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Vector-borne diseases and consequences on human health: A multidisciplinary approach

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Vector-borne diseases and consequences on human health: A multidisciplinary approach

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Editorial: Vector-borne diseases and consequences on human health: a multidisciplinary approach

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KEYWORDS

vector-borne diseases, pathogens, hosts, vectors, climate changes, susceptible human populations

Editorial on the Research Topic
Vector-borne diseases and consequences on human health: a multidisciplinary approach

Emerging and re-emerging vector-borne diseases are among the major public health concerns across the world. They are not directly transmissible among humans in most cases, but are mainly spread under suitable conditions as a result of the interaction of vectors, hosts, climate/environment, pathogens, and vulnerable human populations. Their impact has a significant toll on economies and restricts both rural and urban development. Burden is the highest in tropical and subtropical areas, and they disproportionately affect the poorest population.

This editorial summarizes collections on the Research Topic "*Vector-borne diseases and consequences on human health: a multidisciplinary approach*" which invites the latest research findings on these (re-) emerging diseases. Eleven articles from researchers across the globe (China, India, Japan, Iran, Malaysia, Argentina, Ecuador, Canada, and Sweden) were published.

The effect of global warming on public health and, more particularly, on the seasonal and geographical expansion of pathogens is still increasing due to the propagation of vectors. A systematic literature review and meta-analysis was conducted into the impacts of temperature on dengue vectors and authors revealed various data on the life cycle of *Aedes* mosquitoes at diverse temperatures which may be used for effective dengue management in Malaysia (Halim et al.). In China, the boosting regression tree model was

used to explore the intrinsic relationship of *Scrub typhus* (a bacterial disease transmitted by mites) with meteorological, environmental and social factors. Many counties in the Sichuan Province were evaluated to be receptive to *Scrub typhus*. Their findings may achieve the priorities of disease control and prevention to manage precise and effective allocation of resources (Zhang et al.). In the same context, a literature review focused on the use of risk maps to model distributions of *Borrelia burgdorferi* and its vector, the blacklegged tick *Ixodes scapularis*, in North America to compare variables employed to predict these spatial models (Fellin et al.). Environmental variables were classified to be the most widely used in risk spatial models, specifically temperature. Limited anthropic variables were considered, mainly in studies that predict future risk, although the purpose of these models directly or indirectly concentrate on health intervention strategies. Authors indicated that it is really hard to estimate how reliable these risk maps truly are without including human-related factors and taking into consideration these variables within risk map models.

In a grand challenges paper on vector borne diseases, many authors focused on the incidence and the spread of these (re-) emerging diseases. A countrywide geodemographic epidemiological analysis from 2011 to 2021 of severe Chagas disease (transmitted by triatomine species) in Ecuador was investigated by Vásconez-González et al.. A total of 118 patients have been hospitalized in Ecuador during this period due to Chagas disease. The overall in-hospital mortality percentage was 69.4%. The incidence rate of men was higher than women, despite women having a remarkably higher mortality rate than men which seemed to be more infected due to differences in work and sociocultural habits. Authors mentioned also the role of environmental change in the proliferation of disease-carrying vectors in previously unaffected areas. Several electronic databases were used to select articles treating malaria in pregnancy in India and its results on both mother and child (Foko and Singh). Their results showed that malaria in pregnancy was mostly due to *Plasmodium falciparum* and *P. vivax*, and rarely to *P. ovale* and *P. malariae*. The overall prevalence of malaria in pregnancy was ranging from 0.1 to 57.7% for peripheral malaria and from 0 to 29.3% for placental malaria. According to Wilkman et al., five different mosquito-borne viruses involved in human disease are well-known in Fennoscandia including Sindbis virus, Inkoo virus, Tahyna virus, Chatanga virus, and Batai virus. However, the incidence of mosquito-borne virus infections is still unknown, mainly due to underdiagnosing and lack of control efforts. Early detection of invasive viruses, would be remarkably supported by collaboration between clinicians and other key players applying a One Health approach as recommended by the authors. In the same context of incidence in vector borne diseases, Calvopiña et al. identified the first case caused by the zoonotic *Babesia bigemina* (responsible for a tick-borne disease known as babesiosis) in the Amazon region of Ecuador ensuring the existence of active transmission. The authors highlighted the need for alerting public health decision-making authorities on the emergence of this zoonosis and the need for research to determine strategies to reduce tick exposure.

Substantial progress has been made against many vector-borne diseases during the past decade. In this context, analyses of the epidemiological data during 1989–2020 indicated that Iran has made stable advance and remarkable progress over the past decade, yet persistent challenges exist to decrease the cutaneous leishmaniasis burden in the country (Sharifi et al.). Well-trained staff and experienced clinical practitioners should reinforce country-level capacity-building to support effective control strategies across the healthcare system as indicated by the authors.

Resistance to insecticides persists a major barrier not only for efficient control of malaria but also other vector-borne diseases. Fay et al. reported the first report of knockdown resistance (kdr) mutations in *Aedes aegypti* in the northeast region of Argentina. Authors demonstrate the relevance of kdr information for focused vector control interventions and public health initiatives to minimize both *Aedes aegypti* populations as well as insecticide use/misuse to reduce arboviral disease risks. In Xiamen city of China, a propagation dynamics model was used by Guo et al. to evaluate and estimate the risk of dengue fever. Authors emphasized that imported cases, community population, mosquito density and insecticide resistance have an important role in the transmission of local dengue fever. However, mosquito resistance to insecticide has been identified as the most serious variable for assessing dengue fever communication risks and applying management control systems.

The Research Topic of articles highlights some crucial advances in controlling mosquito-borne diseases, which will contribute to the development of new appropriate methods and tools in vector control. An example of such advances is the report of allose, a rare sugar, to inhibit the development of *Plasmodium* parasites in laboratory-reared *Anopheles* mosquitoes. As a first step to address the inhibition mechanism, Mizushima et al. revealed the non-involvement of the midgut microbiota in the recorded inhibition of *Plasmodium* parasites in the mosquito. This sugar may be a useful material for vector control of malaria as a "transmission-blocking sugar" in the future.

This Research Topic of articles covers ticks, mosquitoes, sand flies, mites, and triatomines bugs-borne pathogens through different continents which indicates their wide distribution and reveals the diversity of hosts and pathogens. An integrated One Health approach would promote a better understanding of the intrinsic complexity of disease transmission emphasizing interactions between human, animal, environmental health, and the importance of transdisciplinary efforts.

In conclusion, we hope that the papers collected in this Research Topic will expand the knowledge base and skills related to vector-borne diseases, particularly in the context of political, socioeconomic, environmental, and climate change factors, enabling the development of practicable solutions both at local and global scales.

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A systematic review and meta-analysis of the effects of temperature on the development and survival of the *Aedes* mosquito

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Introduction: The *Aedes* mosquito species, which are the vectors for the transmission of the dengue virus (DENV) to humans, are becoming increasingly susceptible to the formidable effects of influential factors, especially temperature. However, there are still very few studies that have systematically reviewed the existing literature. Hence, in the present study, a systematic literature review and meta-analysis was conducted into the effects of temperature on dengue vectors.

Method: Several research methodologies were incorporated into the current study, and a review was carried out using PRISMA as a guide. The publications for this study were chosen from two prominent databases, Scopus and Web of Science. All of the studies were assessed, reviewed, and evaluated independently by two reviewers. The meta-analysis tool, Review Manager (RevMan Copenhagen Version 5.4.1), was used to record the extracted data for the meta-analysis. Moran's I^2 and a funnel plot were utilized to measure heterogeneity, and publication bias was investigated. A 95% confidence interval (CI) and overall risk difference (RD) were estimated using a random-effects model.

Result and discussion: As a consequence of the search efforts, a total of 46 articles were selected for inclusion in the systematic review and meta-analysis. This review was divided into five major themes, based on a thematic analysis: (i) hatching rate, (ii) development time, (iii) longevity, (iv) survival rate, and (v) wing morphology. In addition, the development time, survival rate, and wing morphology revealed significantly higher risk differences between the maximum and minimum temperatures (RD: 0.26, 95% CI: 0.16, 0.36; $p = < 0.00001$; RD: 0.10, 95% CI: 0.05, 0.14; $p < 0.0001$; and RD: 0.07, 95%

CI: 0.02, 0.12; $p = 0.006$, respectively). This study makes several substantial contributions to the body of knowledge and to practical applications. Finally, a number of recommendations are made at the conclusion of this research for the future reference of researchers.

KEYWORDS

***Aedes*, dengue (DENV), meta-analysis, temperature, effect**

Introduction

Dengue fever (DF) is an arboviral disease that can spread rapidly through regions and nations, particularly in tropical areas, to cause mild to severe symptoms, including high fever, myalgia, arthralgia, malaise, and pain behind the eyes (1). Based on a 30-fold increase in the incidence of DF over the past five decades, dengue fever is now the most widespread re-emerging mosquito-borne disease and a national hazard. Khetarpal and Khanna (2) discovered that DF is being distributed and is expanding to 128 countries, putting 3.97 billion people at risk annually. Packierisamy et al. (3) initially identified continuous endemic instances of dengue fever (DF) that were harmful to public health in Malaysia in 1902 on Penang Island, while dengue haemorrhagic fever (DHF) was first documented by Kumarasamy (4) in 1962. In addition, since the late 1980s, when the incidence of dengue cases rose sharply, endemic outbreaks of emerging and re-emerging dengue fever (DF) cases have persisted in Malaysia despite disease prevention and control efforts by the government, non-governmental organizations (NGO), and the public.

With the *Aedes* species as the biological vector of DF, there are a number of influential factors that contribute to the incidence of dengue, such as vector density, vector survivability, socioeconomic factors, rainfall distribution, and environmental factors. Rao et al. (1) summarized these into four major factors: epidemiological, virus risk, abiotic risk, and human risk factors. Socioeconomic factors influence the level of dengue awareness of a population (5), whereas Maamor et al. (6) demonstrated that the survival of vectors as a result of their diet and the availability of breeding water may cause a DF outbreak within a 200-m radius of a residential area. In addition, Reinhold et al. (7) hypothesized that an increase in environmental temperature alters the gonotrophic cycle of mosquitoes, resulting in an increase in the rate of disease transmission over a shorter period of time. In light of the fact that everything is directly or indirectly tied to a DF epidemic, which may pose a severe public health risk, preventing, and controlling DF is a challenge.

The ecology of mosquitos is occasionally investigated as they are vectors for DF. Thus far, in Malaysia, studies have confirmed that the ecology of mosquitoes is influenced by rapid unplanned urbanization with defective water supply and solid waste management (8), while artificial light affects the reproductive activities of the Aedes mosquito by lengthening its gonotrophic cycle (9). Other essential elements of the ecology of the mosquito, as referred to by Ahmad et al. (10), include a distinct preference for human habitats and skip oviposition behavior, as well as environmental factors such as heavy rainfall and high temperatures, which are reported to have a strong correlation with the breeding of vector mosquitos and the size of the *Aedes* population (11).

When the mosquito ecology is stratified within a narrow scope, it has been demonstrated that temperature is a crucial determinant influencing the biology of mosquito vectors such as larval development and survivability (9). Shahrudin et al. (12) stated that when exposed to higher constant temperatures, *Aedes albopictus* embryos develop swiftly and their mortality increases, as verified by Rozilawati et al. (13), resulting in a decrease in mosquito fecundity and body mass. Meanwhile, the research by Wang et al. (14) on the impact of a large diurnal temperature range (DTR) on the development of the mosquito population revealed findings that contradicted the findings of many studies that linked temperature with the spread of DF. Despite the contradictory results from studies on temperature and the *Aedes* species, Yeap et al. (15) suggested that normal thermal exposure in field temperatures modifies the morphometric features of the female *Aedes aegypti*. In general, the wings of Aedes mosquitoes are shorter at higher temperatures than at lower temperatures (16), and smaller adults are associated with faster larval development at higher temperatures (17). As a result, smaller Aedes females are better able to select a breeding site and increase the rate of dengue virus infection and spread (18).

Unpredictable weather patterns are becoming more common as a result of climate change. As a result, Brady et al. (19) built a modeling framework on DF cases in relation to the factor of temperature. However, the results showed that the persistence and competence of the *A. aegypti* and *A. albopictus* with regard to the transmission of the dengue virus are not affected by temperature, but rather by the geographical area and type of species. However, temperature is often considered as the main driver that contributes to the development and survival of the *Aedes* species, and it is necessary to explain adequately the variation in temperature that affects these vectors. Temperature range considered suitable for mosquito development, and variations that can positively or negatively affect this development. To test this hypothesis, empirical data

is needed with regard to the development and survival of the mosquitos in response to various ranges of temperature. It may be challenging to collect data on such a wide scale in a single experiment or study. However, a meta-analysis of a collection of published estimates of effects with regard to diverse temperature ranges might be used to approach such a dataset. In this way, the phenotype of the effect on the *Aedes* species in response to temperature may be evaluated across a broader range of situations and geographical boundaries. With this background, a meta-analysis of data from studies on the *Aedes* species was conducted in order to summarize the effects of different temperature conditions on the hatching, length of development, survival rate, longevity, and wing morphology of the *Aedes* mosquito and to determine how they affect each other.

Materials and methods

Literature search and study selection

This section discusses the method that was used to retrieve articles in relation to the effects of temperature on dengue vectors. This method was employed to run the systematic review on two resources (Scopus and Web of Science) with eligibility and exclusion criteria, while the steps in the review process consisted of identification, screening, and eligibility as well as data abstraction and analysis.

This review was guided by the method known as PRISMA or *Preferred Reporting Items for Systematic Review and Meta-Analysis*. It is a published standard protocol for conducting a systematic literature review and meta-analysis. In general, this publication standard is used as a guide in the evaluation and examination of the quality and purity of a review with the necessary and related information provided. Moreover, PRISMA highlights a report of the review, evaluating randomized trials that can also be the basis for other types of research in reporting systematic reviews (20). On the other hand, PRISMA is also suitable for the field of environmental management since it clearly emphasizes on research questions with regard to the need for systematic reviews, and it is also a fact that PRISMA has always been used in medical studies, especially for public health, as it can be a tool to identify the inclusion and exclusion criteria for a particular study (21). Furthermore, PRISMA also extensively examines databases if a literature review is unavailable at a particular time in order to allow searching for the accurate term in relation to the effects of climatic and environmental factors on dengue vectors. In addition, the use of PRISMA enhances the coded information for involvement in future environmental management services. The results of this review were presented following the PRISMA flow diagram as in Figure 1. The protocol for this systematic review and meta-analysis was not registered.

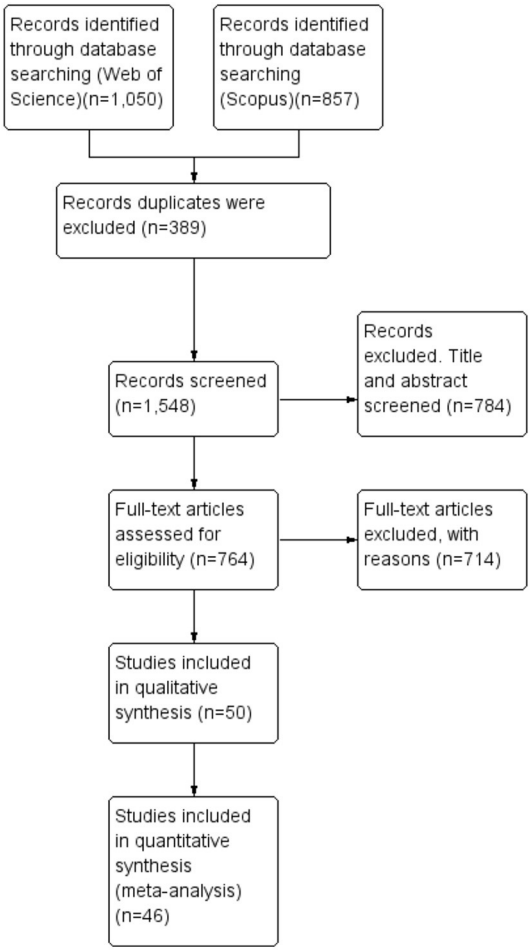

FIGURE 1
Flow diagram of the selection of studies for the review process.

Resources

The method for the present review was conducted using two main databases; (i) Scopus and (ii) Web of Science (WoS). These two databases were used as they are the leaders in systematic reviews among all the databases, and they mostly cover more than 256 fields of study or subject categories, including environmental science. The WoS database consists specifically of more than 33,000 journals and 256 subject categories related to environmental studies, social science, and interdisciplinary social science and development planning issues. It also covers over 100 years of comprehensive back files and citation data established by Clarivate Analysis and ranks them by three components, namely papers, citations, and citations per paper. The second database that was used in this review was Scopus, which is recognized as one of the largest abstract and citation databases of peer-reviewed articles with more than 22,800 journals from 5,000 publishers across the

world covering various subject categories, for instance, social science, environmental science and biological science.

Formulation of research question

In this review, the formulation of a suitable research question was developed using PICO as an assistant tool. PICO is comprised of three components, namely Population or Problem, Interest and Context. Three aspects were covered in this review based on these three components, namely effect (problem), temperature (interest) and dengue vectors (context), which were then used as a guide in the development of the main research question—What are the effects of temperature on the development and survival of the *Aedes* mosquito?

Systematic searching strategies/systematic review process

Three main steps were involved in the systematic searching process to select the relevant articles for this review, namely identification, screening and eligibility. The first stage of identification involved identifying variations of the main keywords. This was followed by the process of searching for any synonyms and related terms based on dictionaries, thesauri, encyclopedia, and relying on past studies and keywords from experts. This process was carried out to obtain more options so as to search for more articles for the review. Next, the existing keywords and search strings were enriched based on Boolean operators, phrase searching, truncations, wild cards and filed code functions on the main databases of Scopus and Web of Science (WoS), which were developed in January 2022 after all the related keywords had been fully accessed.

This review screened all the selected articles retrieved from the identification process by choosing the criteria for the selected articles. This was done automatically based on the sorting function in the database. The first step in the screening process was to remove duplicate articles. During this process, some of the articles were excluded, while the rest were screened for the second step based on several inclusion and exclusion criteria. In this review, the first criterion considered the type of publication, which included only articles with complete empirical data that had been published in a journal as a primary source rather than published in the form of a systematic review, meta-analysis, chapters in books and conference proceedings in order to ensure the quality of the review. Moreover, this review only focused on articles that were published in English to avoid any confusion in understanding. In addition, a timeline of between 2012 to 2021 was included as one of the inclusion criteria. This timeline of 10 years back was chosen since search should be as wide as possible to maximize the likelihood of capturing all relevant data and the publication related to Aedes mosquitoes rise in this period of time. As it was impossible to conduct a search of all the published articles, thus, a fixed period was determined to enable the review to be carried out (22).

All the remaining articles were prepared for the third step, namely, eligibility. During this process, the articles were presented to two experts for a quality assessment to ensure that the contents of the articles were of good quality. Most importantly, the titles, abstracts, and the main contents of the articles were examined clearly to ensure they fulfilled and matched all the inclusion criteria so as to accomplish the objective of the review. Consequently, all the remaining articles selected from the last stage were further analyzed by means of a qualitative analysis.

Quality appraisal/risk of bias assessment

In order to ensure the quality of the contents, the remaining articles from the previous process were presented to two experts for a quality assessment. The experts ranked the articles according to three indicators, namely, low, moderate and high bias. Consequently, only low and moderately biased articles were selected for the review. To determine the quality of the articles, both the experts ranked their quality based on the methodology of the article. Both the authors had to mutually agree that at least a moderate quality article was included in the review. Any argument that came up during the assessment was discussed among them before a decision was made in terms of the inclusion or exclusion criteria for further analysis.

The Joanna Briggs Institute Tool (JBI) tool 29 criteria was used to assess the quality of the articles chosen for this study. There were eight questions on the checklist, namely: (A) Were the criteria for inclusion in the sample clearly defined? (B) Were the study subjects and setting described in detail? (C) Was the exposure measured in a valid and reliable way? (D) Were objective standard criteria used for measuring the conditions? (E) Were confounding factors identified? (F) Were strategies to deal with confounding factors stated? (G) Were the outcomes measured in a valid and reliable way? and (H) Was an appropriate statistical analysis used? The questions were checked against the 46 articles, and answers were given based on the indication of (+) for "Yes" or (–) for "No." The risk of bias in the selected studies was assessed using robvis (visualization tools). Two authors separately evaluated the possibility of bias in each publication. The checklist consisted of five questions. The risk of bias was classified as "low risk," "high risk," or "unclear risk" (Figure 2A). A summary bar chart was constructed using the same dataset (Figure 2B).

Data abstraction

This review relied on an integrative review, a technique that requires the analysis and synthesis of diverse research

FIGURE 2
(A) Summary of risk of bias based on traffic light plot. **(B)** Graph of risk of bias.

designs. This means that quantitative, qualitative and mixed-mode approaches were included in this review. A good approach would be to analyse the integrative data using qualitative or mixed mode techniques so as to enable a comprehensive comparison to be made across the data sources by the authors (23). In this review, all the articles selected in the abstract, results and discussion sections were read thoroughly. The data abstraction process was conducted based on the research question. The data obtained from the reviewed studies were those that were able to answer the research question, and they were, then, summarized in the form of a table. Next, a thematic analysis was performed to determine the themes and sub-themes in order to present the patterns, counting and clustering, and to identify the similarities and associations existing in the summarized data (24). A thematic analysis was considered since it is the most applicable for synthesizing integrative or mixed research designs (25). This kind of descriptive study reduces the flexible mode data that have been merged with other techniques of data analysis (26).

The initial step in the thematic analysis was to generate the themes, and to identify the patterns that appeared in the summarized data of all reviewed articles. Any similarities between the reviewed data were pooled together under one theme. Then, the themes were scrutinized once again and other sub-themes were identified. Next, the process was continued by reviewing the efficiency of the themes. In this step, all the main themes and sub-themes that were generated were scrutinized to ensure the effectiveness and accuracy of the data representation. During this process, some themes might be excluded due to the inclusion criteria that were determined for this study. The next stage was choosing the proper names for the main themes as well as their sub-themes. Within the theme development process, this technique included the involvement of corresponding authors and co-authors in identifying the most relevant themes related to the objective of this review. The group of authors discussed any ideas, inconsistencies, similarities and associations with the data interpretation until a point of agreement was reached on the development of the themes. Following this, the developed themes and their sub-themes were presented to two panel experts, both of whom were experts in qualitative studies. These experts were asked to classify all the themes developed instinctively and decide whether the themes and sub-themes were relevant and appropriate for the review results.

Statistical analysis

The remaining articles were assessed and evaluated. The focus of the research efforts was on specific studies that provided answers to the posed issues. The Review Manager (RevMan Copenhagen Version 5.4.1: The Nordic Cochrane Centre, 2012), which is a meta-analysis tool, was used to record the data extracted from the 46 papers in order to conduct a meta-analysis. Before the data could be submitted into RevMan, a lot of work had to be done. A considerable amount of time was required for the literature search. Since a meta-analysis relies on data from publications, certain data must be transformed. The outcomes of the analysis were represented as forest plots. Subgroup analyses were utilized to stratify the studies that employed distinct or a combination of interventions. Cochrane's Q (Chi-square) and Moran's I^2 (Inconsistency) tests were used to assess heterogeneity. If the p-value was <0.05, then, heterogeneity was considered to be statistically significant, while I^2 values of 25, 50, and 75% were deemed to reflect low, moderate, and high levels of heterogeneity, respectively.

Results

General findings and background of selected articles

More specifically, it should be noted that of the 46 studies that were included, 11 studies were geographically located in the United States, 10 in Argentina, five in Malaysia, three each in Brazil and India, two each in France and Thailand, and one each in China, Germany, Italy, Japan, Mauritius, Mexico, Nepal, South Africa, Taiwan, and the United Kingdom. As mentioned in this study, a combination of experimental and field study designs was mostly selected to determine the goal of the study in relation to dengue vector studies compared to the application of either a field or experimental study design. Furthermore, 21 studies were experimental studies, while only two were field studies. The remaining 23 studies were a combination of both experimental and field studies. It was not the goal of this study to present a list of the best study designs to assess the studies related to dengue vectors in terms of the robustness of the methods. However, on considering the types of research designs that were identified during the analysis, it was noted that there was an interesting balance between experimental and field studies. On the other hand, the results also represented the key elements of the study with regard to the mosquito strains that were selected for further analysis, where 29 articles reported on the *A. aegypti*, nine articles examined the *A. albopictus*, and seven articles considered both the *A. aegypti* and *A. albopictus*, while 1 article studied a combination of the *A. aegypti* and Culex spp., respectively. Table 1 summarizes the findings, research period, location, type of study, study design, and variables observed in the evaluated studies on the effects of climatic and environmental conditions on dengue vectors. Based on the thematic analysis, five themes were developed regarding the effects of temperature on dengue vectors, namely, the hatching rate (%), development time (days), survival rate (%), longevity (days), and wing morphology (mm).

