (Cloete et al., 2019). At the time of specimen collection, all participants appeared to be healthy. However, *Brucella* sp. can cause persistent chronic infections in humans, and a clinical form of the disease may develop due to the individual being immunocompromised (Ulu-Kilic et al., 2013). Participants were made aware of the results and advised to visit their healthcare facilities in the event they feel unwell.

A few studies have been conducted on occupational exposure to *Brucella* sp. amongst healthy individuals in South Africa. These include a survey study of zoonotic diseases contracted by 88 South Africa veterinarians (Gummow, 2003). Out of the 88 veterinarians surveyed, 56 (63.6%) contracted one or more zoonotic diseases, with 7/88 (8%) reporting illness due to brucellosis. In a study conducted on 64 dip-tank workers (people who work at dip tanks) in Bushbuckridge, Mpumalanga, an incidence rate of 0% (0/64) was determined using a Brucellacapt® assay with a reported sensitivity and specificity of 96 and 97.5%, respectively (Simpson et al., 2018). The higher seroprevalence obtained in this study might indicate a higher

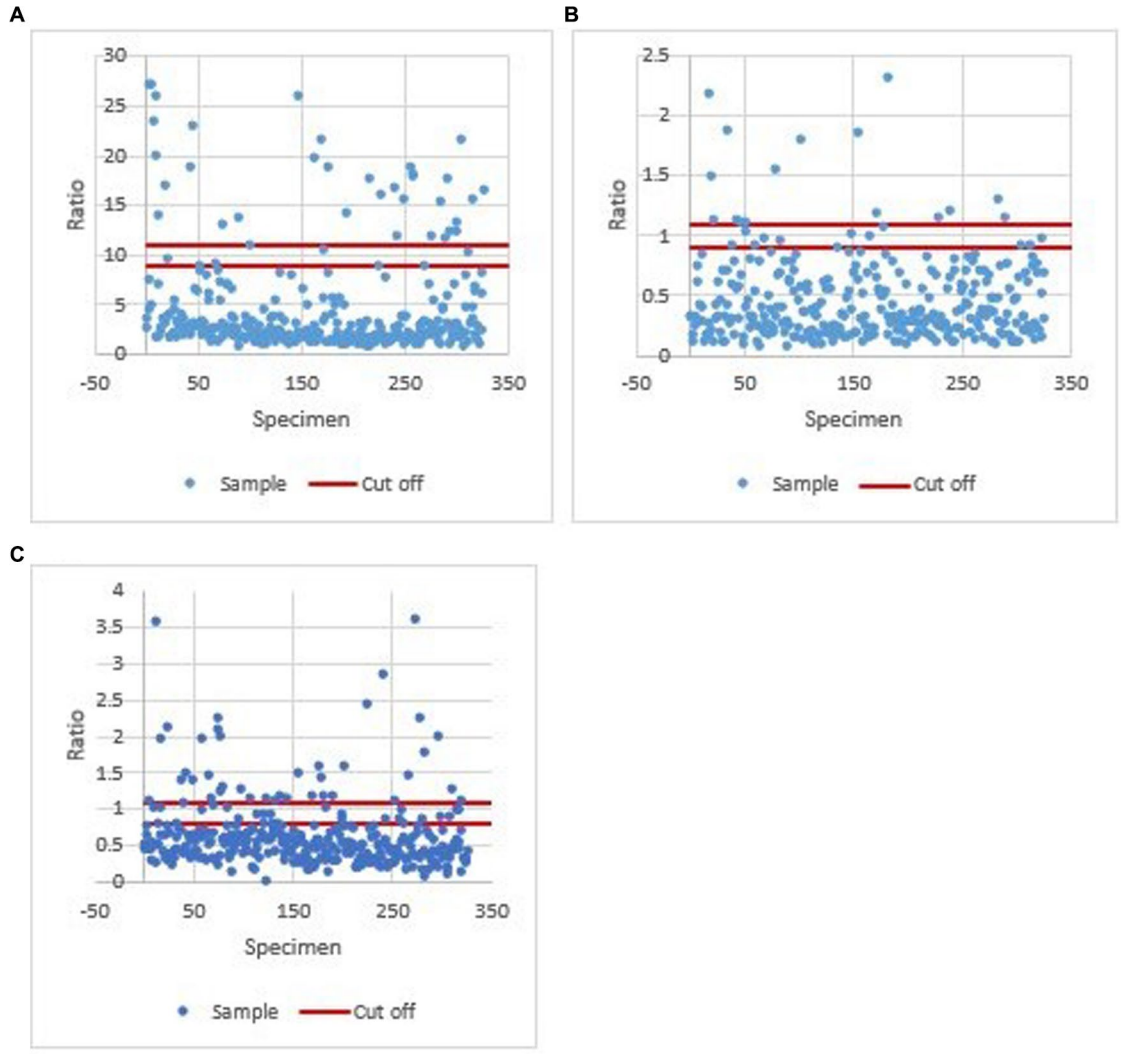


FIGURE 3
Ratio values calculated for each sample in all three assays (*Brucella* sp. *Leptospira* sp., and hantavirus). **(A)** Ratio values calculated for each sample in the *Brucella* sp. IgG ELISA assay. A ratio of <9 was considered negative, 9–11 equivocal, and>11 positive. **(B)** Ratio values calculated for each sample in the *Leptospira* sp. IgM ELISA assay. A ratio of <0.9, 0.9 to 1.1, and >1.1 was defined as negative, equivocal, or positive, respectively. **(C)** Ratio values calculated for each sample in the hantavirus IgG ELISA assay. A value of <0.8 was negative, ≥0.8 to <1.1 equivocal, and ≥1.1 positive.

**TABLE 5** Number of positive *Brucella* sp. IgG, *Leptospira* sp. IgM and hantavirus IgG reactors per occupation group.

| Occupation | Number of positive samples, ratio of >11 or >1.1* (%) | | | |