TABLE 1 Summary of the articles related to effect of temperature on Aedes mosquito.

ID	Author	Study location	Mosquito strain	Study design			Effect on dengue vectors								Independent variables							
			AE	AL	LW	FW	Hatching	Development time	Longevity	Survival	Wing size	Seasonal	Diet	Container	Competence	RH	Wind speed	Density	Altitude	Light	Insecticide
ID1	Alomar et al. (27)			☑			☑			☑											
ID2	Alto and Bettinardi (28)	USA		☑	☑			☑	☑	☑	☑										
ID3	Awang et al. (29)	Malaysia		☑	☑			☑													
ID4	Awang and Dom (30)	Malaysia	☑	☑	☑	☑		☑	☑	☑	☑			☑		☑	☑				
ID5	Bagny Beilhe et al. (31)	France	☑	☑	☑		☑				☑		☑		☑			☑			
ID6	Buckner et al. (32)	USA	☑	☑	☑			☑		☑			☑								
ID7	Byttebier et al. (33)	Argentina	☑		☑		☑							☑							
ID8	Campos et al. (34)	Argentina	☑		☑	☑	☑	☑			☑										
ID9	Carrington et al. (35)	USA	☑		☑	☑			☑	☑	☑										
ID10	Couret et al. (36)	USA	☑		☑		☑	☑					☑					☑			
ID11	De Majo et al. (37)	Argentina	☑		☑	☑	☑	☑		☑											
ID12	De Majo et al. (38)	Argentina	☑		☑	☑	☑	☑		☑											
ID13	De Majo et al. (39)	Argentina	☑		☑	☑		☑	☑	☑	☑										
ID14	Ezeakacha and Yee (40)	USA		☑	☑			☑							☑						
ID15	Farjana et al. (41)	Japan	☑	☑	☑			☑		☑	☑		☑		☑						
ID16	Garzón et al. (42)	Argentina	☑	☑	☑		☑				☑										
ID17	Goindin et al. (43)	France	☑		☑			☑													
ID18	Grech et al. (44)	Argentina	☑		☑	☑		☑		☑											
ID19	Gutiérrez e al. (45)	USA	☑		☑	☑		☑		☑	☑										
ID20	Huxley et al. (46)	UK	☑		☑			☑					☑								
ID21	Iyaloo et al. (47)	Mauritius		☑		☑			☑												
ID22	Kramer et al. (48)	Nepal	☑	☑	☑	☑		☑	☑		☑								☑		
ID23	Krol et al. (49)	S. Africa	☑			☑				☑				☑	☑						

(Continued)

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TABLE 1 (Continued)

ID	Author	Study location	Mosquito strain		Study design			Effect on dengue vectors							Independent variables								
			AE	AL	LW	FW	Hatching	Development time	Longevity	Survival	Wing size	Seasonal	Diet	Container	Competence	RH	Wind speed	Density	Altitude	Light	Insecticide
ID24	Kumar et al. (50)	India	☑		☑			☑						☑							
ID25	Loetti et al. (16)	Argentina	☑		☑			☑	☑	☑	☑									☑	
ID26	Marinho et al. (51)	Brazil	☑		☑	☑		☑	☑												
ID27	Marini et al. (52)	Italy		☑	☑			☑	☑										☑		
ID28	Moura et al. (17)	Brazil	☑		☑				☑		☑										☑
ID29	Moura et al. (18)	Brazil	☑		☑			☑													☑
ID30	Muttis et al. (53)	Argentina	☑		☑	☑	☑	☑	☑												
ID31	Muturi et al. (54)	USA	☑		☑			☑		☑	☑				☑			☑			
ID32	Phanitchat et al. (55)	Thailand		☑	☑	☑	☑	☑													
ID33	Phanitchat et al. (56)	Thailand		☑				☑	☑	☑	☑										
ID34	Reiskind and Zarrabi (57)	USA		☑	☑	☑		☑		☑			☑								
ID35	Reiskind and Janairo (58)	USA	☑		☑	☑		☑		☑			☑	☑							
ID36	Rozilawati et al. (13)	Malaysia	☑	☑	☑	☑		☑	☑		☑										
ID37	Salinas et al. (59)	Mexico	☑	☑	☑	☑				☑											☑
ID38	Sasmita et al. (60)	Taiwan	☑		☑			☑	☑	☑	☑		☑								
ID39	Shahrudin et al. (12)	Malaysia	☑		☑			☑	☑												
ID40	Simoy et al. (61)	Argentina	☑		☑			☑													
ID41	Sivan et al. (62)	India	☑		☑	☑	☑			☑											
ID42	Sivan et al. (63)	India	☑		☑	☑	☑			☑											
ID43	Sukiato et al. (64)	Malaysia	☑		☑		☑	☑		☑	☑									☑	
ID44	Thomas et al. (65)	Germany	☑	☑	☑	☑				☑											
ID45	Upshur et al. (66)	USA	☑		☑			☑	☑		☑		☑								
ID46	Yang et al. (67)	China	☑		☑	☑	☑		☑												

AE, Aedes aegypti; AL, Aedes albopictus; LW, experimental studies; FW, field works; RH, relative humidity.

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Effects of temperature on *Aedes* mosquitoes

Hatching rate

Out of the 46 studies that were analyzed, 11 studies, which gave information about the effect of the hatching rate on dengue vectors using the risk difference between the maximum and minimum temperatures, were included in one meta-analysis (Figure 3). The overall estimate of heterogeneity across the studies was also very low, with $I^2 = 4\%$, and this was probably due to the small number of studies that were included for further analysis. The hatching rate showed a higher risk difference between the maximum and minimum temperatures without any significant effect on the dengue vectors. More specifically, based on the analysis, the studies by Sivan et al. (63), Sivan et al. (62), Phanitchat et al. (55), Yang et al. (67), and De Majo et al. (38) showed no significant higher risk difference between the maximum and minimum. On the other hand, studies by Bagny Beilhe et al. (31), Byttebier et al. (33), Campos et al. (34), De Majo et al. (37), Garzón et al. (42), and Muttis et al. (53) showed that there was no significant lower risk difference between the maximum and minimum temperatures respectively.

Development time

After pooling 34 articles for further analysis on the risk difference between the maximum and minimum temperatures that affects dengue vectors, the development time was also revealed to have a higher significant risk difference between the maximum and minimum temperatures with a high heterogeneity across the studies. More specifically, based on the meta-analysis shown in Figure 4, the studies by Phanitchat et al. (55), Bagny Beilhe et al. (31), Alomar et al. (27), Carrington et al. (35), Couret et al. (36), Ezeakacha and Yee (40), Farjana et al. (41), Huxley et al. (46), Kumar et al. (50), Marinho et al. (51), Muturi et al. (54), Reiskind and Zarrabi (57), Sasmita et al. (60), and Simoy et al. (61) showed a higher significant risk difference between the maximum and minimum temperatures, respectively. Conversely, other studies by Marini et al. (52) and Upshur et al. (66) revealed a significant lower risk difference between the maximum and minimum temperatures. However, the analytical results of other studies by Rozilawati et al. (13), Loetti et al. (16), Garzón et al. (42), Awang et al. (29), Awang and Dom (30), Buckner et al. (32), De Majo et al. (39), Goindin et al. (43), Phanitchat et al. (56), and De Majo et al. (38) presented a higher risk difference that was not significant between the maximum and minimum temperatures. Other studies by Shahrudin et al. (12), De Majo et al. (37), Marini et al. (52), Alto and Bettinardi (28), Grech et al. (44), and Kramer et al. (48) showed a lower risk difference that was not significant between the maximum and minimum temperatures.

Longevity

Out of the total number of studies that were analyzed, 18 studies provided information about the effect of the maximum and minimum temperature on the longevity of dengue vectors in the meta-analysis (Figure 5). The longevity showed no significant higher risk difference between the maximum and minimum temperatures that affected the dengue vectors. However, the overall estimate of heterogeneity across the studies was very high which was probably due to the large number of studies that were included in the analysis. More specifically, based on the analysis, studies by Moura et al. (18), Carrington et al. (35), Huxley et al. (46), Iyaloo et al. (47), Marinho et al. (51), Muttis et al. (53) and Rozilawati et al. (13) revealed a significant higher risk difference between the maximum and minimum temperatures. Also, a significant lower risk difference between the maximum and minimum temperatures was revealed in three studies by De Majo et al. (39), Kramer et al. (48), and Sasmita et al. (60). On the other hand, a few studies by Loetti et al. (16), Phanitchat et al. (55), and Yang et al. (67) presented a higher risk difference with no significance between the maximum and minimum temperatures, whereas a few remaining studies by Shahrudin et al. (12), Goindin et al. (43), Gutiérrez e al. (45), Marini et al. (52), and Upshur et al. (66) demonstrated a lower risk difference without any significance between the maximum and minimum temperatures.

Survival rate

Figure 6 shows the results of the effect of the maximum and minimum temperatures on the survival of dengue vectors after 21 articles were analyzed out of the total number of articles selected for the meta-analysis. Only two studies by Carrington et al. (35) and Farjana et al. (41) showed a significant higher risk difference between the maximum and minimum temperatures. Conversely, studies by Loetti et al. (16), Sivan et al. (63), Bagny Beilhe et al. (31), Garzón et al. (42), Couret et al. (36), Huxley et al. (46), Muturi et al. (54), Reiskind and Zarrabi (57), De Majo et al. (39), Phanitchat et al. (56), Alto and Bettinardi (28), Krol et al. (49), Salinas et al. (59), De Majo et al. (38), and Sasmita et al. (60) revealed a higher risk difference without any significance between the maximum and minimum temperatures. On the other hand, other studies by Sivan et al. (63), De Majo et al. (37), Grech et al. (44), and Reiskind and Janairo (58) demonstrated a lower risk difference without any significance between the maximum and minimum temperatures. The overall estimate of heterogeneity across all the studies was very low which was probably due to the small number studies that were included in the analysis. There was a significant higher risk difference between the maximum and minimum temperatures, thereby indicating their effect on the survival rate of the dengue vectors, while individual researches that had no significant effect of the summary results were excluded.

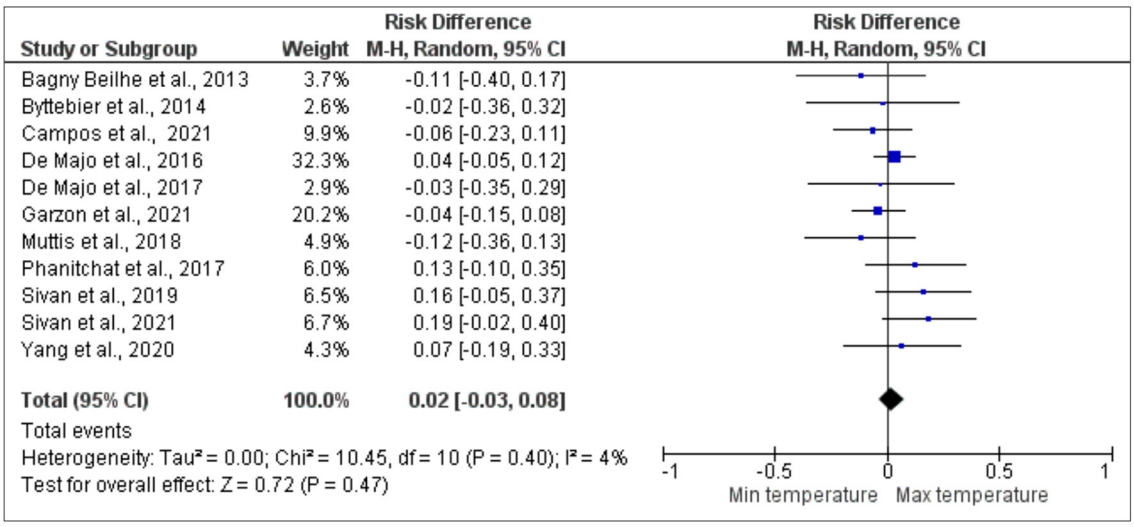

FIGURE 3
Forest plot of comparison of the effect of minimum temperature vs. maximum temperature on the hatching rate of Aedes mosquito.

Wing morphology

After pooling 13 articles for a further analysis of the effect of the risk difference between the maximum and minimum temperatures on the wing morphology of dengue vectors, it was revealed that there was a higher significant risk difference between the maximum and minimum temperatures. However, there was a low heterogeneity across the studies. More specifically, based on the meta-analysis shown on Figure 7, most of the studies by Rozilawati et al. (13), Loetti et al. (16), Moura et al. (18), Alomar et al. (27), Farjana et al. (41), Huxley et al. (46), Muturi et al. (54), Sasmita et al. (60), De Majo et al. (39), Alto and Bettinardi (28), Kramer et al. (48), and Sukiato et al. (64) revealed a non-significant higher risk difference between the maximum and minimum temperatures, whereas only one study by Upshur et al. (66) showed a non-significant lower risk difference between the maximum and minimum temperatures.

Discussion

The present study was carried out to systematically analyse the existing literature on the various effects of temperature on dengue vectors, specifically for the *A. aegypti* and *A. albopictus*, and how these factors affect both these species of mosquitoes. A proper review in relation to the effects of temperature on dengue vectors was conducted on 46 articles sourced from two databases. The findings revealed that these factors affect dengue vectors in various ways. Five themes emerged from the thematic analysis that was performed within the scope of this review. Hence, this section presents further discussions on the developed themes.

Based on this review and meta-analysis, temperature is becoming one of the important factors affecting the biology, population continuation and growth of the mosquito. Before a mosquito reaches adulthood, it goes through a few stages in its life cycle. However, both internal and external factors determine whether each stage is successfully completed. Being poikilothermic (cold-blooded) organisms, mosquitoes are sensitive to changes in the ambient temperature, which directly affects their body temperature. Temperature is therefore one of the most significant elements that might affect the insect survival, adult longevity, immature growth, vectorial capacity and other aspects of the life history of the *A. aegypti* and *A. albopictus* (13, 53, 61, 62, 68).

Hatching rate

Temperature is an essential hatching signal (42, 69). Temperate juvenile *A. aegypti* have successfully acclimated to cooler environments compared to subtropical populations. The eggs of temperate *A. aegypti*, for example, hatch faster at 5°C, 12°C, and 15°C (52). As a result, the spread of the *A. aegypti* to colder regions appears to be possible in the future (8, 48, 63). Another study discovered that during winter the egg hatching rate of the *A. albopictus* at 25°C fell at 11°C (67). Garzón et al. (42) discovered that the percentage of hatching in the *A. aegypti* was higher at 27°C than at 16°C. Most crucially, a temperature threshold of 37°C resulted in a drastically reduced hatching rate for the *A. aegypti*, with the upper limit occurring between 38 and 42°C (70). However, the fact that some larvae

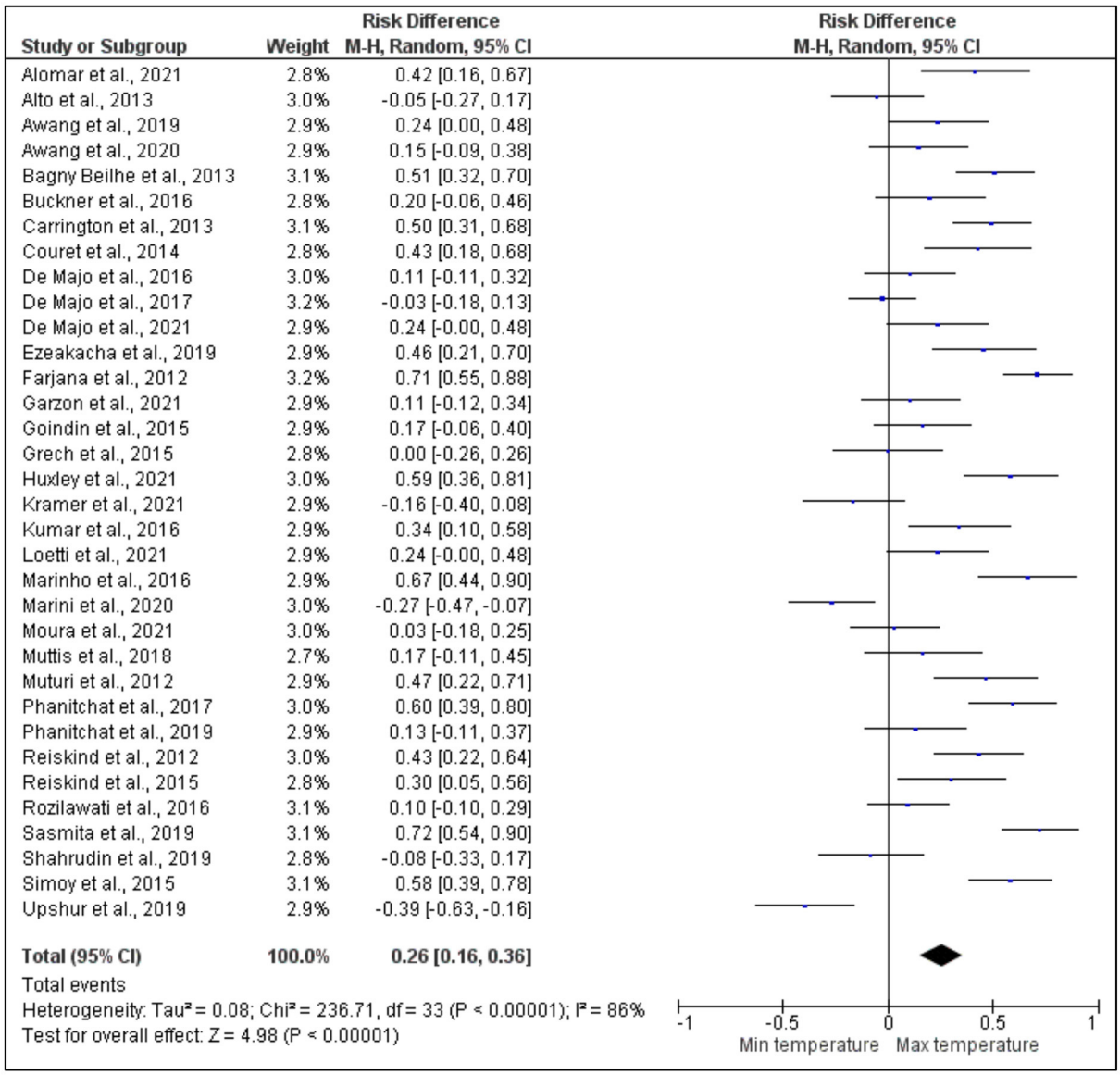

FIGURE 4
Forest plot of comparison of the effect of minimum temperature vs. maximum temperature on the development period of Aedes mosquito.

still hatched suggests a potential physiological adaptation to high water temperatures of above 39°C (51).

Development time

As expected, the development time of the *A. aegypti* and *A. albopictus* mosquito is affected by changes in the temperature of the surrounding environment when it exceeds the lower critical development threshold (71). The results showed that changes in the temperature could alter the stage of development starting from the larva stage until the emergence of the adult (30, 36, 64). Estimates of the development time were strongly temperature sensitive and negatively related to temperatures from 35°C until 40°C. Beyond this point, the rate of development slowed and reproduction ceased. Thus, this situation was very harmful to the mosquitoes and increased the mortality rate in both the *A. aegypti* and *A. albopictus* species (34, 41). Larval development was halted, resulting in larval death of 100% as the mosquito larvae then suffered stress as a result of a rise in temperature, which may have caused abnormalities at the cellular level in the larvae. Higher temperatures also stimulated rapid growth, leading to fewer nutrient reserves, which led to molting failure (50). On the same

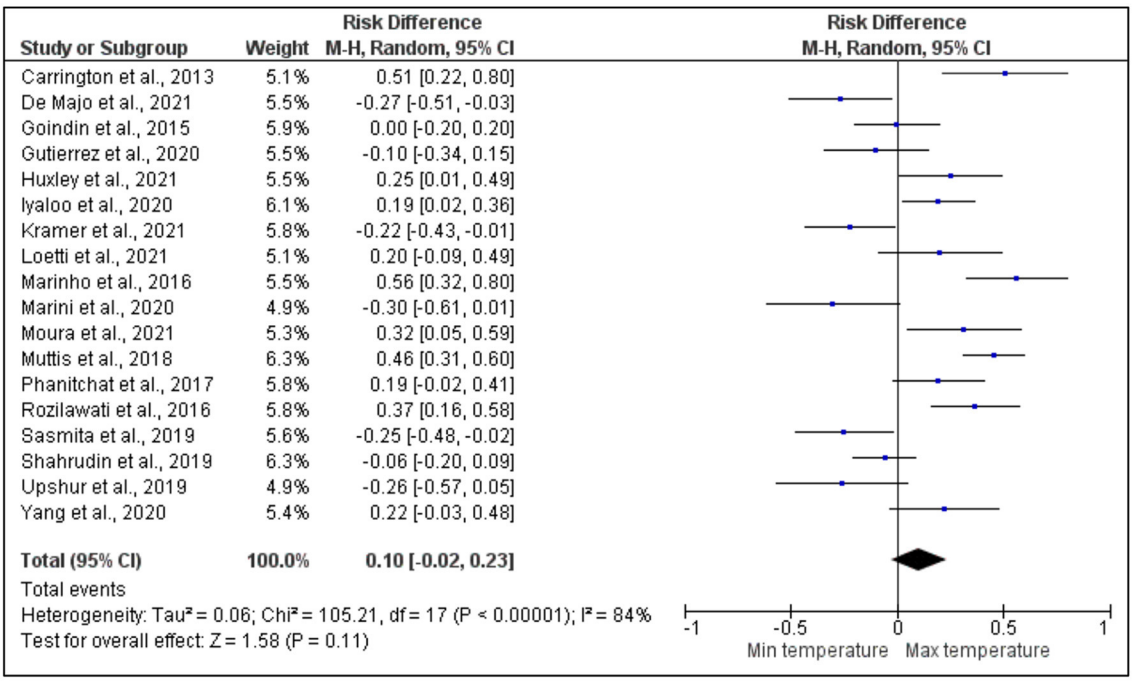

FIGURE 5
Forest plot of comparison of the effect of minimum temperature vs. maximum temperature on the longevity of Aedes mosquito.

note, the detrimental effect of higher temperatures on culicid vector development (68, 72) suggested that this approached the lethal temperature range for the Aedes mosquito (36, 51).

Survival rate and longevity

Temperature also had an effect on the lifespan on the *Aedes* adult (17, 60). Temperature extremes of 16°C and 36°C significantly reduced adult longevity and female fertility (51). In terms of sexes, at the most temperatures, the males have shorter lifespan than female. This pattern could be related to the intensity of oviposition, which is both energetically and likely to limit longevity (51). Male longevity decreases mating possibilities, while female longevity decreases gonotrophic cycles (17). According to Muttis et al. (53), the lifetime of the mosquito is more dependent on temperature than on population-related parameters such as density. As a result, it was anticipated that mosquito larvae would live shorter lives at high temperatures. Other than that, it was inferred that the daily temperature dynamics and ambient temperature are significant in an endemic area, which may help with the selection of mosquitoes that can survive at high temperatures, thereby enhancing their ability to transmit diseases (63).

Wing morphology

Temperature is one of the extrinsic elements that directly influence insect development. Literature suggests that wing and body size are influenced by other environmental conditions such as population density in breeding containers (73, 74) and competence (75). Our results suggest that warmer temperatures also lead to smaller adult sizes and vice versa, a phenomenon known as the temperature-size rule or the "hotter is smaller" rule (76). The estimation of the wing length reveals the mosquito body size that can epidemiologically affect relevant qualities by indicating the responsiveness of the adult mosquito to the development of the juvenile stages. The change in weight and wing length allometry at a particular temperature may be advantageous in a number of different ways. One hypothesis is that the elasticity of wing length and weight may result in lower wing loadings at low temperatures, which could be advantageous at colder operating temperatures due to the increased energy required for a given flying output (57). Measurements of the wing size were performed in order to analyse the impact of variations in the wing size on the ability of the mosquito to select a breeding location and engage in blood feeding in field conditions. The larger size of the female mosquito may indicate that it has a longer flight range than a little mosquito, finds hosts more frequently, feeds successfully on blood, and has superior location skills for oviposition, all of which boost fertility. In light

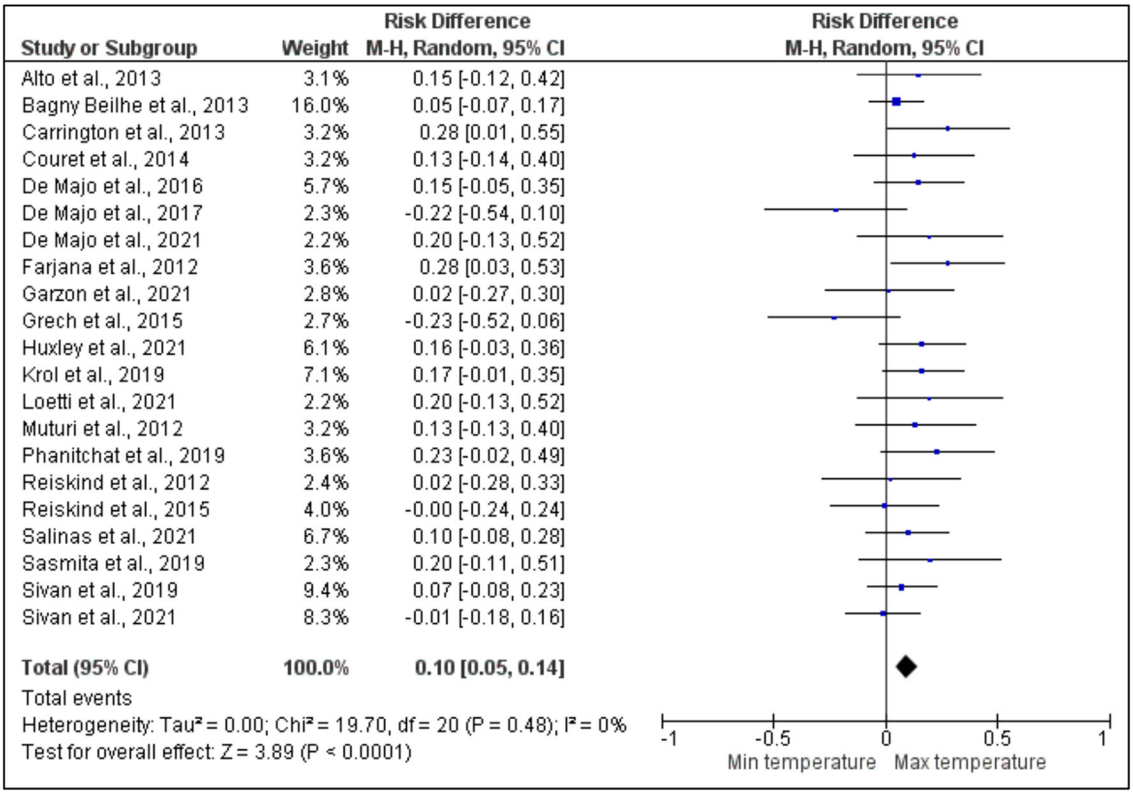

FIGURE 6
Forest plot of comparison of the effect of minimum temperature vs. maximum temperature on the survival of dengue vectors.

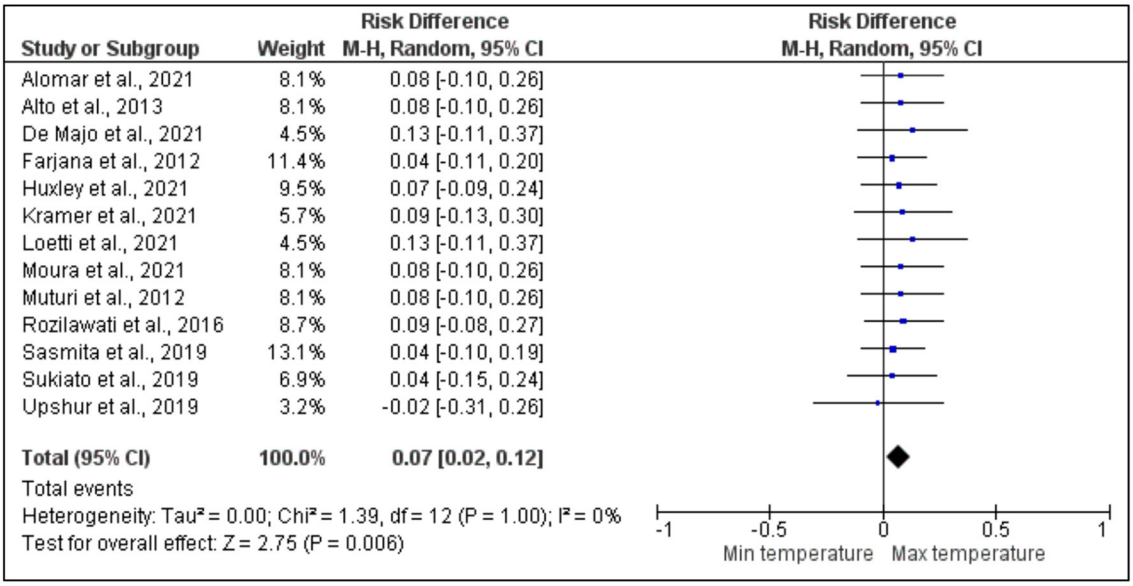

FIGURE 7
Forest plot of comparison of the effect of minimum temperature vs. maximum temperature on the wing morphology of dengue vectors.

of this, it was determined that the fecundity rate and the capacity to fly to an oviposition site are higher at lower temperatures and decrease as temperatures rise (12).

These findings are necessary for the adoption of dengue outbreak systems and minimization of the adverse effects of the transmission of the dengue virus by its two main vectors, the *A. aegypti* and *A. albopictus*. It is critical to have knowledge of what and how environmental and climatic conditions can affect dengue vectors. However, this study had its limitations, and a number of recommendations have been made that may be beneficial for future research while conducting a meta-analysis, such as the fact that only English-language papers were examined, which might have introduced a linguistic bias into the findings. Then, it was discovered that variations in heterogeneity at several stages of the gonotrophic cycle, including hatchability, development time, survival, longevity, and wing shape, might have been responsible for this heterogeneity. This heterogeneity analysis was impacted by the small sample size, which included the number of studies that were taken into account for the review and meta-analysis. It was demonstrated that the development periods of the *A. aegypti* and *A. albopictus* were shortened, larval and pupal mortality were increased, and hatchability was dramatically reduced as a result of rising temperatures. The *Aedes* population will likely increase in the subtropical and temperate zones, and not necessarily in the tropical areas, due to global warming, and the distribution and seasonal duration will increase. Furthermore, new pandemic hotspots will be appearing. This range for this species has already widened to include greater latitudes and altitudes. As a result, epidemics are more prone to occur and spread over wider areas.

Therefore, information on the life cycle of Aedes mosquitoes at different temperatures was important for planning effective dengue control. The ability of Aedes to survive at different water temperatures showed that the vector is highly adaptable to the ever-changing environment. This study also found that development time and wing morphology accelerate when temperature is increased. This Aedes life data could also serve as an advance warning and is necessary from an epidemiological perspective to identify the pattern of results due to seasonal changes throughout the year. Vector control efforts and population dynamics models will be improved if realistic parameter estimates of mosquito populations are incorporated into future surveillance activities and research projects.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

NN, RD, and NC wrote the manuscript. HS collected the data and did the analysis. NP proofread and edited the manuscript. All authors contributed to the article and approved the submitted version.

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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

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Model-based risk assessment of dengue fever transmission in Xiamen City, China

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Background: Quantitative assessment of the risk of local transmission from imported dengue cases makes a great challenge to the development of public health in China. The purpose of this study is to observe the risk of mosquito-borne transmission in Xiamen City through ecological and insecticide resistance monitoring. Quantitative evaluation of mosquito insecticide resistance, community population and the number of imported cases affecting the transmission of dengue fever (DF) in Xiamen was carried out based on transmission dynamics model, so as to reveal the correlation between key risk factors and DF transmission.

Methods: Based on the dynamics model and combined with the epidemiological characteristics of DF in Xiamen City, a transmission dynamics model was built to simulate the secondary cases caused by imported cases to evaluate the transmission risk of DF, and to explore the influence of mosquito insecticide resistance, community population and imported cases on the epidemic situation of DF in Xiamen City.

Results: For the transmission model of DF, when the community population is between 10,000 and 25,000, changing the number of imported DF cases and the mortality rate of mosquitoes will have an impact on the spread of indigenous DF cases, however, changing the birth rate of mosquitoes did not gain more effect on the spread of local DF transmission.

Conclusions: Through the quantitative evaluation of the model, this study determined that the mosquito resistance index has an important influence on the local transmission of dengue fever caused by imported cases in Xiamen, and the Brayton index can also affect the local transmission of the disease.

KEYWORDS

dengue fever, mathematical model, risk assessment, vector investigation, insecticide resistance monitoring

Introduction

Dengue fever (DF) is a viral infectious disease transmitted by *Aedes albopictus* and *Aedes aegypti*. There are four types of serotype for DF virus, namely DENV-1, DENV-2, DENV-3, and DENV-4 (1, 2). People infected with dengue virus can have fever, dengue hemorrhagic fever, and other clinical symptoms. For severe cases, they suffer from dengue shock syndrome and death (3, 4). One recent research indicates that there are 390 million (95% credible interval 284–528

million) new DF cases per year in the world, of which about 12,000 death (1, 5). More researches have estimated that the incidence of DF as increased nearly 30 times compared with 50 years ago, and about one third of the world's population are at risk of DF infection (6–8). At the same time, due to the lack of specific treatment and effective vaccination against DF, the epidemic of DF has become one of the major global public health problem, causing serious disease burden to many countries and regions (2).

In recent years, the annual reported incidence of DF in China has shown an obvious upward trend (9). Besides Guangdong, Guangxi, Hainan, and other areas, which are with high incidence of DF, the central and northern regions of China also have reported cases of DF (10, 11). Xiamen City, as a special economic zone in China approved by the State Council, has frequent personnel exchanges with countries and regions with high incidence of DF cases in Southeast Asia. Therefore, Xiamen City is facing a grim situation of prevention and control of imported DF cases. Meanwhile, located in the subtropical maritime monsoon climate zone, Xiamen City has suitable temperature and humidity for mosquito-borne transmission. Thus, when dengue virus is imported in the right season, the risk of disease transmission was higher. Consequently, the epidemic prevention departments need to quantitatively understand the potential risk factors of DF transmission, accordingly, promote the formulation of more effective control strategies and early warning surveillance.

Prior to this study, there have been many researches on DF by using mathematical models (12–14). A study established a human-vector coupled dynamic model to evaluate the effect of intervention measures taken in DF outbreak (12). In another study, the generalized mixed linear equation was introduced into the SIR dynamics model, which emphasized the influence of meteorological conditions on *Ae. albopictus* and discussed the influence of meteorological factors on DF propagation (13). In the meantime, the researchers, *via* time series regression tree analysis, have found that the timeliness of DF monitoring system in DF transmission in Zhongshan City, China and meteorological factors have influenced the local DF incidence (14). Nevertheless, in the research of potential factors affecting DF communication, there is no quantitative evaluation of DF potential communication risk factors based on communication dynamics model in China.

In this paper, we developed a Hosts-Vectors Susceptible–Exposed–Infectious–Asymptomatic–Recovered (SEIAR) transmission dynamics model to quantitatively evaluate the influence of mosquito insecticide resistance and imported cases on DF transmission in Xiamen City. In addition, through SEIAR model, it will help guiding public health departments in Xiamen City and other areas to put forward scientific strategies and early warning and forecasting systems for controlling DF transmission.

Abbreviations: DF, Dengue fever; SEIAR, Susceptible–Exposed–Infectious–Asymptomatic–Recovered; SEI, Susceptible-Exposed-Infectious; CDC, Center for Disease Control and Prevention; BI, Brayton index; CI, Container index; HDN, Human-baited double net trapping; WHO, World Health Organization.

Materials and methods

Study area

Xiamen (118°04′04″E, 24°26′46″N), located in the southeast of China, is a coastal city with a population of 4.29 million. It is an important city in Fujian Province and occupies an area of 1,700.61 square kilometers. The city has six districts under its jurisdiction, including 26 streets, 12 towns, 361 communities, and 147 villages. The city belongs to is subtropical monsoon climate with a yearly average temperature of 21°C and an average annual rainfall of about 1,315 mm, most of which is mainly concentrated from May to August. According to the dengue surveillance data from 2005 to 2019 in Xiamen City, *Ae. Albopictus* was the only vector species in the city, and imported DF cases were the majority cases (15).

Case definition and case-finding

In the study, an indigenous case was defined as an individual who are infected with DF and have not left this city (current address) within 14 days before the onset of disease. An imported DF cases was defined as an infected patient who had been to DF epidemic regions within 14 days after the onset of disease.

All the DF cases were identified following the diagnostic criteria announced by National Health Commission of the People's Republic of China (WS216-2008) (16):

(a) Suspected case: an individual who had been to DF epidemic regions within 14 days or there has been a DF case around his/her residence or workplace (within a radius of 200 m) within 1 month, along with having one or more symptoms, and no specific diagnosis has been confirmed as other diseases.
(b) Clinically diagnosed case: a suspected case with leucopenia or thrombocytopenia, or a suspected case whose serum specific immunoglobulin IgG or IgM test is positive.
(c) Laboratory-confirmed case: a clinically diagnose case has one or more of the following test results:
    I. Serum tested positive for DENV RNA by real-time PCR;
    II. An IgG titer in the recovery period is 4-fold higher than that in the acute period;
    III. Isolation of the DENV from the blood, tissue or cerebrospinal fluid of a patient with acute infection.

Vector investigation and insecticide resistance monitoring

In order to obtain the relevant parameters in the model, we have assessed the density of *Ae. albopictus* in Xiamen City by three ecological monitoring methods: Brayton index method, Container index method and Human-baited double net trapping method. And mosquito surveillance was performed twice a month in two districts (Huli and Xiang'an) of Xiamen City from May to November in 2020.

Brayton index

An investigation of BI was conducted from May 1st to November 1st in Xiamen City. In four residential areas of different

geographical locations, there were no <100 households selected in each monitoring district. For other habitats, such as park, bamboo forests, old tire dumps, waste collecting stations, 50 households need to be collected. Then we recorded the occurrence of *Ae. albopictus* larvae in all indoor and outdoor water containers (17). For identifying the species, we collected the larvae and brought them back to Center for Disease Control and Prevention (CDC) laboratory for breeding to adult mosquitoes and thereby making the identification (Supplementary Text S1, p. 1). Finally, we use the following formula to calculate the BI:

$$\text{BI} = \frac{\text{Number of positive containers of } Ae.albopictus}{\text{Number of households surveyed}} \times 100\%$$

Container index

The site selected for monitoring Container index is consistent with the above-mentioned site, and should be used to monitoring the CI first. In each monitoring district, it has to be no <100 containers in four residential areas in different geographical locations for 4 days, and the distance between each container is 25–30 m. On the fourth day, the adult mosquitoes were monitored, and the species were identified after larvae grow up (Supplementary Text S1, p. 1, 2). We used the following formula to calculate the CI:

$$\text{CI} = \frac{\text{Number of positive containers}}{\text{Number of effective containers}} \times 100\%$$

Human-baited double net trapping

Adult mosquito monitoring mainly adopts the method of human-baited double net trapping. We have selected four different habitats, each with two nets more than 100 meters apart. In the afternoon (15:00–18:00), when the vector activity was at its peak, the attractor had both legs exposed in an internal closed mosquito net, and the collector was in trousers (18). Mosquito repellent was not used during the monitoring process. An electric mosquito absorber was used to quickly collect vector Aedes that fell on the mosquito net, then then leave as soon as possible. The monitoring lasted for 30 min and the vector Aedes were collected from each mosquito net (Supplementary Text S1, p. 2). We use the formula to calculate the inducement index:

$$\text{HDN} = \frac{\text{Number of female mosquitoes captured}}{\text{Number of mosquito nets} \times 30 \text{ minutes}} \times 60 \text{ minutes/hours}$$

Insecticide resistance monitoring

It was mainly the end of 3rd instar to the beginning of 4th instar larvae that were chosen to monitor the insecticide resistance. And we adopted the impregnation technique recommended by WHO (refer to GB/T26347-2010) to determine LC50 and calculate RR (17). In the meantime, the WHO recommended contact tube method (refer to GB/T26347-2010) was used to monitor the resistance of adult mosquitoes to insecticides, and thereby the mortality of adult mosquitoes at diagnostic dose was determined. We selected 11 different types of pesticides, namely: 0.2% bendiocarb, 0.2% fenitrothion, 0.03% deltamethrin, 0.04% permethrin, 0.5% propoxur, 0.5% malathion, 0.08% beta-cypermethrin, 0.07% lambda-cyhalothrin, 2% chlorpyrifos, 0.4% beta-cypermethrin. See Supplementary Text S1, p. 3–6 for specific experimental methods.

TABLE 1 Variables in the SEIAR-SEI model.

Variables	Description	Unit
S_p	Susceptible individuals	Individuals
E_p	Exposed individuals	Individuals
I_p	Infectious individuals	Individuals
A_p	Asymptomatic individuals	Individuals
R_p	Recovered/removed individuals	Individuals
S_m	Susceptible vectors	Individuals
E_m	Exposed vectors	Individuals
I_m	Infectious vectors	Individuals
A_i/I_i	Imported infectious/asymptomatic individuals	Individuals

Dynamic model of DF transmission

In this study, we has built a dynamics model based on SEIAR to simulate the transmission of the dengue virus (9, 12, 19). And in this model, people were divided into the following five compartments: susceptible (S_p), exposed (E_p), infectious (I_p), asymptomatic (A_p), removed (R_p). A_i/I_i refers to imported DF cases. Vectors were divided into the following three compartments: susceptible (S_m), exposed (E_m), infectious (I_m), see Table 1. And the interaction between the human and vector is presented in Figure 1.

This model is based on the following assumptions:

(1) The model assumes that the propagation coefficient of S_p and I_m after effective propagation is β_{mp}, the transmission rate from A_i/I_i to S_m is β_i, and the transmission rate from S_m to I_p and A_p is β_{pm}. Therefore, at time t, the number of newly infected DF is $\beta_{pm}S_pI_m$, and the number of newly infected vectors is $\beta_iS_mI_i + \beta_{pm}S_m\,(A_p + I_p)$.

(2) The model assumes that the proportion of recessive infection is q and the latency is $\frac{1}{\omega}$, So at time t, the number of individual who changed from E_p to A_p and I_p were $q\omega_pE_p$ and $(1–q)\omega_pE_p$.

(3) The model assumes that the time interval from onset to first diagnosis of case I_p was $\frac{1}{\gamma}$, so at time t, the number of people who changed from I_p to R_p is γI_p. In addition, we assume that the infection period of asymptomatic infected person A is $\frac{1}{\gamma'}$, so at time t, the number of people who changed from A to R is $\gamma' A_p$.

(4) The model assumes that the natural vectors mortality rate is b, the natural birth rate is a, and the vertical transmission ratio of dengue virus by mosquitoes is n.

The mathematical model is described by the following ordinary differential equations (ODE):

$$\begin{aligned}
\frac{dS_p}{dt} &= -\beta_{mp}S_pI_m \\
\frac{dE_p}{dt} &= \beta_{mp}S_pI_m - \omega_pE_p \\
\frac{dI_p}{dt} &= (1-q)\,\omega_pE_p - \gamma I_p
\end{aligned}$$

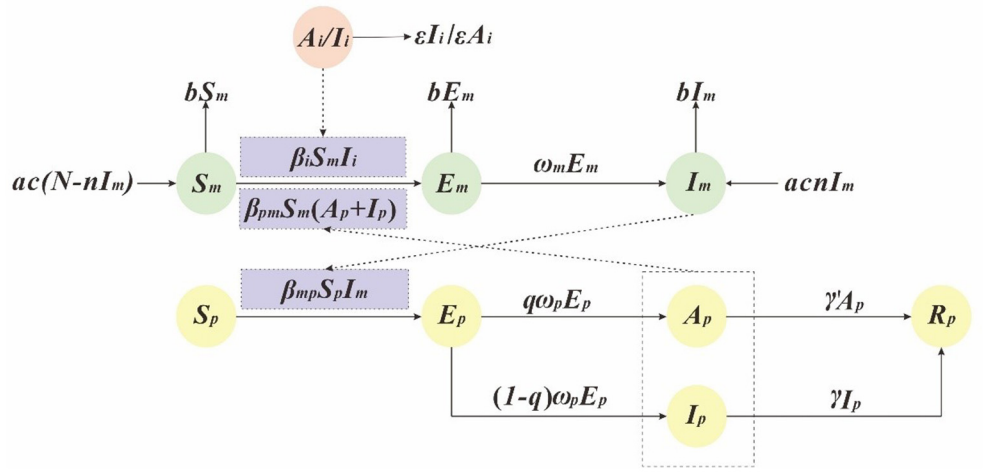

FIGURE 1
The flowchart of the development of the dengue fever transmission model.

$$\frac{dA_p}{dt} = q\omega_p - \gamma' A_p$$
$$\frac{dR_p}{dt} = \gamma' A_p + \gamma I_p$$
$$\frac{dS_m}{dt} = ac\,(N - nI_m) - \left[\beta_i I_i + \beta_{pm}\left(A_p + I_p\right)\right] S_m - b_m$$
$$\frac{dE_m}{dt} = \left[\beta_i I_i + \beta_{pm}\left(A_p + I_p\right)\right] S_m - (\omega_m + b) E_m$$
$$\frac{dI_m}{dt} = \omega_m E_m + acnI_m - bI_m$$
$$\frac{dI_i}{dt} = -\varepsilon I_i - \beta_i S_m I_i$$
$$N_m = S_m + E_m + I_m$$
$$N_p = S_p + E_p + I_p$$

where the seasonality parameter can be modeled by a trigonometric function, according to existing studies (12). We assume the following function for the parameter c:

$$c = cos\,[\frac{2\pi(t-\tau)}{T}]$$

Parameter estimation

There are fifteen parameters in this model (β_i, β_{pm}, β_{mp}, ω_m, ω_p, ε, γ, γ', a, b, c, q, n) according to the literature, see Table 2. The incubation period of dengue virus in human body is usually 4–8 days (9), therefore we selected the value of 6 days as the average, with $\omega_p = 0.1667$. It usually takes 8–12 days for imported dengue virus to enter mosquito host and cause its onset (19), thus the value of 10 days was chosen in the model, with $\omega_m = 0.1000$ per day. The proportion of asymptomatic infections is usually 68.75% (21), so $q = 0.6875$. The infectious period is 3–14 days (19, 20), we selected 7 days in the simulation, thus $\gamma = \gamma' = 0.1429$. Based on a previous study, the initial values of birth and mortality rate of mosquitoes was set as $a = b = 0.0714$ (19). In addition, with the constant change of resistance to mosquito-borne insecticide, we set the value range of a and b to 1/50–1/14 according to our study. The vertical transmission rate of dengue virus DENV-1 through vertical infection ranged from 1.4 to 17.4% (22), which was assumed it to be 10.0%, so $n = 0.1000$.

Evaluate the risk of DF transmission in different conditions

We assessed the transmission risk of DF in Xiamen City through SEIAR model. According to practical experience and previous studies, we included the following factors as transmission risk factors into our research: community population, the number of imported cases, larvae density, adult vector density, mosquito insecticide resistance (23, 24). Consequently, in this study, BI method was used to assess the larvae density of the vector and light-trap method was used to measure assess the adult vector density. The changes in mosquito mortality reflect the intensity of mosquito-borne insecticide resistance.