|---|---|---|---|---|
| | Number of participants (%) | *Brucella* sp. IgG reactors (%) | *Leptospira* sp. IgM reactors (%) | Hantavirus IgG reactors (%) |
| Abattoir workers | 207 (63.3) | 20 (9.7) | 14 (6.7) | 26 (12.6) |
| Veterinarians | 12 (3.7) | 6 (50.0) | 0 (0) | 1 (8.3) |
| Stable groom | 32 (9.8) | 2 (6.3) | 2 (6.3) | 4 (12.5) |
| Recreational hunters | 46 (14.1) | 2 (4.3) | 1 (2.2) | 4 (8.7) |
| Farm workers | 28 (8.6) | 4 (14.3) | 0 (0) | 3 (10.7) |
| Laboratory workers | 2 (0.6) | 1 (50.0) | 0 | 0 (0) |
| Positive rate | | | | 26 (68.4) |
| Total (%) | 327 | 35 (10.7) | 17 (5.2) | 38 (11.6) |

*Depending on manufacturers' instructions.

disease burden in the Free State province. The higher seropositivity is likely due to the participants' occupation and recreational activities, putting them at a higher risk of contracting an infection. Based on the results of this study, two high-risk occupational groups were identified as having a higher *Brucella* IgG seropositive rate compared to the other occupations: laboratory workers ($p = 0.031$) and veterinarians ($p = 0.0006$; Table 5). However, these findings were based on only two samples, and further research is required.

In South Africa, there are sporadic cases of leptospirosis reported by the National Institute for Communicable diseases (NICD). This report identified abattoir workers as a high-risk occupational group for *Leptospira* sp. in the Free State, indicating that *Leptospira* sp. are circulating within the Free State province. The populations screened had possible exposure from various sources, including horses, livestock, and rodent populations as rural residents, hence although not possible to identify the source of infection, the results justify additional investigations to determine the prevalence of *Leptospira* sp. in livestock, domestic animals, rodents and wildlife and to identify the serovars circulating for diagnostic purposes. Abattoirs should also enforce more strenuous preventative measures to reduce infections.