According to the community situation of indigenous cases in Xiamen City in 2019, the community population was set as 10,000, 15,000, 20,000, and 25,000, and the parameter of imported cases were 1–60. Our previous studies on insecticide resistance have determined that the mosquito birth and mortality rate would be set as 1/50, 1/38, 1/26, and 1/14 (12, 23). Simultaneously, the number of susceptible mosquito-borne could be reflected by changing the mosquito density, which was 1, 2, 3, 4, and 5, respectively, in BI. Therefore, we have set the following scenarios to quantitatively assess the transmission risk of DF in Xiamen City:

Scenario 1: We set the community population as 10,000, and BI values were 1, 2, 3, 4, and 5. The mortality rates of mosquitoes were set as 1/50, 1/38, 1/26, and 1/14, respectively. And to see how many dengue cases were imported, which could cause the spread of indigenous secondary cases.
Scenario 2: We set the community population as 15,000, BI values were 1, 2, 3, 4, and 5, respectively; The mortality rates of mosquitoes were set as 1/50, 1/38, 1/26, and 1/14, respectively. And to see how many dengue cases were imported, which could cause the spread of indigenous secondary cases.

TABLE 2 Parameter definitions and values.

Parameter	Description	Unit	Value	Range	Method
β_i	Transmission relative rate from input case to mosquitoes	1	5.11892×10^{-6}	≥0	Curve fitting
β_{pm}	Transmission relative rate from human to mosquitoes	1	8.01142×10^{-6}	≥0	Curve fitting
β_{mp}	Transmission relative rate from mosquitoes to human	1	1.24107×10^{-5}	≥0	Curve fitting
ω_m	Incubation relative rate of mosquitoes infection	Day^{-1}	1/10	0.0833–0.1250	(19)
ω_p	Incubation relative rate of human infection	Day^{-1}	1/6	0.1250–0.2500	(9)
ε	Input case recovery ratio	1	0.1429	0.0714–0.3333	Curve fitting
γ	Removed relative rate of infectious individuals	Day^{-1}	1/7	0.0714–0.3333	(19, 20)
γ'	Removed relative rate of asymptomatic individuals	Day^{-1}	1/7	0.0714–0.3333	(19, 20)
a	Daily birth rate of mosquitoes	Day^{-1}	1/14	0–1/14	(19, 20)
b	Daily mortality rate of mosquitoes	Day^{-1}	1/14	0–1/14	(19, 20)
c	Seasonality parameter of the mosquitoes population	1	See text	0–1	Curve fitting
τ	Simulation delay of the initial time in the whole season	Day	242	≥0	Analysis on the reported data
T	Duration of the cycle	Day	365	≥0	Analysis on the reported data
q	Proportion of human asymptomatic infection	1	0.6875	0-1	(21)
n	Proportion of transovarial transmission	1	0.1	0.0140–0.1740	(22)

Scenario 3: We set the community population as 20,000, BI values were 1, 2, 3, 4, and 5, respectively; The mortality rates of mosquitoes were set as 1/50, 1/38, 1/26, and 1/14, respectively. And to see how many dengue cases were imported, which could cause the spread of indigenous secondary cases.
Scenario 4: We set the community population as 25,000, BI values were 1, 2, 3, 4, and 5, respectively; The mortality rates of mosquitoes were set as 1/50, 1/38, 1/26, and 1/14, respectively. And to see how many dengue cases were imported, which could cause the spread of indigenous secondary cases.

Simulation and statistical analysis

We used Berkeley Madonna ver.8.3.18 (developed by Robert Macey and George Oster of the University of California at Berkeley, CA, USA) for parameter fitting and model simulation. The goodness-of-fitting was assessed by least root-mean-square error between simulated and observed number of new indigenous cases per day between August 24th and November 5th. The simulation method was the Runge–Kutta method of order four. Differential equations were solved by the step of 0.02. Meanwhile, the goodness of fit was judged by the coefficient of determination (R^2) value.

Results

Epidemiological characteristics of DF in Xiamen City

In 2019, a total of 138 cases of DF were reported in Xiamen City, and there were 19 indigenous cases and 119 imported cases. The distribution of all DF cases in Xiamen City is shown in Figure 2, among which indigenous cases were mainly reported in Huli District, Siming District and Jimei District. But the imported cases were reported in all districts of Xiamen City. The number of new cases in Xiamen City per month is shown in Figure 3. The first indigenous case was reported in the Jinshan community of Huli District on August 24th, 2019. The peak of DF incidence was from July to October, and the number of DF cases reported in these 4 months accounts for 75% of the total reported cases in the whole year. The population distribution of DF cases in Xiamen City in 2019 is shown in Table 3. Male patients predominate in imported cases, while females outnumber males in local cases. Meanwhile, most of the reported cases of DF were most reported in people aged between 20 and 50 years old, with no cases reported in people younger than 10 years old. In addition, a higher proportion of cases were reported in commercial services and domestic activities.

Investigation on mosquito-borne ecology and insecticide resistance monitoring

We conducted a household survey of *Ae. Albopictus* between May and October 2020. A total of 9,060 *Ae. Albopictus* were captured, and 693 Aedes larvae were positive in water accumulation of Aedes larvae were found, see Figure 4A. The average value of BI is 7.6, among which the average value of BI in Huli District is 9.3 and that in Xiang'an District is 6.1. The monitoring results of BI are shown in Table 3. It is noticeable that the BI index of Xiamen City is higher than the threshold value of 5 from May to October 2020. Besides, from May to October 2020, we have placed a total of 9,840 mosquito container in Xiamen City. Among them, 8,893 were recovered with rate of 90.4%. The results showed that there were 518 positive traps, and the average index of CI was 5.8, which was higher than the safety level of 5 from June to

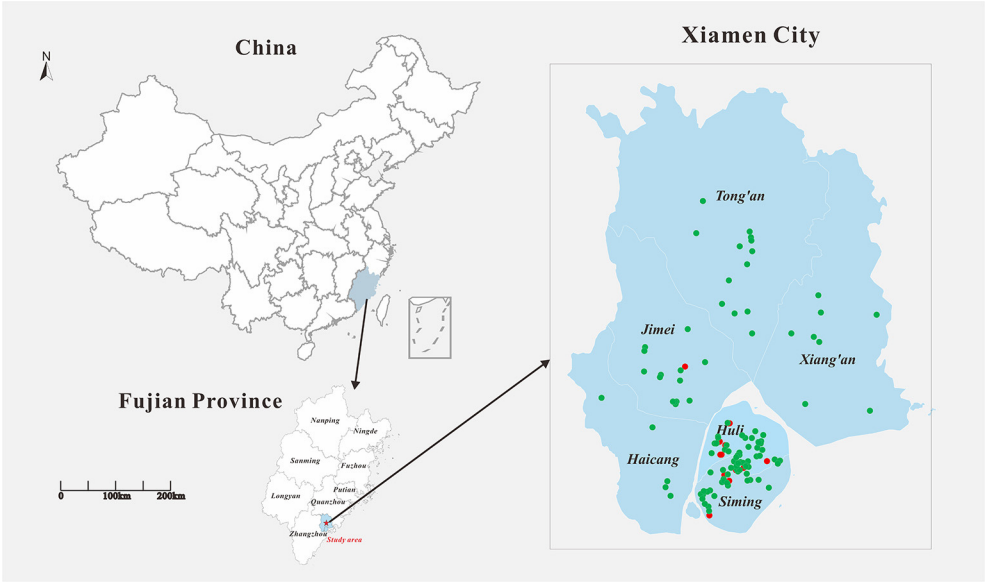

FIGURE 2
Geographical location of the study area and distribution of DF cases in Xiamen in 2019. Filled red circle represents the indigenous cases and filled green circle represents the imported cases.

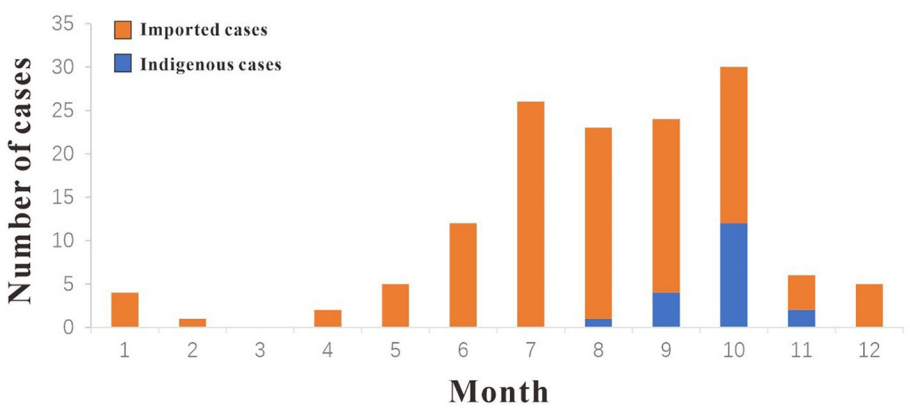

FIGURE 3
Reported cases of DF in Xiamen City, P.R. China, in 2019.

October. Therefore, it can be found that the change trend of CI is similar to that of BI, its the value is generally lower than BI, see Figure 4B.

For the monitoring of adult mosquito, we have the Human-baited double net trapping technique. From May to October, a total of 276 double-layered accounts were deployed. Finally, it was calculated that 1.7 mosquito nets were detected per hour, of which the highest was 2.2 in July, and the threshold value was higher than 2 in both June and July, see Figure 4C.

Therefore, we monitored the insecticide resistance of *Ae. albopictus* in Xiamen City, the results demonstrated that knock-down rate of mosquitoes at 1 h, the corrected mortality rate for 24 h and the insecticide resistance are shown in Table 4. Furthermore, the resistance times and resistance result of *Ae. albopictus* larvae exposed to parathion, prepoxur and pyriproxyfen are shown in Table 5. The adult *Ae. albopictus* in Huli District is sensitive to insecticides such as oxymoron, fenitrothion, residual carbofuran, malathion and chlorpyrifos, and high resistance to pyrethroid insecticides such as deltamethrin, high efficiency cypermethrin and high efficiency cyfluthrin. The larvae of *Ae. albopictus* are sensitive to disulfiram, with low resistance to residual carbofuran and medium resistance to pyriproxyfen. Adult *Ae. albopictus* is sensitive to oxytetracycline, fenitrothion and chlorpyrifos, possibly resistant to residual chlorpyrifos and malathion, and highly resistant to deltamethrin, cypermethrin, deltamethrin, cypermethrin and other pyrethroid insecticides in the Xiang'an District. Larvae of *Ae. albopictus* are sensitive to disulfoton and residual chlorpyrifos and moderately resistant to imidacloprid.

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TABLE 3 The population distribution of DF cases in Xiamen City in 2019.

		Indigenous cases	Imported cases
Gender	Male	8	94
	Female	11	25
Age	≤10	–	–
	11–20	2	4
	21–30	6	39
	31–40	2	39
	41–50	5	34
	51–60	1	2
	≥61	3	1
Occupation	Commercial service	7	21
	Student	2	4
	Staff member	3	1
	Worker	2	2
	Housework and unemployment	4	18
	Farmer	–	1
	Catering food industry	–	7
	Others	1	64

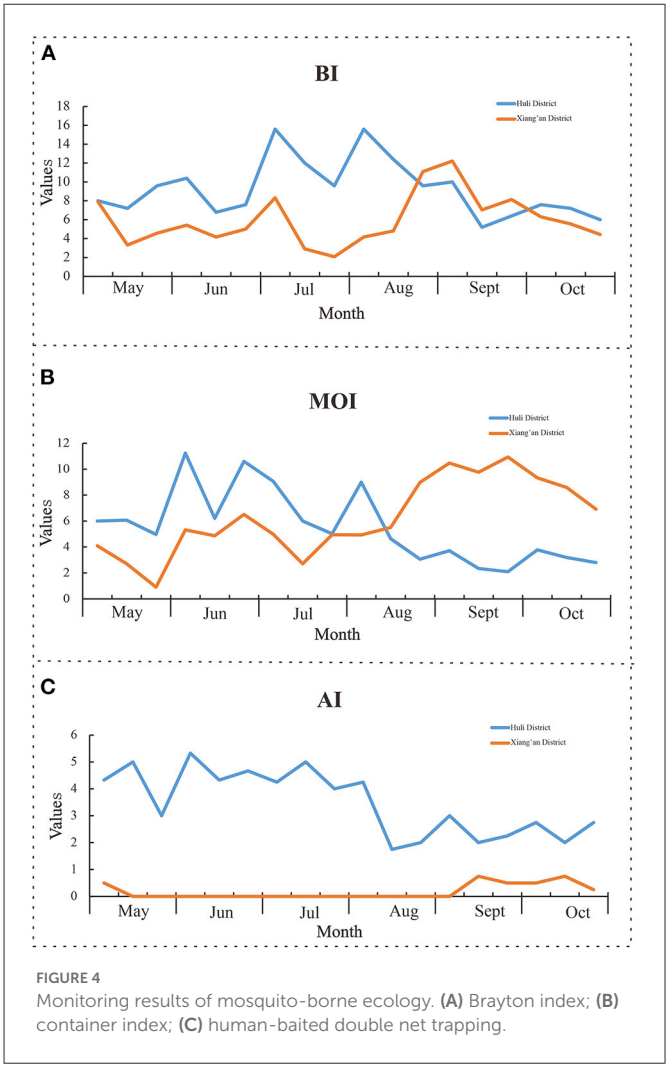

FIGURE 4
Monitoring results of mosquito-borne ecology. **(A)** Brayton index; **(B)** container index; **(C)** human-baited double net trapping.

Risk assessment of different transmission models

In this study, by adjusting different parameters in the model, we observed whether different imported cases can cause the transmission of indigenous DF cases, and quantitatively assessed the transmission risk of DF in Xiamen City, as shown in Figure 5. In scenario 1, we set the community population to 10,000. At this point, by changing BI value, birth rate and mortality rate of mosquitoes, we found that 58 DF cases imported from the community can cause indigenous DF cases when mosquito mortality rate is 1/14 and BI value is 1. With the BI values changing from 2, 3, 4–5, the imported case parameters are set to 29, 19, 14, and 11 cases, respectively, which can generate indigenous secondary cases. On the other hand, when the BI value is fixed at 1, the mosquito mortality rate changes to 1/50, so it takes 10 imported cases to cause the spread of indigenous cases. However, when changing the birth rate parameters, the impact on the model is limited, and the input of dengue cases within the set range cannot produce local secondary cases. As mosquito mortality declines and BI value increases, so did the number of imported cases that can cause the spread of local dengue cases, see Figure 5A.

In scenario 2, the community population is 15,000. At this time, the BI value is set to 1, the mosquito mortality rate is 1/14, and 25 imported cases are needed to produce local secondary cases. However, when the mortality rate is 1/14 and the BI value is 5, or when the mortality rate is 1/50 and the BI value is 1, only five imported cases are needed to produce local secondary cases. At the same time, we also found that when the BI value increased, the mortality dropped to a certain range, and only one imported case could cause local dengue fever cases, and the risk of DF transmission was relatively high at this time, see Figure 5B.

In Scenarios 3 and 4, the community population reaches 20,000 and 25,000, respectively. The results showed that under the same BI value and mosquito mortality conditions, the larger the community population, the smaller the number of imported cases needed to cause local dengue fever transmission, and the higher the transmission risk. Moreover, we changed the BI value and mosquito mortality, and found that the lower the mortality rate, the higher the BI, the higher the transmission risk of dengue fever. Especially when the BI value is above 3 and the mortality rate is <1/26, only one dengue fever case is needed to produce an indigenous DF case, see Figures 5C, D.

Discussion

DF cases was first reported in Foshan city in China in 1978. The dengue virus then spread widely to Guangdong, Guangxi, Hainan and Fujian province, causing hundreds of thousands of patients (25). As a main economic city in Fujian Province, Xiamen has a suitable climate and a large population mobility, resulting in a heavy public health burden caused by DF. In this study, we used the transmission dynamics model to quantitatively evaluate the

TABLE 4 Insecticide resistance of *Ae. albopictus*.

Pesticides	(Adjusted) mortality rate		Population determine	
	Huli	Xiang'an	Huli	Xiang'an
0.2% bendiocarb	100%	100%	Sensitive species	Sensitive species
0.2% fenitrothion	96%	99.24%	Likely resistant species	Sensitive species
0.03% deltamethrin	93%	24.07%	Likely resistant species	Resistant species
0.04% permethrin	64%	53.57%	Resistant species	Resistant species
0.5% propoxur	100%	95.71%	Sensitive species	Likely resistant species
0.5% malathion	99%	99.31%	Sensitive species	Sensitive species
0.08% beta-cypermethrin	38%	30.89%	Resistant species	Resistant species
0.07% lambda-cyhalothrin	75%	66.67%	Resistant species	Resistant species
2% chlorpyrifos	100%	100%	Sensitive species	Sensitive species
0.4% beta-cypermethrin	43%	68.87%	Resistant species	Resistant species

TABLE 5 Insecticide resistance of *Ae. albopictus* larvae.

Pesticide	Population	Regression equation	LC50 (mg/L)	95% CI	Resistance multiple
Disulphion	Sensitive strain	$y = 21.47 + 8.71x$	3.43×10^{-3}	3.31×10^{-3}, 3.55×10^{-3}	
	Huli	$y = 9.65 + 4.1x$	4.42×10^{-3}	4.12×10^{-3}, 4.71×10^{-3}	1.29
	Xiang'an	$y = 9.17 + 4.13x$	5.98×10^{-3}	5.57×10^{-3}, 6.48×10^{-3}	1.74
Propoxur	Sensitive strain	$y = 7.23x - 0.15$	1.05	0.95, 1.13	
	Huli	$y = 4.62x - 2.38$	3.27	3.08, 3.49	3.12
	Xiang'an	$y = 3.33x - 1.64$	3.11	2.84, 3.42	2.97
Pyripropoxyfen	Sensitive strain		1.01×10^{-5}	6.24×10^{-6}, 1.45×10^{-5}	
	Huli	$y = 1.907 + 0.531x$	$2.57 \times 10 \times 10^{-4}$	1.54×10^{-4}, 4.03×10^{-4}	25.55
	Xiang'an	$y = 2.837 + 0.740x$	1.48×10^{-4}	9.4×10^{-5}, 2.24×10^{-4}	14.71

influence of community population, mosquito density and mosquito-borne insecticide resistance on DF transmission in Xiamen City, so as to promote the formulation of more effective prevention and control strategies.

Population risk assessment

In 2019, the distribution of DF cases in Chinese mainland has expanded significantly, with 1,066 regions reporting imported cases and 550 regions reporting indigenous cases (26). From 2005 to 2019, the imported cases of DF in Xiamen City showed a rapid upward trend. In 2019, Xiamen reported a total of 119 imported cases from multiple regions and countries, mainly distributed in Huli and Siming District. The public health and epidemic prevention departments of these two administrative districts need to pay extra attention to the transmission of imported cases to secondary cases in the local areas. The indigenous cases were reported mainly from August to October, for the main reason may be that the temperature and humidity in summer are suitable for mosquito-borne growth and reproduction, leading to the local spread of dengue virus (12, 27). Imported cases have also been reported in 11 months except March. We believe that Xiamen City, as a coastal city, has frequent communication with Southeast Asian countries and regions, and there are many imported cases reported during the high incidence of DF in summer. Accordingly, the customs and epidemic prevention departments should pay attention to the movement of personnel from areas with high incidence of DF and do a good job of port education on DF prevention, which can effectively reduce the local spread and even outbreak of DF. The reported cases are mainly concentrated in the middle-aged and young people aged 21–50, and the majority of them are professional engaged in commercial services. According to the study, these people are more susceptible to DF infection due to their active work. Therefore, the education and medical investigation of these people should be strengthened as part of daily prevention work.

Mosquito vector risk assessment

According to our research, we selected Huli District and Xiang'an District of Xiamen City to investigate mosquito-borne ecology and monitor insecticide resistance. The results showed that the average value of BI and CI in Huli District were 9.3, 5.3, then 6.1 and 6.4 in Xiang'an District, all of which were higher than the safety level of 5. In this cases, larvae proliferated rapidly, increasing the risk of dengue virus transmission (27). At this point, when imported cases occur in the jurisdiction, DF might spread or even spread locally. At the same time, we measured the number of adult mosquitoes by HDN method, and the results showed that the average of HDN in

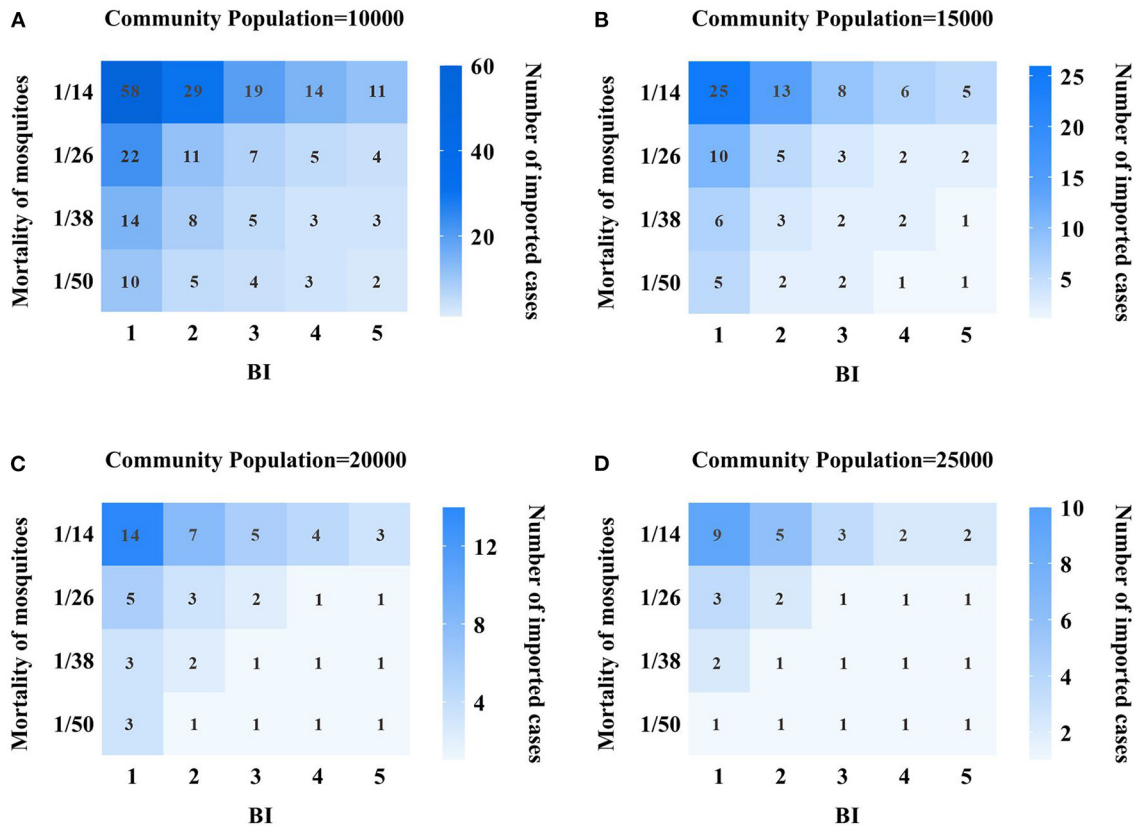

FIGURE 5
Quantitative assessment results of DF transmission risk in Xiamen City. **(A)** 10,000 population community; **(B)** 15,000 population community; **(C)** 20,000 population community; **(D)** 25,000 population community; the vertical index represents the mortality of mosquitoes, and the value is 1/15, 1/26, 1/38, and 1/50 respectively, and the horizontal indicator represents the value of BI, which are 1, 2, 3, 4, 5, respectively. The values in each grid represent the number of dengue cases that need to be imported to cause local transmission, and the darker the grid, the more dengue cases need to be imported.

Huli District was 3.4, which gradually decreased after August, while the HDN in Xiang'an District was always at a low level, just similar to the number of indigenous cases. As previously monitored, *Ae. albopictus* in Xiamen is sensitive to malathion and propoxur, and has high resistance to pyrethroid insecticides such as deltamethrin, beta-cypermethrin, and beta-cypermethrin. Thus, we selected mosquitoes in two districts to carry out insecticide resistance tests. The results illustrated that *Ae. albopictus* in Huli District was sensitive to warfarin, fenitrothion, propoxur, malathion, and chlorpyrifos, and had higher resistance to other insecticides. *Ae. albopictus* in Xiang'an District was only sensitive to warfarin, fenitrothion, and chlorpyrifos, showing different resistance levels to other insecticides. Based on previous studies, mosquito resistance to chemical insecticides is inevitable, so we need to avoid using chemical insecticides blindly (28, 29). The local public health authorities can scientifically assess the risk of mosquito-borne transmission on the basis of experiments, rationally select sensitive and low-resistance pesticides, and use them in crop rotation on an annual basis. This can effectively present the increase of resistance and achieve the purpose of reducing mosquito density.