Hantaviruses are usually transmitted from environmental exposure, and the presence of hantaviruses in South Africa has, to date, not been confirmed. The hantavirus IgG seroprevalence data are similar to data obtained in other studies (Klempa et al., 2013, Witkowski et al., 2014). Although no conclusions can be drawn from this limited study in the absence of confirmatory assays such as neutralization tests, more extensive serosurveillance studies are justified to provide more information regarding the presence of hantaviruses in the country. Hantaviruses have not previously been associated with disease in Africa however, medically significant rodent borne hantaviruses belonging to various genera circulate in Asia, Europe and North and South America. The presence of potential rodent hosts in Africa suggest that they are likely to occur and hence warrant investigation as a potential zoonotic pathogen among at risk populations.

In conclusion, South Africa has a large proportion of the human population dependent on animals for their livelihood, whether as a source of food, trade, companionship, or services, and the importance of a One Health approach to zoonotic pathogens should be encouraged. Therefore, more emphasis should be placed on populations at higher risk of contracting zoonotic infections regarding epidemiological investigations. Identifying high-risk populations for different zoonotic diseases across other geographical regions will ultimately aid in implementing effective preventative measures and assist clinicians in diagnosing undifferentiated febrile illness patients.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

CW, FB, and JM created the study concept and design. CW, FB, NH, WZ, TA, and JM performed data collection and analysis. NH drafted the first manuscript. All authors contributed to the article and approved the submitted version.

## Funding

This work was supported by the Department of Science and Technology, National Research Foundation (NRF) Thuthuka research grant [UID107432 to JM], South African Research Chairs Initiative (SARChI) Vector-borne and zoonotic pathogens research [U98346 to FB].

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## References

Ameni, G., Vordermeier, M., Firdessa, R., Aseffa, A., Hewinson, G., Gordon, S. V., et al. (2011). *Mycobacterium tuberculosis* infection in grazing cattle in central Ethiopia. *Vet. J.* 188, 359–361. doi: 10.1016/j.tvjl.2010.05.005

Arnot, L. F., and Michel, A. (2020). Challenges for controlling bovine tuberculosis in South Africa. *Onderstepoort J. Vet. Res.* 87, e1–e8. doi: 10.4102/ojvr.v87i1.1690

Cassidy, J. P. (2006). The pathogenesis and pathology of bovine tuberculosis with insights from studies of tuberculosis in humans and laboratory animal models. *Vet. Microbiol.* 112, 151–161. doi: 10.1016/j.vetmic.2005.11.031

Chisi, S. L., Perret, K., Marageni, Y., Naidoo, P., Zulu, G., and Mothloung, T. (2014). The KwaZulu Natal Provincial Bovine Brucellosis Sero-Prevalence Survey in Communal Cattle. Proceedings of the 12th Annual Congress of the South African Society for Veterinary Epidemiology and Preventative Medicine, 20-22 August 2013.

Cloete, A., Gerstenberg, C., Mayet, N., and Tempia, S. (2019). Brucellosis knowledge, attitudes and practices of a South African communal cattle keeper group. *Onderstepoort J. Vet. Res.* 86, e1–e10. doi: 10.4102/ojvr.v86i1.1671

Department of Agriculture, Forestry and Fisheries of SA (DAFF) (2016). Bovine Tuberculosis Manual, accessed 16 January 2019. Available at: https://www.nda.agric.za/vetweb/pamphlets&Information/Policy/Tuberculosis%20in%20Cattle%20Interim%20Manual%20for%20the%20Veterinarian%20&%20AHT%20-%20Sept2….pdf

Gizamba, J. M., Paul, L., Dlamini, S., and Odayar, J. (2022). Incidence and distribution of human leptospirosis in the Western Cape Province, South Africa, (2010-2019): a retrospective study. *medRxiv*. doi: 10.1101/2022.01.05.22268774

Godfroid, J., Garin-Bastuji, B., Blasco, J. M., Thomson, J., and Thoen, C. O. (2004). "*Brucella melitensis* infection" in *Infectious Diseases of Livestock*. eds. J. A. W. Coetzer, G. R. Thomson and R. C. Tustin, Oxford University Press. 1535–1541.