Model evaluation

In this study, the ODE dynamics model was used to investigate the impact of potential risk factors such as community population, number of imported cases, mosquito vector density and mosquito insecticide resistance on the local transmission of DF in Xiamen. We set up four scenarios with simulated community population of 10,000, 15,000, 20,000, and 25,000, and input different numbers of DF cases to quantitatively evaluate the spread of indigenous DF cases. In the model, the density of mosquito vectors is reflected by the change of BI index, while the insecticide resistance of mosquitoes is assumed to change the birth rate and mortality rate of mosquitoes. According to our simulation results, when the community population is 10,000, the mortality rate of mosquitoes is 1/14 of the highest value in the set range, and the BI index is 1, both insecticide resistance and mosquito vector density of mosquitoes are at the lowest value, so 58 imported DF cases are needed to cause the spread of indigenous DF cases. As the density of mosquito vectors gradually increases, the number of imported cases required for indigenous DF transmission gradually decreases. Similarly, when the mortality rate of mosquitoes is reduced to 1/50, that is, mosquito resistance to insecticide is very high, the results show that only 10 imported cases can result in secondary local cases. This result suggests that public health departments should do a good job in killing mosquito vectors and choose sensitive and low-resistance chemical. In addition, in the model, we found that the birth rate of mosquitoes has little influence on the model, when we change the birth rate parameters, a certain number of dengue cases are input within the set range, which cannot cause the spread of indigenous cases.

When the community population gradually increased to 15,000, 20,000, and 25,000, the results of model simulation showed that when the mosquito mortality rate was 1/14 and BI value was 1, the number of imported cases needed to cause indigenous DF cases was 25, 14, and 9, respectively, that is, when mosquito-borne insecticide resistance and mosquito-borne density were consistent, the community population base had a great influence on local transmission of DF. Therefore, CDC should do well in health education and health prevention and control in large communities to effectively improve the prevention and control effect of DF. In the meantime, we found that when the community population is more than 20,000, the mosquito density is more than 3, and the mortality rate of mosquitoes is $<1/38$, only one case can be imported, which can cause the spread of indigenous DF. At this time, the risk of transmission is very high, so mosquito prevention and control work mainly in large communities is needed to prevent the synergistic effect of these key factors from causing the spread of indigenous DF.

The prevention and control of dengue fever is a social work, that requires the participation of health and epidemic prevention departments and the public. For places where DF is less threatening, we can mobilize the masses to carry out environmental prevention and control measures through community departments, so as to reduce the mosquito density in the external environment. For places where DF is a great threat, we suggest that public health departments should adopt chemical control during the high incidence period of DF (July to October) to reduce the risk of DF transmission, and select sensitive and low-resistance chemical insecticides to reduce the risk of DF transmission.

Limitation

It is worth noting that there are some limitations to our research. First of all, infectious disease outbreak assessment includes three steps and two factors, but in this study, we did not discuss the impact of environmental factors on mosquito vectors. Secondly, because DF is transmitted through vector, we can't evaluate the transmission ability of dengue fever by calculating R_0 (the basic reproduction number) or R_{eff} (the effective reproduction number). In the future work, we will also explore a feasible way to solve these problems.

Conclusions

In this study, propagation dynamics model was used to assess and predict the risk of DF. We emphasize that imported cases, community population, mosquito density and insecticide resistance play a key role in local DF transmission. Mosquito insecticide resistance has been identified as the most critical factor for evaluating DF communication risks and implementing management control measures. So public health authorities in China should pay more attention to mosquito control. The change of mosquito-borne birth rate and mortality rate can be regarded as indicators of mosquito-borne insecticide resistance. We suggest that detection of threshold effects of the number of imported cases, mosquito density and the changes of birth rate and mortality rate of local mosquito vectors tested by the transmission dynamics model can be used to predict and evaluate the risk of dengue fever epidemic. The identified factors are beneficial to the establishment of early warning and monitoring system of infectious diseases.

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

TC, ZG, WL, and XL designed research. ZG, WL, XL, BA, SiW, BD, TY, JH, LLe, and ZZ analyzed data. WL, ZG, BA, LLu, and SiW completed the experiment. TC, ZG, WL, XL, BA, BD, LLe, ZZ, ZL, PL, CL, and LLu conducted the research and analyzed the results. TC, ZG, WL, XL, BA, and LLu wrote the manuscript. All authors read and approved the final manuscript.

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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

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Mosquito-borne viruses causing human disease in Fennoscandia—Past, current, and future perspectives

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Five different mosquito-borne viruses (moboviruses) significant to human disease are known to be endemic to Fennoscandia (Sindbis virus, Inkoo virus, Tahyna virus, Chatanga virus, and Batai virus). However, the incidence of mosquito-borne virus infections in Fennoscandia is unknown, largely due to underdiagnosing and lack of surveillance efforts. The Fennoscandian moboviruses are difficult to prevent due to their method of transmission, and often difficult to diagnose due to a lack of clear case definition criteria. Thus, many cases are likely to be mis-diagnosed, or even not diagnosed at all. Significant long-term effects, often in the form of malaise, rashes, and arthralgia have been found for some of these infections. Research into mobovirus disease is ongoing, though mainly focused on a few pathogens, with many others neglected. With moboviruses found as far north as the 69th parallel, studying mosquito-borne disease occurring in the tropics is only a small part of the whole picture. This review is written with the objective of summarizing current medically relevant knowledge of moboviruses occurring in Fennoscandia, while highlighting what is yet unknown and possibly overlooked.

KEYWORDS

arbovirus, mosquito-borne virus, mobovirus, epidemiology, Fennoscandia, Sindbis virus

Introduction

During the last decade, emerging viral diseases have increased considerably in incidence in regions all over the world, many of these previously neglected as sporadically occurring viruses of limited importance to public health. Of these viral diseases, arboviruses (**ar**thropod-**bo**rne) have seen the greatest global spread in recent times, with dengue, chikungunya, Zika and yellow fever seeing unprecedented spread from 2015 onwards (1). The global burden of vector-borne viruses cannot be overstated; with 80% of the population at risk for one or more vector-borne disease, and already causing 17% of the global burden of communicable disease, including 700,000 deaths annually, their impact on human society is clear (1).

Arboviruses are defined as viruses replicating within arthropods and are then spread to vertebrate hosts. These viruses, belonging to a group of between 500 and 600 viruses including the families of *Peribunyaviridae*, *Phenuiviridae*, *Flaviviridae*, *Reoviridae*, *Rhabdoviridae*, and *Togaviridae*. Viruses belonging to *Peribunya-*, *Phenui-*, *Flavi-*, and *Togaviridae* are known to be transmitted by hematophagous (i.e., blood-feeding) insects and other arthropods ranging from ticks to mosquitoes (2–4). For example, most flaviviruses in Fennoscandia are transmitted by ticks with a key example being the tick-borne

encephalitis virus (TBEV). This review focuses on the viruses spread by mosquitoes, known as **mo**squito-**bo**rne **viruses**, or moboviruses.

While the number of mosquito-borne diseases and their incidence in Europe is far lower than those of tropical regions, they are nevertheless a substantial contributor to infectious disease morbidity (5). With changing climates, future outbreaks may occur in areas where populations are immunologically naïve, and public health systems are unprepared (1, 3, 6, 7). Historically, mosquito-borne pathogens have been a major source of disease in Europe, with malaria being widespread as far north as Fennoscandia until the mid to late nineteenth century (8, 9). In more recent times, moboviruses belonging to the California encephalitis group of genus *Orthobunyavirus*, family *Peribunyaviridae* have been isolated as far north as the Finnmark region of Norway, in the village of Masi (69°26′N) (10). With appropriate, competent vectors found throughout Fennoscandia, it may only be a matter of time before mosquito-borne disease returns to be the major public health concern it once was in Fennoscandia, and currently is in more temperate regions.

Fennoscandia is the geographical peninsula that includes Scandinavia, in addition to Finland, Karelia and the Kola peninsula. This is distinct from Fenno-Scandinavia, which is simply Finland and Scandinavia. For the purposes of this review the term Fennoscandia, as well as the region it represents was used because of the relative ecological unity in the subarctic climate of the area in respect to which vectors are present, and which viruses they carry. This ecological unity would not be as clear if Iceland or Denmark were included, or Finland omitted.

The mosquitoes (*Culicidae*) have ever since they were first identified as the vectors of yellow fever (11), been at the center of medico-entomological research due to their significance as efficient vectors of both human and animal disease (4). Mosquitoes are very adaptable vectors, capable of thriving in a large variety of environments; from lakes to water-filled footprints, there's hardly any aquatic habitat unsuitable for mosquito larvae (4). Large areas in Fennoscandia are covered by forests and there are a multitude of wetlands and water bodies. This in combination with a relative high precipitation makes the area favorable for mosquitoes (12). Finland features 43 different recorded blood-sucking mosquito species, and Norway 38. In Sweden there are around 50 different species, with most of the additional species being found further south than the southernmost regions of Finland and Norway (12–15).

In our changing climate, it is possible for invasive species of mosquitoes to spread disease previously unknown to that area, as was observed in Europe when the chikungunya outbreak occurred in Italy in 2007 (16). Further autochthonous cases have been detected in southern Europe since then, especially in areas where the invasive species *Ae. albopictus* has been established (7, 17).

As previously highlighted, there are many gaps in the present knowledge of the transmission of moboviruses, and the diseases they cause in humans. By furthering the research into these viruses, and the way they are spread, better diagnostic criteria and new treatment options may be developed. Through this, cases of human infection may be detected earlier, and morbidity as well as mortality reduced. Through improved early surveillance, case series may be prevented before they develop into outbreaks. Together, all these steps may lead us onto our ultimate goal, reducing patient suffering, by coming up with useful therapeutics and vaccines for the management and prevention of disease spread.

Viral genetics and structure

The Fennoscandian arboviruses known to be human pathogens fall under the families *Peribunya-*, *Flavi-*, and *Togaviridae*. Of these, the ones spread by mosquitoes all fall under either the genera *Orthobunyavirus*, in the family *Peribunyaviridae*; or *Alphavirus*, the sole genus in the family *Togaviridae*, while the flaviviruses are transmitted by ticks. (18, 19). Accordingly, only these two genera will be further described in this review.

Alphaviruses are enveloped viruses with a single-stranded positive sense RNA genome of 10–12 kb housed in spherical virions, around 70 nm wide. They are characterized by their impressive host range, including vertebrate hosts such as humans, non-human primates, equids, birds, amphibians, rodents, and pigs, as well as sea mammals and fish. Sindbis virus is the sole member of *Alphavirus* found in Fennoscandia (2, 20).

Orthobunyaviruses have a segmented genome, with three segments of negative-sense RNA (S, M and L) of 12.4 kb in total; housed in enveloped, spherical virions of 80–120 nm in diameter. It is the largest and most diverse genus in the family, with a wide range of vertebrate hosts including squirrels, bats, rabbits, ungulates, sloths, and birds. The orthobunyaviruses found in Fennoscandia are Chatanga virus, Inkoo virus, Tahyna virus, and Batai virus. Of these four, all but Batai virus falls under the California serogroup, grouped together by their similarity to the prototypical California encephalitis virus (CEV) (21, 22). The phylogeny of relevant viruses in the genus *Orthobunyavirus* is summarized in Figure 1.

Regarding viral characteristics of transmission, the two genera differ in their method of genetic recombination. The positive sense single-stranded genomes of alphaviruses undergo frequent recombination, associated with genetic diversity (25). The segmented genome of *orthobunyaviruses* allow for reassortment to occur, while recombination is rare. This has been implied to play an important role in *Orthobunyavirus* emergence and virulence, especially though interspecies transmission. (26).

Epidemiology and disease

Sindbis virus

Sindbis virus (SINV) is the most clinically important of the Fennoscandic arboviruses (19), and is the sole member of the genus *Alphavirus* present in Fennoscandia. SINV was first isolated in 1952 near the village of Sindbis in Egypt, with the first case of human disease reported in 1961 (27). In Sweden, SINV was first isolated in the Swedish village of Ockelbo in 1982 and therefore called Ockelbo virus. It is very closely related to SINV-I, the only genotype out of six recognized (I-VI) associated with human disease (28), in both structure, pathogenicity, and antigenicity. It differs from the other SINV strains found in mainland Europe in that it is most closely related to the South African, suggesting that SINV was likely introduced to Fennoscandia by migratory birds, which have been observed to act as amplifying hosts to SINV, rather than spreading *via* mainland Europe (29, 30). The South African origin has however been challenged, due to a suggested origin in Central Africa found by Ling et al. (28).

The human disease caused by SINV, known as 'Ockelbo fever' (Sweden), 'Pogosta disease' (Finland), 'Karelian fever' (Russia), and generally as 'Sindbis fever'. Other terms include "August–September

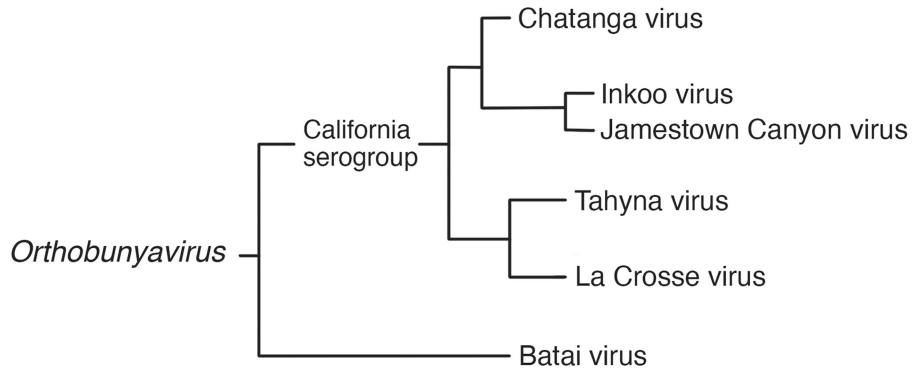

FIGURE 1
Phylogram of selected viruses from the *Orthobunyavirus* genus, including the three Fennoscandian moboviruses in the genus (19, 21, 23, 24).

disease" (*augusti-september-sjukan*) and "berry-pickers disease" (*bärplockarsjukan*, *bærplukkersyken*), named after the time-period and population in which it is mostly diagnosed (31). SINV causes occasional epidemics in Fennoscandia, characterized by a febrile maculopapular rash with myalgia and polyarthritis, of which the last symptom often persists the longest (32). Despite the avian SINV seroprevalence being widespread in Australasia and Africa, human SINV infection only appears clinically apparent in Northern Europe (mainly 60°–64°N) and South Africa, where the SINV-I genotype is dominant (27, 30, 33, 34).

SINV has an incubation period of 5–7 days before the onset of symptoms, after which IgM and IgG are detectable within 8 and 11 days respectively, resulting in a delay of 2–3 weeks between initial virus acquisition and the time a serological diagnosis can be made (35), at which point the extra articular symptoms have usually diminished. The articular symptoms may however persist for several months, in some cases even several years (36, 37). In Fennoscandia, SINV is mainly vectored by the ornithophilic mosquitoes *Aedes communis, Ae. cinereus, Ae. excrucians, Coquillettidia richiardii, Culex pipiens, Cx. torrentium*, and *Culiseta morsitans*. Additionally, it has also been found in *Anopheles maculipennis sensu* lato (18, 38–41). Of these species, *Ae. communis*, *Ae. cinereus*, *Ae. exrucians*, *Cq. richiardii* also readily feed on humans (42).

The present literature is unclear whether the variants of human disease (Ockelbo fever, Pogosta disease, and Karelian fever) represent separate entities caused by different strains or are simply local variants in nomenclature. Though fever and rash are present in all three, some characteristics vary between the diseases, suggesting that they may indeed be separate; such as paresthesia being reported only in Ockelbo disease, while Karelian fever rarely features chronic arthritis or arthralgia (2). Sequence analysis of regional samples has not proven the three diseases to be caused by distinct viral strains, see Figure 2. While human infection tends to be subclinical more often than evident, the ratio between subclinical to clinical infection appears to vary by region, with ratios of 20:1–40:1 reported in Sweden and 17:1 in Finland (27). It is worth noting that in Fennoscandia, SINV is a notifiable disease only in Finland (28). In the clinical setting, diagnosis may be made more difficult by a non-specific array of these symptoms, where the rash might be confused for one caused by rubella virus in Pogosta disease, and parvovirus B19 in Ockelbo disease (2, 30, 45).

Incidence rates also vary between countries, with reported figures ranging from 2.9/100000 for Ockelbo fever, 2.7/100000 for Pogosta disease, and 18/100000 for Karelian fever in their respective regions, see Table 1 (30, 34). Seroprevalence is similar, 2.9% in northern Sweden, and 2.5% for Finland; the studies also found increasing seroprevalence with age (34, 46, 47). The SINV infections are all more commonly observed in late summer to early fall. Pogosta disease features an additional pattern of larger endemic outbreaks every 7 years, a pattern not reported in the other regional variants. This remarkable pattern of cyclical outbreaks held true from the first outbreak in 1974 until 2009, when case numbers, although slightly higher than previous years, were significantly lower than those 597 seven cases reported in 2002 (30, 48). The cyclical pattern has not yet returned, it is not yet known what caused the cyclical pattern, or what caused it to cease. Several previously held theories were disproven by the cycle breaking, however grouse populations, a non-migratory bird and important vertebrate host for SINV in Finland, were at a record low in 2009, and did not recover until 2018, which might offer a partial explanation (32). The pattern may have returned, as a new major outbreak occurred in the fall of 2021, when 556 cases were recorded in Finland (49).

Inkoo virus

Inkoo virus (INKV), as well as the later mentioned viruses, all belong to the *Orthobunyavirus* genus, INKV is within the California serogroup, a group of serologically and genetically related orthobunyaviruses, all of which are presumed arboviruses, and many recognized human pathogens (50). INKV seropositivity has been recorded in northern Sweden as being significantly higher in men (46.9%) than in women (34.8%), weighted average 40.9% (51). The equivalent figure in Finland was 51.3% (52). Seroprevalence in Norway has not been extensively studied in humans, though a 1985 survey of seroprevalence in Norwegian soldiers 22% of the recruits tested displayed antibodies to California serogroup viruses (53). High IgG seroprevalence has been reported in reindeer (22). Human disease caused by INKV is often asymptomatic, with evident disease characterized by an influenza-like illness. INKV has been linked to cases of neuroinvasive disease in both adults and children, were the latter appeared to have a more severe form of the disease (23, 54). First isolated in *Aedes communis* and *Ae. punctor* in Finland in 1964, INKV has later been found in *Ae. hexodontus, Culex torrentum, Cx. Pipiens* and *Culiseta morsitans* as well. *Ae. communis* and *Ae. punctor* have been observed to have the capacity to act as vectors (23, 27, 38, 55).

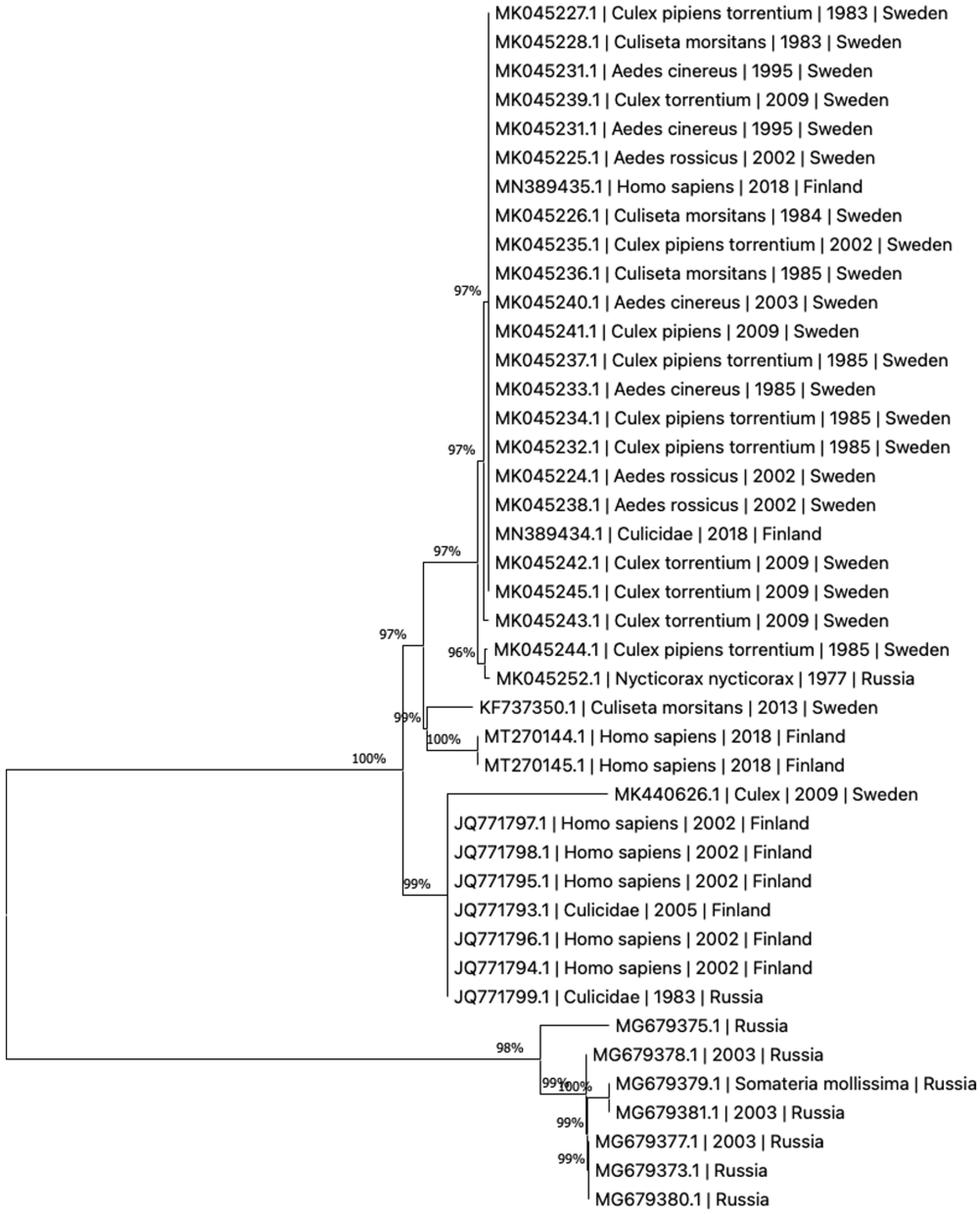

FIGURE 2
Phylogenetic tree based on whole genome sequences of Sinbis virus (SINV) isolates in Finland, Sweden and Russia. The sequences were downloaded from NCBI Virus (43). Analysis was performed by ClustalW alignment and phylogram constructed by a maximum likelihood algorithm, both implemented in MEGA11 (44).

INKV, along with the related Tahyna virus, are two of the most common mosquito-borne California group viruses in Eurasia, and have therefore been recommended for close public health surveillance by the World Health Organization (3) Despite of this, INKV is not highly represented in the literature, with only 27 results in PubMed in the last 20 years.[1]

Tahyna virus

Tahyna virus (TAHV) is closely related to La Crosse virus (LACV), first isolated in the Ťahyňa and Križany villages in what is now Slovakia in 1958 (56), the first mosquito-borne virus to be isolated in Europe (57, 58), TAHV now occurs in most countries of continental Europe (18, 55), with high TAHV antibody prevalence (60%–80%) in human populations in endemic regions, suggesting it to be widespread (59). Yet, only a few isolates have been produced in Fennoscandia (10, 18, 27, 39). Human disease caused by TAHV, known as 'Valtice fever' has been documented to cause an influenza like illness, characterized by fever and respiratory symptoms, and in rare cases central nervous

1 PubMed search:" Inkoo OR INKV," results by year: 2002–2022. Retrieved 2022-07-05.

TABLE 1 Epidemiology of mosquito-borne viruses in Fennoscandia summarized.