Govindasamy, K. (2020). Human brucellosis in South Africa: A review for medical practitioners. *S. Afr. Med. J.* 110, 646–651. doi: 10.7196/SAMJ.2020.v110i7.14538

Govindasamy, K., Thompson, P. N., Harris, B. N., Rossouw, J., Abernethy, D. A., and Etter, E. M. C. (2021). Bovine Brucellosis in Gauteng, South Africa: Seroprevalence

amongst Cattle Handlers and Variables Associated with Seropositive Cattle Herds, 2014–2016. *Pathogens*, 10: 1547.

Gummow, B. (2003). A survey of zoonotic diseases contracted by South African veterinarians. *J. S. Afr. Vet. Assoc.* 74, 72–76. doi: 10.4102/jsava.v74i3.514

Haake, D. A., and Levett, P. N. (2015). Leptospirosis in humans. *Curr Top Microbiol Immunol* 387, 65–97. doi: 10.1007/978-3-662-45059-8_5

Hlokwe, T. M., Van Helden, P., and Michel, A. L. (2014). Evidence of increasing intra and inter-species transmission of *Mycobacterium bovis* in South Africa: Are we losing the battle? *Prev. Vet. Med.* 115, 10–17. doi: 10.1016/j.prevetmed.2014.03.011

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., et al. (2008). Global trends in emerging infectious diseases. *Nature* 451, 990–993. doi: 10.1038/nature06536

Klempa, B., Koulemou, K., Auste, B., Emmerich, P., Thomé-Bolduan, C., Günther, S., et al. (2013). Seroepidemiological study reveals regional co-occurrence of Lassa-and Hantavirus antibodies in Upper Guinea, West Africa. *Tropical Med. Int. Health* 18, 366–371. doi: 10.1111/tmi.12045

Lai, S., Zhou, H., Xiong, W., Gilbert, M., Huang, Z., Yu, J., et al. (2017). Changing epidemiology of human brucellosis, China, 1955–2014. *Emerg. Infect. Dis.* 23, 184–194. doi: 10.3201/eid2302.151710

LeDuc, J. W., and Barry, M. A. (2004). SARS, the First Pandemic of the 21st Century. *Emerg. Infect. Dis.* 10:e26. doi: 10.3201/eid1011.040797

Madzingira, O., Fasina, F. O., Kandiwa, E., Musilika-Shilongo, A., Chitate, F., and van Heerden, H. (2020). A retrospective sero-epidemiological survey of bovine brucellosis on commercial and communal farming systems in Namibia from 2004 to 2018. *Trop. Anim. Health Prod.* 52, 3099–3107. doi: 10.1007/s11250-020-02332-4

Matekwe, N. (2011). Seroprevalence of Brucella Abortus in Cattle at Communal Diptanks in the Mnisi Area, Mpumalanga, South Africa (Doctoral Dissertation, University of Pretoria).

Michel, A. L., Bengis, R. G., Keet, D. F., Hofmeyr, M., De Klerk, L. M., Cross, P. C., et al. (2006). Wildlife tuberculosis in South African conservation areas: implications and challenges. *Vet. Microbiol.* 112, 91–100. doi: 10.1016/j.vetmic.2005.11.035

Michel, A. L. (2008). Mycobacterium fortuitum infection interference with Mycobacterium bovis diagnostics: natural infection cases and a pilot experimental infection. *J. Vet. Diagn.* 20, 501–503.

Moolla, N., Adler, D., Blumberg, L., Glass, A., Grobbelaar, A., le Roux, C., et al. (2022). First report of an imported case of haemorrhagic fever with renal syndrome in South Africa. *S. Afr. Med. J.* 112, 519–525. doi: 10.7196/SAMJ.2022.v112i8.16540

Müller, B., Dürr, S., Alonso, S., Hattendorf, J., Laisse, C. J., Parsons, S. D., et al. (2013). Zoonotic *Mycobacterium bovis*-induced tuberculosis in humans. *Emerg. Infect. Dis.* 19, 899–908. doi: 10.3201/eid1906.120543