Virus	First isolate in Fennoscandia[a]	Known distribution	Main symptoms or effects of human disease	Seroprevalence in population (%)	Incidence/ year (per 100,000)	Incubation period (days)
SINV	1982 (Sweden)	Norway, Sweden, Finland	Fever, rash, arthralgia	2.5 (Finland, Sweden)	2.7 (Finland)	5–7
INKV	1964 (Finland)	Norway, Sweden, Finland	Fever, influenza-like illness; encephalitis	51 (Finland) 41 (Sweden)	-	-
TAHV	1978 (Norway)	Norway, Sweden	Influenza-like illness; respiratory disease, meningoencephalitis	-	-	3–7
CHATV	2007 (Finland)	Finland	Fever, influenza-like illness; encephalitis	-	-	-
BATV	1985 (Sweden)	Norway, Sweden, Finland	Influenza-like illness	<1 (Norway, Sweden, Finland)	-	-

SINV, Sindbis virus; INKV, Inkoo virus; CHATV, Chatanga virus; BATV, Batai virus; TAHV, Tahyna virus.
[a]First isolates in mosquitoes.

system involvement in the form of meningoencephalitis. The disease appears more common in children than adults (18, 55, 56, 59). An incubation period of 3–7 days has been recorded (60).

According to the literature, cases appear limited to mainland Europe, with no reported cases of human disease caused by TAHV reported within Fennoscandia. Due to limited data, it is unclear whether this variance is due to limited TAHV spread, or factors related to the virus itself. TAHV is mainly spread by *Aedes* spp., principally *Ae. vexans* or *Ae. cantans*, though it has also been isolated in other species, such as *Culiseta annulata* and *Culex modestus*. The anthropophilic nature of *Ae. vexans* has been proposed to account for the high antibody rates found in human populations living in endemic areas (3, 54, 57).

Chatanga virus

Originally isolated in Russia in 1987, Chatanga virus (CHATV) was first isolated in Fennoscandia from *Aedes* spp. mosquitoes collected in Finland in 2007 (52). It has been conjectured that CHATV may be more widespread than previously indicated, due to its antigenic similarity to the more widely studied INKV, which has been reported to have a significant seroprevalence in endemic regions (52). CHATV is described very little in the literature,[2] but is suspected to behave similarly to INKV in human disease. Putkuri, et al. reported two patients hospitalized with symptoms of fever, headache and nausea were confirmed to be CHATV infections by plaque reduction neutralization testing (PRNT) (23). The patients showed no signs of neuroinvasive disease, as seen in INKV infection.

Batai virus

Batai virus (BATV) is notable amongst the European orthobunyaviruses in that, unlike the others, it is not a member of the California serogroup, but rather the Bunyamwera serogroup (61). Originally isolated in Malaysia in 1955, BATV was first isolated in Sweden from *Ae. communis* mosquitoes collected in central Sweden 1983–1985 (38). BATV is one of the most geographically widespread orthobunyaviruses, ranging from Malaysia, and India, to most countries in central Europe. Evidence for Fennoscandic circulation has been reported in Norway, Sweden and Finland, with a seroprevalence of around 1% (61). The role of BATV in human disease is not entirely clear, with some cases of disease similar to other orthobunyaviruses being reported in China, though these were identified to involve naturally occurring reassortments with S and L segments from Bunyamwera virus (2, 62). In Africa and Asia, BATV has been described as a non-specific febrile illness, while European cases have been influenza-like in character (63). Fever, bronchopneumonia, tonsilitis and gastritis have all been associated with human BATV infection in former Czechoslovakia (27, 64).

Pathophysiology of mosquito-borne viral arthralgia and neuroinvasion

As previously discussed, the morbidity of these viral infections in humans is primarily caused by arthralgia, as well as the potential long-term effects of neuroinvasive disease. In many cases the mechanisms behind these effects are poorly understood and understudied, with research in these areas having been mostly focused on the arthralgia of Chikungunya virus (genus *Alphavirus*) and the neuroinvasive disease of West Nile virus (genus *Flavirus*). (65, 66). Several mechanisms have however been identified. These include direct invasion of the joint, joint involvement by immune complex formation, and immune modulation causing a chronic inflammatory response occurring after the transient viremia of the acute phase of the disease. The specific mechanism varies by virus (66). For alphaviruses, who all share a similar mechanism of infection and replication, much of the inflammation is thought to be caused by pro-inflammatory cytokines and matrix metalloproteinases released by infected macrophages in the articular synovium. An inflammatory cycle is then continued by resident cells, who in are in turn infected themselves (67). In sequence alignment studies, another mechanism has been proposed, by which structural proteins in arthritogenic alphaviruses

2 PubMed search "(Chatanga OR CHATV) AND virus," returned 6 results. Retrieved 2022-07-06. Ovid MEDLINE search "(Chatanga OR CHATV) AND virus, returned 2 text results. Retrieved 2022-07-06.

are able to activate T cells similar to endogenous proteins implicated in rheumatoid arthritis (68).

Neuroinvasive disease requires the virus to evade the innate and adaptive immune response such that it may gain entry to the central nervous system (CNS); this is possible through either the neural route, the olfactory route, or the blood–brain barrier. (66) Neural transmission along the axon has not been associated with any mosquito-borne virus. The olfactory route, through infection of olfactory receptor cells in the nasal cavity, has however been demonstrated for both, alphaviruses such as the Venezuelan equine encephalitis virus and the Semliki Forest virus, and orthobunyaviruses such as the La Crosse virus (69, 70). Invasion of the blood–brain barrier, either by adherence to erythrocytes or by pinocytosis has been observed for several alphaviruses, including the Semliki Forest virus (69). Once within the CNS, neurons serve as the main target cells for both encephalitic alphaviruses and orthobunyaviruses (69). This infection triggers an inflammatory response in the neurons, releasing inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosing factor (TNF)-α, potentially exacerbating the neuroinflammation (71).

Transmission dynamics and vector competence

For an arbovirus to be successfully transmitted, a complicated series of events and interactions involving the virus, arthropod vector, vertebrate reservoir host and human, all depending on environmental and ecological factors must take place. It is these factors that limit the physiological ability of a species to act as a vector for a specific virus, it is this ability that is known as *vector competence*. In the prospective vector, a series of events of just as complex must take place. Virions acquired by the female mosquito during blood-feeding must pass through the gastro-intestinal system, first entering by passing the midgut infection barrier, through the midgut escape barrier, and into the hemolymph, through which it spreads into the other organs, ultimately into the salivary glands where it may replicate see Figure 3. If it successfully replicates and can pass through the salivary gland escape barrier, it may then be passed onto the vertebrate from which the mosquito takes its next blood meal. If the virus fails to pass through any of these barriers, the mosquito is simply a dead-end vessel for the virus, a so-called *mechanical vector* (58, 72). Because of this, one must keep in mind that a mere virus detection in a species does not necessarily mean that the species is a competent vector. A vector is only truly competent if the ingested virus passes through the organism as previously described such that viremia occurs in, and escapes from, the salivary glands. Thus, vector competence can only truly be determined if viremia in the saliva is measured, detection of virus in any other way might only indicate that the mosquito ingested a blood meal from an infected host (58). Therefore, confirming a species as a vector of a certain virus with certainty is a scientifically rigorous process requiring an infection study of several stages of *in vivo* and *in vitro* study. Vector competence studies have been performed on SINV with wild caught mosquitoes in Sweden (73–75), though no other studies have been performed yet in the rest of Fennoscandia, or on any of the orthobunyaviruses. Another special case is the so-called reservoir host. This occurs when an organism is infected by a vector but shows little susceptibility to the pathological effects of the virus. This organism may then develop sufficient viremia for a new vector to acquire the virus during blood feeding, without developing significant disease itself. Reservoir hosts may then maintain the endemic state of a virus, acting as sources for a virus even if the vectors are not themselves carrying the virus, such as during periods of climate to harsh for the mosquito vectors to survive in aduult form. This creates an ecological system, in which the virus may survive indefinitely (76).

Host-vector transmission as above requires that the infected mosquito was to feed first on an infected host, and then take a second blood-meal from a new host, with enough time in between to develop sufficient viremia itself. There are however a number of viruses capable of being transmitted from the infected female to the next generation (known as *transovarial transmission*) whereby only a single

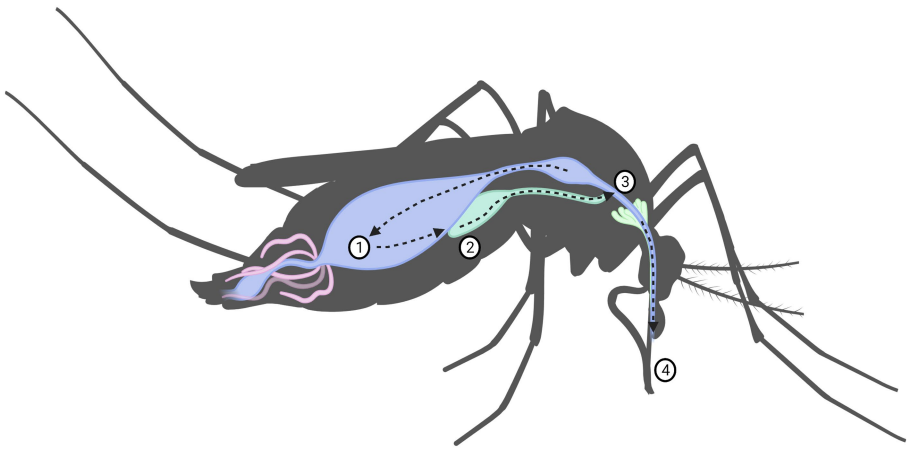

FIGURE 3
Virus replication in mosquito including the mechanical barriers designated: (1) midgut infection barrier, (2) midgut escape barrier, (3) salivary infection barrier and (4) salivary escape barrier.

blood-feeding would be sufficient. This will also amplify the effect, as it is not only a single mosquito that is infected, but possibly all of the brood which it lays. This also allows the virus to overwinter in species where the adults do not survive the winter. Of the 18 California serogroup viruses known, 9 have so far been shown to be transovarially transmitted by their mosquito vectors, including the aforementioned La Crosse virus, California encephalitis virus, Jamestown Canyon virus, Tahyna virus, Inkoo virus, as well as the Snowshoe hare virus, Keystone virus, Trivittatus virus, and Morro Bay virus (24, 50). This has also been demonstrated in SINV (77).

Diagnosis

As previously mentioned, for most of these mosquito-borne viruses, the diagnostic criteria that are to be used when assessing whether to include a suspected infection amongst to differential diagnoses are practically non-existent. These principles of inadequate diagnostic criteria are relevant even outside the scope of Fennoscandian arbovirus infections. The La Crosse virus (LACV) is a major cause of pediatric viral encephalitis in the United States, with a reported case fatality of up to 1.9% of confirmed cases (78). LACV encephalitis has been reported to often be mistaken for herpes simplex (HSV) or meningoencephalitis due to similar case presentations and can so often evade correct diagnosis (23, 78). Other viruses in the same California serogroup, e.g., Jamestown Canyon Virus, have also been reported to cause neuroinvasive disease, though these are reported more rarely, and are studied even less than LACV (23).

Diagnostics should be performed in a two-tiered approach, first testing for common and/or treatable etiologies, as well as those suspected based on specific risk factors. The second tier consists of broader, more invasive tests if previous diagnostic measures remain unsuccessful. Available methods for collecting material for laboratory diagnosis (Polymerase chain reaction; PCR, PRNT, antibody panels etc.) are sampling of blood, CSF by lumbar puncture when involvement of the central nervous system is suspected, serological testing for general malaise, and puncture of vesicles with sampling of exudate in vesicular rashes (79). These tests are then usually sent away for laboratory analysis at larger hospital laboratories. Although simpler kits for viral analysis are available, they are for research use only, and not rated for diagnostic use.

Due to the close genetic and antigenic relationships the orthobunyaviruses form, especially those in the California serogroup, cross reactivity in serology diagnostics makes differentiation between the viruses difficult, necessitating the use of neutralization assays (e.g., PRNT). These assays are not routinely used in clinical practice, except for in certain cases, eg. when certain notifiable diseases are suspected, as they are expensive and usually only available at some university hospitals (22). Some of these infections have well developed serological criteria, such as SINV, in which sensitivities of IgM and IgG enzyme immunoassays were reported as 97.6 and 100%; specificities were 95.2% and 97.6%, respectively (80).

Indeed, in many cases the diagnoses is only possible after the fact, often once an outbreak has already occurred and an increase in similar case presentations appear, a phenomenon observed in the SINV outbreak in Sweden in 2013. IgG may then be used to confirm seroprevalence. (36).

Treatment

Treatment of the acute infection is of these moboviruses are strictly limited to the symptomatic, as no specific treatment is available. Also, no vaccine or prophylactic medication has of yet been made available (81).

Though most of the human infections caused by these viruses are generally mild in symptoms, the long-term effects may cause significant morbidity. In a follow-up study of patients with serologically confirmed Pogosta disease, only 50% of patients were found to be symptomless 2.5 years after onset (82). In a study conducted in northern Sweden, seropositivity for SINV was found to be an independent predictor of having had a stroke, odds ratio 4.3 (36, 46).

It is not clear why only some patients infected with orthobunyaviruses develop symptoms. In the case of INKV, it has been theorized that underlying disease or trauma may be a prerequisite for causing entry across the blood–brain barrier, whereby neuroinvasive infection may then occur (23).

So far, there has been little research into the treatment of infections caused by these moboviruses. Limited by few cases being identified in the early stages, very little has been done in situations resembling randomized-control trials. As such, not much is known about optimal treatment, as most infections in the literature were treated either symptomatically, or as the more common disease the infection was mistaken for.

Insights from a clinician's perspective

When discussing the subject of this review with Fennoscandian colleagues in medicine, not specifically engaged in infectious medicine, the first question is inevitably: "Hold on, are there mosquito-borne diseases around here?"

The true incidence of California serogroup virus infections in Fennoscandia is unknown, largely due to underdiagnosing and lack of surveillance efforts (23). It is therefore impossible to estimate the effects of these viruses in the human population, as many cases are likely to be misdiagnosed, or even not diagnosed at all. One could easily imagine a case of INKV going undetected, as most patients will not seek medical care for a fever without complications. Should a patient in their early 60's develops arthritis, one could easily imagine that the symptoms would just be thought of as "a part of normal aging" and no more would be thought of that. In comparing the reported seroprevalence with incidence of human disease for SINV, it becomes evident that the disease is either unreported in many areas, or the disease may take a different form itself. With present diagnostic criteria and case reporting, it is not clear which, if any, is closer to the truth.

It is especially important for general practitioners, who are most likely to see these cases first, to pay attention to infections presenting as fever, rash and/or joint symptoms, especially during late summer to early fall, i.e., from August to September in the Northern Hemisphere. As previously discussed, these infections lack clear case criteria, and can often mask as more commonly seen infections, such as parvovirus B19 or other virus infections with rash in younger patients. In these younger patients, mosquito borne infections are generally not considered as readily, due to the reputation many of

these infections have of only occurring in women of late middle-age, which is not the case (2, 83). As many of these infections, particularly SINV, generally occur in outbreaks, being aware of how many patients in the area have presented with similar symptoms may be of great value.

Future perspectives

One must keep an eye open for the possibility of novel moboviruses being established in Fennoscandia, with viruses such as the Usutu virus and antibodies to West Nile virus already having been found in avian hosts in Sweden (84, 85), human cases may appear soon, although no autochthonous cases has been recorded yet. Early detection of invasive moboviruses, would be greatly aided by cooperation between clinicians and other key players, such as medical researchers, entomologists, veterinarians, and policy makers, using a One Health perspective.

Only with increased awareness of the diseases may the morbidity caused by prolonged infections be reduced. These developments, when in place, will also make the healthcare system more adaptable through preparedness, should a previously unknown virus become endemic in the future or indeed, an endemic mobovirus may, e.g., find more potent vectors and/or more suitable climate, and cause outbreaks (12).

Data availability statement

The original contributions presented in this study are included in the article, further inquiries can be directed to the corresponding author.

Author contributions

LW, CA, ME, and OL conceived, designed, and coordinated the writing of the whole manuscript. LW and OL contributed to data collection. All authors contributed to critically revised and approved the final version of this manuscript.

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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

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Severe Chagas disease in Ecuador: a countrywide geodemographic epidemiological analysis from 2011 to 2021

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Background: Chagas disease is a neglected and often forgotten tropical disease caused by the *Trypanosoma cruzi.* This parasite can be transmitted through the direct contact of human skin with feces and urine of the triatomine insect. According to the World Health Organization (WHO), an estimated 6–7 million people are infected worldwide, killing at least 14,000 every year. The disease has been reported in 20 of the 24 provinces of Ecuador, with El Oro, Guayas, and Loja being the most affected.

Methodology: We analyzed the morbidity and mortality rates of severe Chagas disease in Ecuador on a nationwide, population-based level. Hospitalization cases and deaths were also examined based on altitude, including low (<2,500m) and high (>2,500m) altitudes, according to the International Society. Data was retrieved from the National Institute of Statistics and Census hospital admissions and in-hospital mortality databases from 2011 to 2021.

Results: A total of 118 patients have been hospitalized in Ecuador since 2011 due to Chagas disease. The overall in-hospital mortality rate was 69.4% (*N*=82). Men have a higher incidence rate (4.8/1,000,000) than women, although women have a significantly higher mortality rate than men (6.9/1,000,000).

Conclusion: Chagas disease is a severe parasitic condition that primarily affects rural and poorer areas of Ecuador. Men are more likely to be infected due to differences in work and sociocultural activities. Using average elevation data, we conducted a geodemographic analysis to assess incidence rates by altitude. Our findings indicate that the disease is more common at low and moderate altitudes, but recent increases in cases at higher altitudes suggest that environmental changes, such as global warming, could be driving the proliferation of disease-carrying vectors in previously unaffected areas.

KEYWORDS

Chagas disease, parasites, neglected disease, high altitude, tropical, *Trypanosoma cruzi* (*T. cruzi*)

1. Introduction

Chagas disease (CD) is a neglected and often forgotten anthropozoonotic disease, first described by Chagas (1). This vector-borne disease is caused by a protozoan euglenoid parasite named *Trypanosoma cruzi*, which enters the human body after close contact with feces or urine from its vector, the triatomine insect also known as the "kissing bug" (2). Triatomes are arthropods from the Reduviidae family, comprising 128 recognized species classified as 17 genera in five tribes (3–5).

This disease, also called American trypanosomiasis, is endemic to Latin America and has been described in at least 21 countries. Although some reports suggest that CD has spread, rarely in the United States, Canada, Europe, and the Eastern Mediterranean region (4), the cases reported in these countries come mostly from emigrants from endemic countries and from people who traveled to countries where the disease is endemic and ended up catching it (6, 7). According to the World Health Organization (WHO), approximately 70 million people live in areas of high risk of infection; at least 6 to 7 million people are infected by this parasite each year, and roughly 30,000 new cases, including 9,000 newborns are diagnosed during prenatal and post-natal periods and around 14,000 deaths are attributed to this disease each year (2).

Transmission of CD occurs through direct contact with feces and urine from the triatomine insect (8). Once this type of arthropod bites a human, it defecates and urinates near the lesion (9). The parasites enter the body when the human instinctively scratches the bite and tears the skin's integrity, allowing the insect's feces to enter the inner tissues (10). Although other forms of transmission have been described, such as vertical transmission (congenital), through blood transfusion and organ donation, and even via contaminated food, the most common mechanism is vector-borne in endemic countries (11–15).

Ecuador is one of the South American nations with the worst CD burden, according to WHO (16). It has been estimated that 2.5% of the population in Ecuador has CD, which is higher than the 1.6% regional average (2, 17). In this sense, it is crucial to study CD in Ecuador not only because, according to the WHO, more than 370,000 people could be at risk of being infected by this parasite but also because of the impact on public health and its consequences on the cardiovascular and digestive systems are evident (2, 17).

Between 2013 and 2019, and based on the data of the Epidemiological Surveillance System (SIVE) of the Ministry of Public Health, 439 confirmed cases of Chagas disease were reported in Ecuador, with chronic cases ($n = 331$, 75.4%) being more common than acute cases ($n = 108$, 24.6%) (18). The disease has been reported in 20 of the 24 provinces, with El Oro ($n = 104$, 23.69%), Guayas ($n = 64$, 14.58%), and Loja ($n = 60$, 13.67%) being the most affected (17). Chagas disease has a high incidence in rural communities, with houses featuring thatched roofs, adobe or cracked walls, and no electricity, providing a suitable environment for the triatomine insect to reproduce (19). In a study of *Triatoma dimidiata* in the southern coastal region of Ecuador, 72% of the insects were infected with trypanosomes, and 95:77 were collected inside homes (20).

This study aimed to analyze the epidemiological characteristics of severe Chagas based on hospital admission data as a proxy for the incidence and mortality of CD in Ecuador.

2. Materials and methods

2.1. Study design

We conducted a cross-sectional countrywide study to determine the demographic and spatial distribution patterns of CD in Ecuador using hospital discharge and in-hospital mortality data as a proxy of incidence and mortality from 2011 until 2021.

2.2. Sample and setting

The study was conducted in Ecuador, the smallest country in the Andean region in South America. The country is divided into four geographical regions: the coast, mountains, Amazon, and Galapagos Islands. The political division contains 24 provinces and 221 cantons (cantons are political subdivisions of a province). Despite its small geographic size, Ecuador has an important climatic diversity characterized by a humid tropical climate in transition zones toward the coast and the Amazon, semi-humid to humid in the inter-Andean zone, hot and dry in the inter-Andean valleys and cold in the high mountains above 3,000 m altitude (21). According to the National Institute of Statistics and Census (INEC) in 2022, the population of Ecuador was 18,034,344 inhabitants (22).

The hospitalization cases of CD were defined according to the operational definitions outlined by the Ministry of Health of Ecuador, which can be found in Supplementary file 1 (23).

2.3. Data source and description

All hospitalization cases of CD B560 (Trypanosomiasis due to *Trypanosoma cruzi*), B561 (South American trypanosomiasis), B572 (American trypanosomiasis), B5721/I412 (American trypanosomiasis with heart injury/American trypanosomiasis with specified organ damage, Not classified elsewhere), and B575 (Brazilian trypanosomiasis) according to the 3-digit ICD-10 classification, were retrieved from National Institute of Statistics and Census (INEC).

Continuous and categorical data were acquired at a national level. In addition, when available, data was collated for the above-diagnosed cases during 11 years from the 24 provinces and the 221 cantons in the country. The data in our study includes information from both public healthcare providers (such as those with universal coverage and social security pension plans) and private healthcare providers (including both for-profit and non-profit entities).

2.4. Data analysis

We analyzed the following variables: age, sex, place of residence, and year of hospital admission. The incidence, mortality, and case-fatality rates were sex-and-age-standardized using projection data by canton and province according to the 2010 census. Incidence was calculated by dividing the number of hospitalization cases per year by the total population at risk each year for every age group. The incidence and mortality rates were computed by age, sex, geographic location, and corresponding population. All cases were classified across 17 age groups. The incidence and mortality of CD by the altitude of residence were also

analyzed. The classification of low altitude <2,500 m and high altitude >2,500 m was used as a cut-off point for exposure to altitude. The analysis was also carried out using the classification offered by the International Society of Mountain Medicine low altitude (<1,500 m), moderate altitude (1,500 m – 2,500 m), high altitude (2,500–3,500 m).

3. Results

One hundred eighteen patients were hospitalized in Ecuador from 2011 to 2021 due to CD. The overall in-hospital mortality rate was 69.4% ($N = 82$). Men have a higher incidence rate (4.8/1,000,000) than women, although women have a significantly higher mortality rate than men (6.9/1,000,000).

3.1. Age and sex analysis

The average age of analyzed cases was 61.49 years (SD 23.84) for men and 60.58 years (SD 25.14) for women. From 2011 to 2021, in Ecuador, the incidence of CD has had an oscillating behavior without a clear predominance for one of the sexes, represented by incidence rates ranging from 3/ 1,000,000 in the years with fewer cases to 12/1,000,000 in 2017 with the highest number of cases.

3.2. Incidence rates

The overall sex-specific adjusted incidence rate was higher for men (4.8/1,000,000). In the analysis by age group, among women, the most affected were those over 75 years of age; on the other hand, among men, it is observed that CD predominantly occurs in younger groups (65 years and older) (Table 1).

3.3. Mortality rate

The overall mortality rate shows that women are more affected by CD [6.9/1,000,000 (5.1–8.7)]. However, among young populations (0–49 years), males are the only ones who suffer death from the disease. On the other hand, both groups share an increase in mortality rates as the age of the patients increases, reaching the highest rates among those older than 80 years (Male Mortality Rate = 12/1,000,000 (10.7–13.4); Female Mortality Rate = 18.4/1,000,000 (17.1–19.6).

3.4. Geographic distribution

3.4.1. Trends by province

Ecuador's provinces with the highest Chagas adjusted incidence rates per 1,000,000 inhabits per the patient's recorded residence are Zamora Chinchipe, El Oro, and Orellana, with 145.3, 116.3, and 78.2, respectively. On the contrary, Ecuador's provinces with the lowest adjusted incidence rates are Pichincha, Guayas, and Manabí, with 5.3, 5.6, and 15.0, respectively (Figure 1 and Supplementary file 2).

According to mortality due to CD, Ecuador's provinces with the highest adjusted mortality rates per 1,000,000 inhabits per the patient's recorded residence are Orellana, El Oro, Santo Domingo De Los Tsachilas with 199.5, 156.2, and 58.8/1,000,000, respectively. On the contrary, Ecuador's provinces with the lowest adjusted mortality rates

TABLE 1 Incidence and mortality rates per 1,000,000 population by sex and age–specific groups in Ecuador, from 2011 to 2021.

Age (years)	Women			Men		
	Cases (n)	Incidence rate	Mortality rate	Cases (n)	Incidence rate	Mortality rate
<1	–	–	–	1	5.8	–
1–4	1	1.5	–	2	1.4	–
5–9	–	–	–	2	1.2	–
10–14	3	1.9	–	1	1.2	–
15–19	1	1.3	–	1	1.3	1.3
20–24	1	1.4	–	1	1.5	1.4
25–29	1	1.5	–	1	1.4	–
30–34	–	–	–	1	1.6	1.8
40–44	2	1.8	–	3	3.5	2.3
45–49	3	2.2	–	9	3.8	2.4
50–54	1	2.4	2.5	2	3.0	2.7
55–59	3	3.0	3.1	6	4.6	5.0
60–64	6	7.2	3.4	4	4.3	8.1
65–69	3	4.6	4.2	3	8.3	5.6
70–74	2	5.9	11	6	11.3	11.6
75–79	4	8.8	9.0	14	18.4	13.5
>80	12	11.7	12.0	18	20.0	18.4
Total	43	3.7	6.9	75	4.8	5.5

are Pichincha, Guayas, and Los Rios, with 5.9, 8.1, and 17.3 per 1,000,000, respectively (Figure 1 and Supplementary file 2).

3.4.2. Trends by canton

Ecuador's cantons with the highest CD incidence rates per 1,000,000 population, as per the patient's recorded residence, are Chinchipe, Atahualpa, and Chaguarpamba, with 687.1, 512.8, and 341.9, respectively. In contrast, Ecuador's cantons with the lowest incidence rates are Quito, Guayaquil, and Cuenca, with 6.2, 9.6, and 7.3 per 1,000,000, respectively.

Ecuador's cantons with the highest CD mortality rates per 1,000,000 population as per the patient's recorded residence are Balsas, Portovelo, and Atahualpa, with 716.4, 532.72, and 509.16, respectively. On the contrary, Ecuador's cantons with the lowest mortality rates are Quito, Guayaquil, and Cuenca, with 6.7, 12.5, and 49.9, respectively.

3.5. Altitude analysis

3.5.1. Classic classification

Ecuador's altitude regions with the highest CD adjusted incidence rates per 1,000,000 inhabits as per the patient's recorded place of residence, were located at low altitudes (< 2,500 m) adjusted incidence rate (AIR): 33.88/1,000,000 compared to the highlanders (> 2,500 m) respectively (Table 2).

Related to deaths, the highest adjusted mortality rates per 1,000,000 inhabits were found in low altitudes (< 2,500 m) with adjusted mortality rate (AMR): 51.45/1,000,000 (Table 2).

3.5.2. ISMM (International Society of Mountain Medicine) classification

Within this analysis, Ecuador's altitude groups with the highest adjusted incidence rates per 1,000,000 inhabits were Moderate altitude (1,500–2,500 m) and low altitude (< 1,500 m), with 73.42 and 36.49, respectively. On the other hand, according to deaths, the adjusted mortality rates per 1,000,000 inhabits rates showed a trend like an incidence with AMR: 83.68 in Moderate altitude (1,500–2,500 m) and AMR: 57.51 in low landers (< 1,500 m) (Table 3).

4. Discussion

Chagas disease is a significant public health issue in Latin America, with an estimated 6–7 million people infected and approximately 65 million at risk of infection (2). The disease is endemic in 21 Latin American countries, where poverty, poor housing

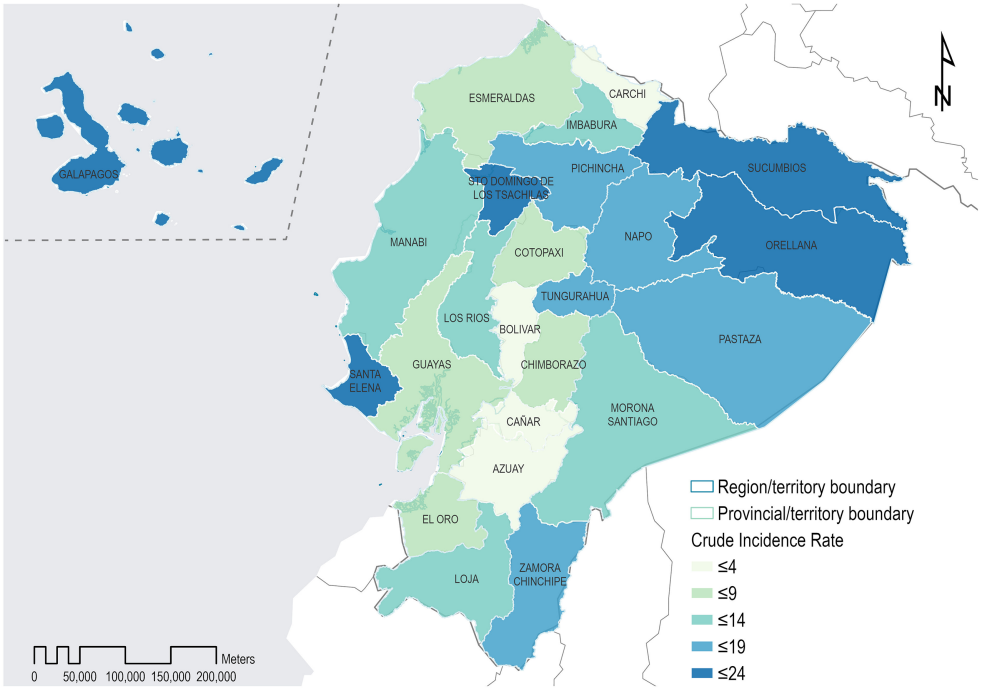

FIGURE 1
Geographical distribution of incidence rates of hospitalization cases for Chagas disease in the provinces of Ecuador, from 2011 to 2021.

TABLE 2 Distribution of incidence, and mortality rates per 1,000,000 inhabits according to altitude ranges from 2011 to 2021.

Altitude range	Cases (n)	CIR	AIR	Deaths (n)	CMR	AMR
Low altitude <2,500 m	105	63.7	338.8	82	84.9	514.5
High altitude >2,500 m	13	16.4	137.0	5	8.1	19.9

CIR, crude incidence rate; AIR, adjusted incidence rate; CMR, crude mortality rate; AMR, adjusted mortality rate.

TABLE 3 Distribution of incidence, and mortality rates per 1,000,000 inhabits according to ISMM altitude classification from 2011 to 2021.

Altitude range	Cases n	CIR	AIR	Deaths n	CMR	AMR
Low altitude (< 1,500 m)	98	61.5	364.9	76	83.4	575.1
Moderate altitude (1,500–2,500 m)	7	116.8	734.2	6	110.1	836.8
High altitude (2,500–3,500 m)	13	16.4	136.9	5	8.1	20.0

CIR, crude incidence rate; AIR, adjusted incidence rate; CMR, crude mortality rate; AMR, adjusted mortality rate.

conditions, and inadequate healthcare infrastructure contribute to its high prevalence (24). In Ecuador, limited epidemiological research has been conducted despite active monitoring by the Minister of Public Health. While some studies have analyzed CD distribution in specific regions and provinces of the country, few comprehensive analyses are available. Our research underscores the ongoing burden of CD in Ecuador, particularly as revealed through hospitalization and mortality data recorded in the Ecuadorian Institute of Statistics and Census (INEC) public databases. Despite previous studies dating back over 60 years (25), there remains a dearth of research investigating the epidemiological impact of this vector-borne disease.

Our analysis revealed that Chagas disease hospitalizes an average of 15 patients annually in Ecuador. Notably, the incidence of hospitalization is highest among adults and older adults, likely due to the chronic and long-term effects of the disease on the heart and gastrointestinal system (26, 27). Regarding the severity of hospitalization cases, our findings suggest that most severe cases, as indicated by hospital mortality rates, occur in individuals over 70. In addition, our study revealed a higher mortality rate among adult men than women, a trend observed in other studies (28).

Additionally, our study highlights that CD cases are highest in provinces with warm climates and located at low or moderate altitudes, even after adjusting for the patient's place of residence. On the other hand, provinces located in the highlands of Ecuador have lower rates of both incidence and mortality. These findings provide important insights for targeted interventions and resource allocation to address the burden of CD in Ecuador.

Research conducted in Ecuador, particularly in the field of entomology, has identified at least 17 species that have the potential to transmit *T. cruzi*. Among these species, nine are of particular concern, including *Triatoma dimidiata, Triatoma carrioni, Rhodnius pictipes, Rhodnius ecuadorensis, Rhodnius robustus, Panstrongylus rufotuberculatus, Panstrongylus chinai, Panstrongylus geniculatus*, and *Pastrongylus howardi* (29–31). These species are widely distributed throughout the country, taking advantage of Ecuador's equatorial location and favorable climate conditions in the tropic of cancer. These findings underscore the importance of continued surveillance and control measures to prevent the spread of CD in Ecuador.

Castelle et al. reported that CD in Ecuador had been successfully controlled, as no new cases were reported in children under five in 2010 (32), However, subsequent studies indicate a different trend. Between 2013 and 2019, 10 cases were reported in children aged 1–4 years old and 11 cases in children aged 5–9. Additionally, two cases were reported in infants under 1 year old between 2015 and 2016 (33); Moreover, a study conducted in the Ecuadorian Amazon from 2015 to 2018, which analyzed the seroprevalence of CD, found nine cases in children aged 10 years old (34). In another study by Quinde-Calderón et al., which analyzed data from 2004 to 2014, a total of 915 human cases of CD were reported in Ecuador. Notably, there was a significant increase in reported cases over the years, followed by a decrease in 2013 and 2014 (35).

Our study observed that the number of hospitalizations for CD in Ecuador showed an increasing trend until 2017, followed by a decrease in subsequent years. However, our findings are inconsistent with the data from the Ministry of Public Health's epidemiological surveillance system, which reported an increase in the number of CD cases from 2018 to 2021, with 74, 167, 113, and 170 cases reported each year, respectively (32). This suggests that there may be a gap between hospitalization data and disease surveillance data and highlights the need for further investigation into the epidemiology of CD in Ecuador.

Our study revealed higher incidence rates of CD among men in several age groups, consistent with the results of a similar study conducted in Mexico in 2018 (3) This trend can be explained by the higher involvement of men in agricultural activities during their productive years. However, our analysis also revealed that the overall mortality rate per 1,000,000 inhabitants was higher among women, despite higher mortality rates among men in most age groups. Previous studies in neighboring countries have yielded conflicting results on this variable. For instance, da Nóbrega et al. (36), found that 86% of deaths from CD in Brazil occurred in men and were associated with heart disease, while a 40-year investigation in Colombia showed that the highest mortality rates from CD were found in men over 65 years old (37). On the other hand, a systematic review with a meta-analysis conducted in 2016 did not find a significant difference in CD mortality related to sex (38).

We conducted a novel analysis to investigate the incidence and mortality of CD at different altitudes. Ecuador's unique topography, with 221 cities situated at varying elevations ranging from sea level to 4,300 m, provides an exceptional opportunity to study the burden of diseases at different altitudes (39–41). Our study found that the majority of CD cases ($n = 107$) were concentrated at elevations below 2,500 m. To provide a more nuanced analysis, we also employed the International Society of Mountain Medicine classification, which classifies altitudes into different categories, and observed that the highest incidence and mortality rates were found at low and moderate altitudes. We hypothesize that this pattern may be attributed to the warmer temperatures that promote the growth and survival of Triatomes, as documented in prior research (42, 43). While our investigation did not explicitly aim to explore the relationship between climate change and the emergence of CD, our data suggest that the likelihood of discovering Triatomes at higher elevations may increase as temperatures continue to rise.

Our study highlights that ongoing efforts are crucial to control the spread of CD in Ecuador, particularly in provinces with warmer climates and low to moderate altitudes, where the disease incidence is concentrated. Strengthening disease control measures, such as implementing vector control strategies and health education programs, could reduce the burden of CD in these areas. Moreover,

increasing funding and resources for research and surveillance programs could enhance our understanding of the disease and facilitate the development of more effective prevention and control measures (44–46).

This study establishes crucial precedents regarding the cost of caring for patients suffering from serious cardiovascular and gastrointestinal problems related to CD, such as cardiomegaly or megacolon, by using hospitalization data. Although several published studies in Ecuador exist, analyzing the epidemiological burden of different clinical presentations of CD is significant because it enables us to estimate the healthcare expenses incurred by the health system for treating these patients. On average, the Ecuadorian public healthcare system incurs a cost of over $300 per day of hospitalization, with patients staying in the hospital for three to 6 days, resulting in substantial financial losses for the Ministry of Public Health of Ecuador (47).

5. Limitations

When interpreting the results, one must consider several limitations of our study. These limitations include the observational and ecological study design, which limits the ability to establish causal relationships for the entire population. Additionally, the lack of information on the type of complication experienced by each hospitalized patient and the treatment given to them restricts the ability to draw specific conclusions about the disease burden of CD. Moreover, the nature of the data analyzed prevents the distinction between re-hospitalizations and single occasion hospitalizations.

Another limitation of our study is that we only included hospital discharge data and did not account for cases of CD that were mild or moderate and did not require hospitalization. This exclusion may have led to an underestimation of the actual disease burden and could have limited the generalizability of our findings to the entire population.

Despite these limitations, we believe that our study offers valuable insights into the burden of CD in Ecuador, particularly in terms of the incidence and mortality rates among different age and gender groups and at different altitudes. However, we need further research to obtain a more comprehensive understanding of the disease burden and to develop effective prevention and treatment strategies.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ecuadorencifras.gob.ec/camas-y-egresos-hospitalarios/.

Author contributions

JV-G, JI-C, and EO-P contributed to the conception and design of the entire project, gained full access to data from the National Statistical Institutes in Ecuador, was primarily responsible for all aspects of the work, and ensure the completeness and accuracy of the investigation. RF-N, JI-C, EG-R, AT-D-l-T, GG-C, CR-S, and EO-P contributed to data acquisition and review of the available literature and initial writing of the manuscript. RF-N, JI-C, and EO-P contributed to the statistical analysis and internal validity of the study. JV-G, EG-R, AT-D-l-T, GG-C, and CR-S developed the draft version of the manuscript. JI-C and EO-P critically reviewed and edited the manuscript to its final complete version and provided input to the data report and its interpretation. All authors reviewed and approved the final version of the manuscript.

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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

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Pyrethroid genetic resistance in the dengue vector (*Aedes aegypti*) in Posadas, Argentina

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Pyrethroids are extensively used to control adult populations of the arboviral vector *Aedes aegypti*, raising concerns regarding the increasing frequency and distribution of insecticide resistance mutations (kdr: knock-down resistance) in the voltage-gated sodium channel gene (*Nav*). The widespread use of pyrethroids imposes a threat to the success of mosquito control and the environment. In this study, we investigated the presence of two kdr mutations (V1016I and F1534C) in the *Nav* gene and their distribution across four neighborhoods in Posadas, Argentina, with different *Ae. aegypti* abundance and contrasting socioeconomic status (SES). Alleles at each locus were interrogated using TaqMan SNP genotyping assays in DNA extracted from adult females collected in a longitudinal study. We report the presence of both pyrethroid resistance alleles (kdr 1016I = 29.08%; kdr 1534C = 70.70%) among adult females. The frequency of combined kdr genotypes reveals that approximately 70% of local adult females have enhanced resistance to pyrethroids. Both, the proportion of resistant adult females (with at least one kdr allele in each locus) and *Ae. aegypti* abundance showed an uneven distribution between neighborhoods with different SES ($p < 0.001$). In high-SES neighborhoods, we found more mosquitoes and a higher frequency of pyrethroid resistance, possibly as a consequence of different public health interventions, social habits, and insecticide use. This is the first report of kdr mutations in *Ae. Aegypti* in the northeast region of Argentina. Our results focus on the need for within-population (city) distribution analyses of kdr mutations and highlight the relevance of incorporating insecticide resistance monitoring within the Integrated Vector Management initiative.

KEYWORDS

***Aedes aegypti*, pyrethroid resistance, knock-down resistance (kdr), arboviral vector, dengue vector surveillance, vector abundance**

Introduction

Aedes aegypti, the primary vector of arboviral diseases such as Zika, dengue, yellow fever, and chikungunya, is unquestionably adapted to anthropogenic environments, and it is one of the most threatening species to human health worldwide (1). Hundreds of millions of dengue infections every year inflict a huge public health burden in tropical and subtropical countries, a consequence of an ever-expanding range of arboviral infections and vector populations during the last 50 years (2–4). During the 2019–2020 dengue epidemic, Misiones Province,

located in the northeast region of Argentina (NEA), registered most of the cases (5). An arboviral survey during 2019–2021 confirmed the circulation of three dengue virus serotypes in the main districts of Misiones, but Zika or chikungunya viruses were not found (6). The recent report of more than 2,000 chikungunya cases 10 miles away across the Paraná River (7) and the circulation of all dengue serotypes in the bordering countries (8), represent major risk factors in Misiones Province considering the high local *Aedes aegypti* larvae index, egg counts, and active fed females documented year-round (9).

Arboviral disease prevention and mitigation strategies are primarily addressed to control the vector by insecticide spraying and eliminating breeding sites and, more recently, by incorporating biotechnologically modified vectors. The rather intense and widespread use of pyrethroid insecticides led to the development of insecticide resistance due to the dissemination of genetic resistance in vector populations worldwide (10–12). Pyrethroids interrupt the mosquito's nerve function by binding to the voltage-dependent sodium channel proteins (11). Its neurotoxic activity is diminished by non-synonymous knock-down-resistant (kdr) mutations introducing amino acid changes in the pyrethroid target protein (13, 14), a predominant mechanism of genetic insecticide resistance.

Several kdr mutations have been identified in *Ae. aegypti*, two of them consistently associated with pyrethroid resistance, i.e., a Val-to-Ile amino acid change at protein position p.1016 (kdr V1016I) and the Phe-to-Cys amino acid change at p.1534 (kdr F1534C) (1, 15). Studies validated the association between these mutations and permethrin resistance (14, 16), showing that at both positions, homozygous kdr genotypes (II/CC) are more resistant than the wild-type homozygous kdr genotypes (VV/FF), with heterozygous genotypes displaying a range of intermediate resistance phenotypes (1, 17, 18). Reports also showed that the kdr V1016I mutation may synergize with the kdr F1534C in generating more resistant phenotypes (1, 15, 17).

Countries in the Americas, i.e., Venezuela, Mexico, USA, Costa Rica, and Brazil, reported increasing kdr V1016I and F1534C frequencies during the last decade, with kdr alleles reaching fixation in some cities (1, 11, 19–21). However, the distribution of kdr alleles within cities remains to be explored, particularly in different demographic and socio-economic settings.

Two insecticide sensitivity studies in Argentina showed incipient resistance in *Ae. aegypti* larvae and adults (22, 23); however, assessments of vector genetic resistance to pyrethroids have not been included in the local Integrated Vector Management programs. The presence, frequency, and distribution of pyrethroid kdr alleles using direct genotyping methods have not been reported in NEA to date. Within a range of 600 miles, only a Brazilian extensive study reported high kdr allele frequencies (11).

Monitoring frequencies of kdr alleles as an indicator of genetic resistance in *Ae. aegypti* populations gives public health authorities a direct assessment tool to evaluate the efficiency of control strategies and means to guide targeted interventions. In the context of the local entomological and arboviral vigilance program, we investigated the presence and distribution of alleles associated with resistance to pyrethroids, demonstrating for the first time the presence of kdr mutations in *Ae. aegypti* populations in Misiones, Argentina. Resistant (kdr) genotypes showed a non-random spatial distribution between contrasting socioeconomic status (SES) districts, distinguishing neighborhoods with increased pyrethroid resistance degree and *Ae. aegypti* abundance.

Methods

Experimental setting, study site, and biological material

The collection of adult specimens of *Ae. aegypti* was carried out as part of an international research collaboration network (RADAM-LAC) to investigate determinants of *Ae. aegypti* density at the household level, including factors related to household characteristics (i.e., crowding, water and waste management, and protection against mosquitoes) (9). Sampling was performed on a household basis simultaneously in three cities, i.e., Manta (Ecuador), Bage (Colombia), and Posadas (Argentina), following the same experimental design and methodology (9).

Adult *Ae. aegypti* were collected from 372 houses during a longitudinal study (January to December 2019) carried out in Posadas, NEA (27°22′00″S 55°53′49″O), a city of 4,00,000 inhabitants on the banks of the Paraná River, 120 m.o.s.l., with recurrent dengue epidemics. Four neighborhoods were grouped according to their population density, household crowding, household wealth, type of housing, and public services availability (water and sewage): two neighborhoods into "high" socioeconomic status (SES), privileged longstanding residential areas close to the city center [Villa Sarita (VS) and Barrio Palomar (BP)]; and two into "low" SES, less favored recent housing areas in the periphery [San Lorenzo (SL) and Nueva Esperanza (NE)] (Figure 1). Three adult mosquito traps (including one BG-Sentinel 2, one CDC miniature light trap, and one resting trap) were set in each of the 93 selected houses per neighborhood, and mosquitoes were also aspirated from indoor/outdoor surfaces using a Prokopack 1419 Aspirator [for details, see (9)]. Adult *Ae. aegypti* were sorted (males, fed females, and unfed females) and dissected, one piece individually stored at −20°C and the other part placed in pools of 20 individuals for subsequent molecular analyses. For the kdr assessment, 25 adult *Ae. aegypti* females were randomly selected from adult mosquitoes captured in different houses within each neighborhood during the highest prevalence sampling period (November–April).