Musoke, J., Hlokwe, T., Marcotty, T., Du Plessis, B. J., and Michel, A. L. (2015). Spill-over of *Mycobacterium bovis* from wildlife to livestock, South Africa. *Emerg. Infect. Dis.* 21, 448–451. doi: 10.3201/eid2103.131690

Naidoo, K., Moseley, M., McCarthy, K., Chingonzoh, R., Lawrence, C., Setshedi, G. M., et al. (2020). Fatal rodent-borne leptospirosis in prison inmates, South Africa, 2015. *Emerg. Infect. Dis.* 26, 1033–1035. doi: 10.3201/eid2605.191132

Rahman, A. K. M. A., Saegerman, C., Berkvens, D., Melzer, F., Neubauer, H., Fretin, D., et al. (2017). *Brucella abortus* is prevalent in both humans and animals in Bangladesh. *Zoonoses Public Health* 64, 394–399. doi: 10.1111/zph.12344

Salyer, S. J., Silver, R., Simone, K., and Behravesh, C. B. (2017). Prioritizing zoonoses for global health capacity building—themes from One Health zoonotic disease workshops in 7 countries, 2014–2016. *Emerg. Infect. Dis.* 23, S55–S64. doi: 10.3201/eid2313.170418

Schrire, L. (1962). Human brucellosis in South Africa. *S. Afr. Med. J.* 36, 342–349.

Simpson, G. J., Quan, V., Frean, J., Knobel, D. L., Rossouw, J., Weyer, J., et al. (2018). Prevalence of selected zoonotic diseases and risk factors at a human-wildlife-livestock interface in Mpumalanga Province, South Africa. *Vector Borne Zoonotic Dis.* 18, 303–310. doi: 10.1089/vbz.2017.2158

Tilahun, Z., Reta, D., and Simenew, K. (2013). Global epidemiological overview of leptospirosis. *Int. J. Microbiol. Res* 4, 5–9. doi: 10.5829/idosi.ijmr.2013.4.1.7134

Ulu-Kilic, A., Metan, G., and Alp, E. (2013). Clinical presentations and diagnosis of brucellosis. *Recent Pat. Antiinfect. Drug Discov.* 8, 34–41. doi: 10.2174/1574891X11308010007

Van Rhijn, I., Godfroid, J., Michel, A., and Rutten, V. (2008). Bovine tuberculosis as a model for human tuberculosis: advantages over small animal models. *Microbes Infect.* 10, 711–715. doi: 10.1016/j.micinf.2008.04.005

Vawda, S., Goedhals, D., Bester, P. A., and Burt, F. (2018). Seroepidemiologic survey of Crimean-Congo hemorrhagic fever virus in selected risk groups, South Africa. *Emerg. Infect. Dis.* 24, 1360–1363. doi: 10.3201/eid2407.172096

Wahba, N. M., Elnisr, N. A., Saad, N. M., Nasr, S. M., and Ali, W. M. (2011). Incidence of Nocardia species in raw milk collected from different localities of Assiut City of Egypt. *Vet. World.* 4:201. doi: 10.5455/vetworld.2011.201-204

Wang, H., Naghavi, M., Allen, C., Barber, R. M., Bhutta, Z. A., Carter, A., et al. (2016). Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 388, 1459–1544. doi: 10.1016/S0140-6736(16)31012-1

Witkowski, P. T., Klempa, B., Ithete, N. L., Auste, B., Mfune, J. K., Hoveka, J., et al. (2014). Hantaviruses in Africa. *Virus Res.* 187, 34–42. doi: 10.1016/j.virusres.2013.12.039

World Health Organization (2017). Roadmap for zoonotic tuberculosis.

# Frontiers in Microbiology

**Explores the habitable world and the potential of microbial life**

The largest and most cited microbiology journal which advances our understanding of the role microbes play in addressing global challenges such as healthcare, food security, and climate change.

---

## Discover the latest Research Topics

See more →


**Frontiers**

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

**Contact us**

+41 (0)21 510 17 00
frontiersin.org/about/contact