Identification of knock-down resistance mutations

Individual DNA that was isolated (Wizard Genomic DNA Purification Kit, Promega) from 100 dissected adult *Ae. aegypti* (50 from each SES group) was used as a template for a direct genotyping of two kdr mutations in the *Nav* gene (kdr V1016I and kdr F1534C) using validated TaqMan SNP assays (Thermo Fisher) as published in Melo Costa et al. (11). Positive DNA controls for resistant genotypes were kindly provided by Dr. Adriana E Flores, UANL, Mexico. A separate, non-template reaction was used as a

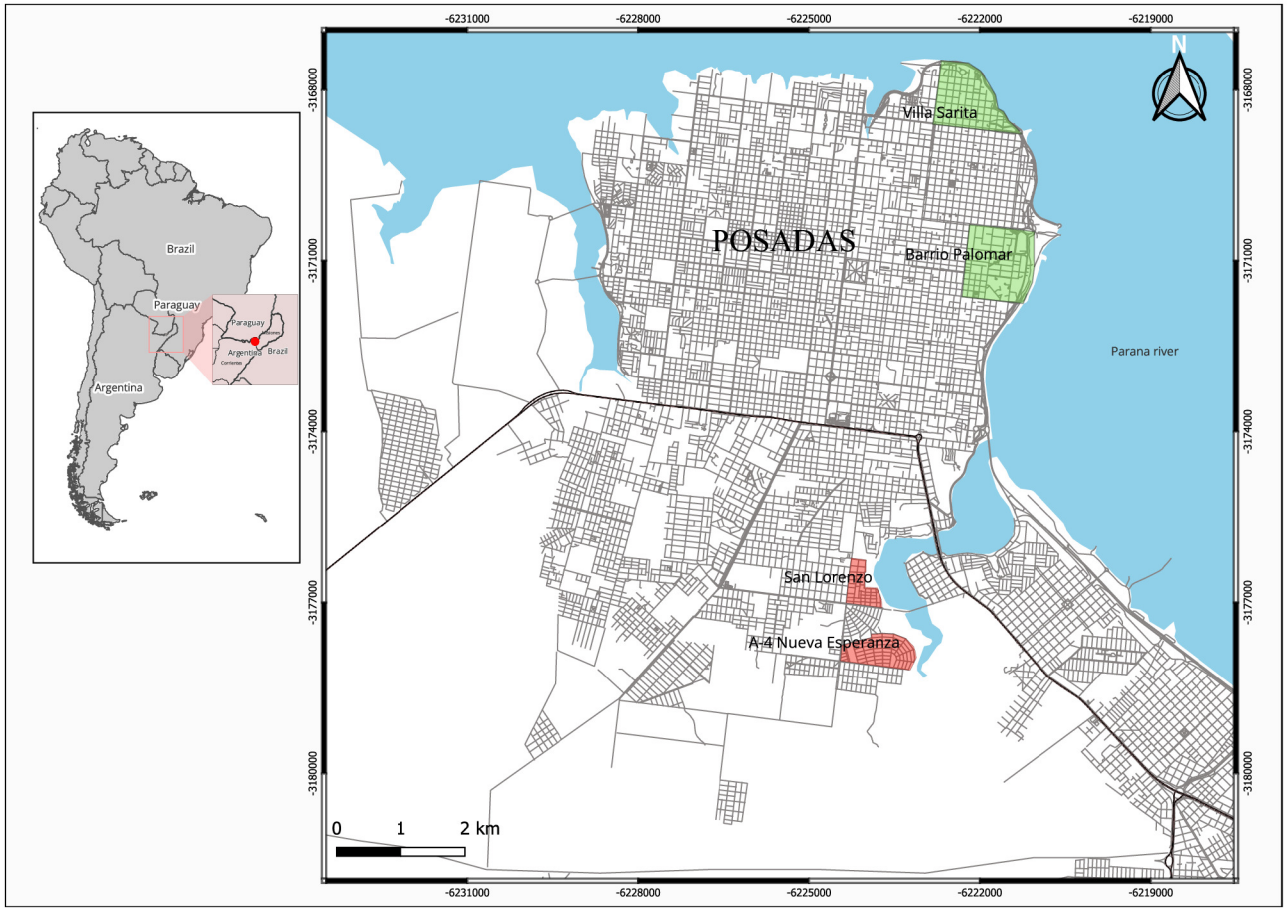

FIGURE 1
Distribution of *Aedes* collection sites on low (red) and high (green) socio-economic status (SES) neighborhoods in Posadas, Argentina.

negative control. Primer and probes sequences and reaction assays are detailed in Supplementary Table 1.

Statistical analyses

Allelic and genotypic frequencies were estimated by direct count, individually at each position (i.e., *Nav* p.1016: VV, VI, II; and *Nav* p.1534 FF, FC, CC). The frequency of combined genotypes, V1016I +F1534C, was also estimated (i.e., VV/FF, VV/FC, VV/CC, VI/FF, VI/FC, VI/CC, II/FF, II/FC, II/CC), considering that they are physically very close in the *Ae. aegypti* genome and therefore in the linkage disequilibrium (19, 24). We used the Mann–Whitney U-test to assess the differences in adult females *Ae. aegypti* count in low- vs. high-SES areas and Z-score to evaluate the proportion of resistant and wild-type genotypes in these two areas.

Results

The 2019 longitudinal study assessed 372 houses in four neighborhoods (two high and two low SES) in Posadas, Argentina, collecting mosquitoes indoors and in the peridomicile area using four validated trapping methods in each site. In total, we collected 4,391 *Culex* spp. and 1,020 *Ae. aegypti* adult mosquitoes. High-SES neighborhoods, Villa Sarita + Barrio Palomar (VS+BP), accounted for a higher number of adult *Ae. aegypti* per household and *Ae. aegypti* abundance, showing significant differences in the proportion of captured *Ae. aegypti* (z-score: −16.48225, $p < 0.001$) (Table 1).

TaqMan genotyping individual mosquitoes for two kdr mutations, V1016I (n = 98) and F1534C (n = 99), showed a high frequency of pyrethroid resistance alleles in local *Ae. aegypti* populations, whereas kdr V1016I mutation was present in 49% of analyzed adults, kdr F1534C was observed in 92% of individuals. Frequencies of the resistant alleles at both positions were substantially different (kdr 1016I = 0.2908; kdr 1534C = 0.7070). For each position, all three genotypes (i.e., *Nav* p.1016: VV, VI, II; *Nav* p.1534 FF, FC, CC) were identified in the assayed sample. The resistant homozygous kdr 1534C genotype (CC) was the second most frequent. Observed genotypic frequencies (Table 2) are in Hardy–Weinberg equilibrium.

The combined genotypes considering both the 1016 and 1534 *Nav* sites and their frequencies found in this study are listed in Table 3. Combined genotypic frequencies showed that 92.63% of all individuals carried at least one resistant allele and 50% at least one resistant allele in each position, i.e., VI/FC, VI/CC, and II/CC (Table 3). VI/FF, II/FC, and II/FF genotypes were not observed.

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TABLE 1 Adult *Ae. aegypti* captured in low (NE+SL) and high (VS+BP) socioeconomic status (SES) neighborhoods.

	Neighborhood	*Ae. aegypti* abundance	Number of adult Aedes per household
Low SES	NE	226	2.43
	SL	176	1.89
	Total	***402****	***2.16***
High SES	VS	313	3.36
	BP	305	3.28
	Total	***618****	***3.32***

*Significantly different $p < 0.01$. The bold values indicate totals or sub totals.

TABLE 2 Frequency of knock-down-resistant (kdr) alleles and genotypes found at *Nav* p.1016 and *Nav* p.1534 positions in *Aedes aegypti* from Posadas, Argentina.

kdr position	Allele	Frequency (%)	Genotype	Frequency (%)
1016	**V**	70.91	**VV**	51.02
			VI	39.80
	I*	29.08	**II**	9.18
				n = 98
1534	**F**	29.29	**FF**	8.08
			FC	42.42
	C*	70.70	**CC**	49.49
				n = 99

*Knock-down-resistant alleles at Nav p.1016 and Nav p.1534C.

The genotype counts in low- and high-SES neighborhoods were not proportional to their distribution in the whole sample ($p < 0.001$, Table 3). There was a higher prevalence of genotypes carrying at least one resistant allele in both positions (VI/FC, VI/CC, and II/CC) in mosquitoes collected from high-SES (67%) compared with low-SES neighborhoods (34%). In addition, the susceptible double homozygous genotype (VV/FF) was absent in high-SES districts but represented 14% of mosquitoes collected from the low-SES districts.

Discussion

The arboviral vector mosquito *Aedes aegypti* is a major threat to human health worldwide. Its control represents a vital challenge for public health authorities in subtropical and tropical countries. Genetic resistance to insecticides is well documented in several species, including *Ae. Aegypti,* and has been largely validated in physiological and molecular studies (8, 22), showing that mutations in the *Nav* gene are responsible for weakening the pyrethroid knock-down efficacy. Investigations have extensively portrayed the effect of kdrV1016I and kdrF1534C mutations and their fluctuating distribution in the Americas (1, 11, 25).

Here, we report for the first time the presence and distribution of *Ae. aegypti* kdr mutations in the northeast region of Argentina. We assessed the two main pyrethroid resistance markers in adult mosquitoes, kdrV1016I and kdrF1534C, using TaqMan SNP genotyping assays, the most widely used genotyping system. Unlike other methods, a TaqMan assay executes direct genotyping, reducing adjustments and additional confirmatory reactions to non-specific amplifications (17).

In total, 92.63% of all tested mosquitoes had at least one resistant allele. The high kdr allelic frequencies observed in this study, particularly in kdr F1534C (70.7%), derived from the predominance of homozygous resistant genotypes identified in approximately 50% of mosquitoes (Table 2). In the absence of previous *Ae. aegypti* kdr data gathered in Argentinian nearby regions (within 600 miles), our report gains relevance as a reference for future studies, particularly to assist local vector control strategies. The closest kdr survey reported was carried out in a Brazilian border city 210 miles away, showing even higher frequencies in both kdr mutations, i.e., V1016I = 70% and F1534C = 91% (11).

Co-occurrence of kdr mutations (V1016I+F1534C) presenting at least one copy of the resistant allele at each kdr site is common, reaching up to 90% in Central and South American districts (1), possibly as an outcome of the intense use of pyrethroids. According to the hypothesis of the synergistic contribution of kdr V1016I + kdr F1534C, homozygous resistant genotypes at both positions, i.e., Nav.p1016/p.1534 (II/CC), are more permethrin resistant than the VI/CC, and these, in turn, more than the VV/CC (15), demonstrating the individual contribution of kdr alleles. Our data show that VI/CC and II/CC genotypes represent ~30% of combined genotypes observed in Posadas' neighborhoods. Furthermore, almost 50% of the assayed adult *Ae. aegypti* presented at least one kdr allele in both loci, indicating a currently extensive degree of pyrethroid resistance. Combined genotypes II/FC and II/FF were not found in Posadas, in line with their exceptionally

TABLE 3 Frequency (%) of combined kdr genotypes (V1016I + F1534C) in *Aedes aegypti* from Posadas, Argentina.

Combined genotypes*	Whole sample ($N = 95$)	Low-SES neighborhood ($N = 50$)	High-SES neighborhood ($N = 45$)
V/V/FF	7.37	14.00	0.00
VV/FC	23.16	26.00	20.00
VV/CC	20.00	26.00	13.33
VI/FC	20.00	14.00	26.67
VI/CC	21.05	16.00	27.67
II/CC	8.42	4.00	13.33
		0.34**	**0.67****

*VI/FF, II/FC–II/FF genotypes absent.
**Significant different ($p < 0.001$). The bold values indicate totals or sub totals.

low frequency (0.0004) reported by the comprehensive survey conducted in 123 Brazilian cities (11). Hernandez et al. (1) suggested that the low frequency of genotypes II (Nav p.1016) could be associated with their subsequent evolution after F1534C, as well as with a lower fitness of these genotypes. Selection pressure due to the extensive use of pyrethroids is also a likely scenario.

Examining the distribution of combined resistant genotypes carrying at least one kdr mutation in each position (VI/FC, VI/CC, and II/CC) among neighborhoods showed a significantly increased proportion of kdr genotypes in high-SES areas (box in Table 3). This indicates an increased degree of pyrethroid resistance in privileged SES areas in Posadas, which is confirmed by the absence of a double homozygous susceptible genotype (VV/FF) in this area. Notably, the abundance of *Ae. aegypti* captured in high-SES neighborhoods is also higher ($p < 0.01$, Table 1). Hence, in high-SES neighborhoods, we found not only more mosquitoes but also more resistant ones. The increased *Ae. aegypti* counts alongside prevalent resistant genotypes in high-SES areas may result from two main factors, namely (a) easier access to and more intense and widespread use of household pyrethroid insecticides in high-SES districts and (b) comparative success of usual vector control strategies mostly associated with the elimination of breeding sites and debris removal actions in low-SES areas (corroborated by City Public Health intervention frequency). In line with this, the resistance to insecticides for domestic use and the selection pressure associated with this extensive practice have already been demonstrated in *Ae. aegypti* (11, 26, 27). In Brazil, a non-random distribution of kdr genotypes among cities, with the absence of the wild-type in localities where chemical control inside houses played a fundamental role, was observed (11). Also, an increased frequency of kdr V1016I mutation was associated with the use of surface pyrethroid aerosols in homes in Mexico (26). Extensive chemical control operations have been performed in the north of Argentina since 1998, and systematic actions began in 2003 in Misiones, Argentina. In recent years, local health authorities have implemented controls with type 1 pyrethroids [(1-RS)-cis-trans permethrin].

In this context, the consideration of *Ae. aegypti* abundance without information on the frequency of the pyrethroid resistance alleles in vector populations would have led to making inappropriate decisions on vector control management in high SES. Crucially, the available kdr data offer the opportunity to redirect strategies and reduce the amount and/or type of pesticide spraying by a precision-guided use of insecticides within cities. Considering the lack of regional kdr data associated with *Ae. aegypti* abundance, this study contributes to the first reference dataset for local vector vigilance and insecticide resistance management programs. Continuous evaluation of permethrin resistance across neighborhoods by monitoring V1016I + F1534C kdr mutations and insecticide bioassay testing will allow the analysis of the spatiotemporal evolution of the resistance phenomenon, a chance to effectively plan localized control strategies including the use of alternate insecticides and non-chemical interventions.

This study provides evidence regarding two premises that have been poorly addressed that represent key issues in the design and implementation of vector control strategies. First, (a) between-neighborhood (subpopulations) differences in kdr genotypic frequencies: studies mostly evaluate kdr data from each city (sample), comparing results between cities in country-wide or statewide studies. In addition to the actual relevance of those analyses, here we found skewed kdr genotype frequencies between two groups of neighborhoods within the same city. Second, (b) the complex relationship among *Ae. aegypti* abundance, pyrethroid resistance, and socio-economic (cultural) determinants. Both require further experimental analyses and monitoring in different eco-epidemiological settings.

Frequency estimates are based on genotyping 100 *Ae. aegypti* adults, representing only ∼10% of the captured samples. This could be interpreted as a limitation of this study; however, increasing the sample size would not imply significant changes as allele frequencies at both positions are moderate to high, and genotypic frequencies are under Hardy–Weinberg equilibrium. Notwithstanding this, the spatial expansion of the sampling by including all neighborhoods in Posadas would generate a more complete understanding of the local *Ae. aegypti* population structuring concerning kdr mutations.

In conclusion, this study demonstrates the relevance of kdr data for precise and focused vector control interventions and public health initiatives to reduce both *Ae. aegypti* populations and insecticide use/misuse to mitigate arboviral disease risks.

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.

Author contributions

JFa, KL, FZ, MK, JFe, CA, and MM contributed to the conception and design of the study. JFa, MBo, SE, and MBl performed the laboratory work and analysis of results. JFa, JFe, and MM wrote the first draft of the manuscript. All authors contributed to the revision of the manuscript, read, and approved the submitted version.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1166007/full#supplementary-material

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Cutaneous leishmaniasis situation analysis in the Islamic Republic of Iran in preparation for an elimination plan

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Iran has invariably been under the growing public health threat of cutaneous leishmaniasis (CL), a significant barrier to local development that hinders the prevention and control efforts toward eliminating the disease. So far, no comprehensive and in-depth epidemiological analysis of the CL situation has been carried out nationwide. This study aimed to employ advanced statistical models to analyze the data collected through the Center for Diseases Control and Prevention of Communicable Diseases during 1989–2020. However, we emphasized the current trends, 2013–2020, to study temporal and spatial CL patterns. In the country, the epidemiology of CL is incredibly intricate due to various factors. This fact indicates that the basic infrastructure, the preceding supports, and the implementation plan related to preventive and therapeutic measures need crucial support. The leishmaniasis situation analysis is consistent with desperate requirements for efficient information on the control program in the area. This review provides evidence of temporally regressive and spatially expanding incidence of CL with characteristic geographical patterns and disease hotspots, signifying an urgent need for comprehensive control strategies. This information could be a suitable model and practical experience in the Eastern Mediterranean Region, where over 80% of CL is reported.

KEYWORDS

cutaneous leishmaniasis, situation analysis, prevention, elimination plan, Iran

1. Introduction

Leishmaniasis is among the most emerging and re-emerging zoonotic diseases that have long been recognized, evolved, remarkably increased, and gradually expanded beyond the traditional geographical range and progressively dispersed among different hosts and numerous vectors (1). This disease frequently occurs, spreading greater and faster than before (2). In 2018, over 253,000 new cutaneous leishmaniasis (CL) cases were reported to World Health Organization (WHO). The region accounts for over 80% of the CL caseloads worldwide, only from the Eastern Mediterranean Region (EMR) countries, representing a primary "hotspot" eco-epidemiological region (3). Cutaneous leishmaniasis is a multidimensional entity posing a severe public health concern in the WHO EMR in which 18/22 (82%) of countries and territories were endemic for CL. Several epidemiological and clinical forms indicate a challenge in managing, controlling, and eliminating the disease (4). Leishmaniasis is still the third most vector-borne disease and is frequently fatal if it remains untreated in 101 tropical and subtropical countries and territories (5, 6). Although visceral leishmaniasis (VL or kala-azar) is the deadliest type, CL is the most common form encompassing three-fourths of the total burden (7).

Over the past three decades, despite tremendous efforts to control CL in Iran, it seems that the burden of the disease is still high in some areas. This fact indicates that the basic infrastructure and the preceding support and implementation plan related to diagnostics, drugs, and vaccines are inadequate nationally and abroad (8). The leishmaniasis situation analysis and the suggested framework is consistent with desperate requirements for efficient information on the range of the control program in the area as previously outlined by the WHO Resolution (9). This resolution called for conditions that would allow WHO to assume a leadership role in scientific and technical cooperation to strengthen, initiate, maintain, and expand the leishmaniasis control program. The outcome has been streamlined, and integrated approaches have produced extraordinary gains for public health improvement. Iran is among the forefront affected countries that, together with Brazil, Peru, Columbia, Algeria, Afghanistan, Syria, and Pakistan, constitute the eight most endemic countries (5). Like the Old World, *Leishmania major* and *L. tropica* are responsible for zoonotic (ZCL) and anthroponotic CL (ACL) in Iran, respectively (10). In the country, the epidemiology of CL is incredibly intricate due to the complexity of the rural and urban life cycles, the implication of numerous reservoir hosts and varying species of phlebotomine sandfly vectors, variable response to standard medications, complex confounding factors, mixed species circulating in the endemic foci, various clinical features and recurrent emerging epidemics and main challenges and gaps (1, 11–14).

No similar comprehensive and in-depth epidemiological analysis of the CL situation has been carried out at the national level. This study analyzed the national data collected through the Center for Diseases Control and Prevention of Communicable Diseases (CDC) during 1989–2020. However, we emphasized the current trends, 2013–2020, to study temporal and spatial CL trends and pinpoint hotspots. Identification of the persistent and high-incidence areas could help the program prepare an elimination plan for future control planning, as requested by the CDC, Ministry of Health and Medical Education (MOHME), and WHO. Herein, we provide evidence of temporally regressive and spatially expanding incidence of CL with characteristic geographical patterns and disease hotspots, signifying an urgent need for intensified, effective, and comprehensive control strategies, particularly in the hotspot settings. The information presented in this study could be a suitable model and practical experience in EMR countries and worldwide.

2. Data sources and scope

The primary data source was those reported monthly by the health surveillance system to the CDC in the country and via literature review in the area of interest relevant to the characteristics of the CL cases. We have presented the current situation of CL and reviewed various operational aspects of the current and preceding control strategies, including implementing prophylactic and therapeutic measures in humans, animal reservoirs, and sandfly vectors. We have likewise summarized significant challenges and gaps, present status, and finally listed a framework of strategic approaches and essential needs for conducting a preliminary control program toward a CL elimination plan. Furthermore, a leishmaniasis expert team comprising experienced staff and academic members affiliated with the universities at the provincial and national levels was established. During a 24-month work, 23 meetings were held together with a literature review, interviews, visits, and discussion of various leishmaniasis issues, particularly those which might interfere with the future control program. Furthermore, relevant challenges and gaps analyses linked to strengths, weaknesses, opportunities, and threats (SWOT), and a list of essential needs were presented.

3. Geographical areas

Iran consists of 31 provinces, 85 million population, and 1,648,000 km^2 areas. This country possesses one of the longest land borders (5,894 km) of any country in western Asia, neighboring Afghanistan, Pakistan, Turkey, Armenia, Azerbaijan, Turkmenistan, and Iraq. Iran is a vast country with inconstant and different climates: mild and relatively wet on the coast of the Caspian Sea, continental and arid in the plateau, cold in high mountains, and desert, and hot on the southern coast in the southeast. In the northwest, winters are cold with heavy snowfall and subfreezing temperatures. Spring and fall are relatively mild, while summers are dry and hot. In the south, winters are mild, and the summers are sweltering, having average daily temperatures in July exceeding 38°C. On the Khuzestan plains, the summer heat is accompanied by high humidity (15, 16). In general, Iran has a continental climate where most of the relatively scant annual precipitation falls from October through April (17). Such a diverse range of temperatures, climatic zones, and fertile land creates an opportunity for growing all kinds of crops and vegetables. These conditions brought about fertile grounds for providing multiple risk determinants and suitable breeding environments for propagating sandflies and reservoirs, contributing to an excessive number of CL cases in a geographically vast area of the country (18, 19).

4. Epidemiological characteristics

4.1. Case–definition

A confirmed CL case refers to a patient who shows clinical signs (skin lesions) along with parasitological confirmation of the organism

(positive smear or culture obtained from the edge of the skin lesion). The confirmed CL cases are routinely diagnosed to the genus level (20); however, the investigators seeking research purposes are exceptions for those identified to the species phenotype.

4.2. Surveillance and case detection

Cutaneous leishmaniasis is a notifiable disease in Iran and is integrated into the primary health surveillance system (PHC). The CL cases are reported monthly from peripheral areas to district-province-national levels. In routine detection, passive case finding consists of screening for CL at health clinics, and rural or urban centers, while in emergencies, passive recognition, and active case detection of CL cases through house-to-house visits are carried out.

4.3. Clinico-epidemiological forms

Two main CL types are present in Iran, either alone or mixed, depending upon the endemic locality (21). Zoonotic CL (ZCL) is the most predominant and widespread form caused by *L. major*. The *Phlebotomus papatasi* female sandflies primarily transmit this species from small gerbils to humans, presumably in 80% of the foci (22) in 842 districts inhabiting 2.4 million at-risk populations. This form is endemic in many rural and municipal areas of 19 out of the 31 provinces of Iran, including Tehran, Isfahan, Kashan, Qom, Semnan, Khorasan Razavi, Khorasan Shomali, Golestan, Khorasan Jonoobi, Fars, Ilam, Lorestan, Bushehr, Khuzestan, Hormozgan, Kerman, Kermanshah, Yazd, and Sistan/Baluchestan (10, 23). The global foci of ZCL are vast, including numerous geographical areas in the Middle East, north-western China, and North Africa (10, 24). While anthroponotic CL (ACL) is caused by *L. tropica* and transmitted by the female *Ph. sergenti* sandflies from human to human, often in an urban life cycle. This form is the minor type (nearly 20%) present, more likely in 205 large and medium-sized cities and suburban settlements entailing Mashhad, Neyshbour, Sabzevar., Kashan, Yazd, Isfahan, Kerman, Bam, and Shiraz, within the country (20).

4.4. Reservoir hosts

Four species of small rodents belonging to the family Cricetidae are designated as the principal reservoir hosts for ZCL in several parts of Iran. *Rhombomys opimus* (the great gerbil) is the primary host of ZCL in central and northeast Iran, while *Meriones libycus* (the Libyan Jird) is the primary reservoir host of ZCL in the central and south of the country. *Tatera indicia* (the Indian gerbil) is the primary reservoir host of ZCL in the southeast of Iran. *Meriones hurrianae* (the desert gerbil) is implicated as the reservoir host of ZCL in Baluchistan, in southeastern Iran (25), while prominent infection has been described in *Nesokia indica* as well. Summary of presence probabilities for three main species (e.g., *R. opimus*, *M. libycus*, and *T. indica*) exposed to favorable environmental niches in widespread areas of 16/31 provinces (26–31). Anthroponotic CL caused by *L. tropica,* is restricted to humans, although sporadic dogs have been infected in some foci. It is assumed that dogs might be implicated in the epidemiology of the disease and play a secondary role in transmitting the *L. tropica* parasite (32, 33).

4.5. Biological vectors

Only a marginal of sandflies are biological vectors of CL in Iran. *Phlebotomus papatasi* is the primary and well-known sandfly vector of ZCL among humans and gerbils. Natural *L. major* promastigote infection has been detected in 0.2–22% of *Ph. papatasi* from rodent burrows. Some reports indicate that *Ph. salehi* is the secondary vector transmitting ZCL infections in an endemic focus of ZCL in central Iran (34–46). In contrast, ACL is found in long-lasting foci in medium and large-sized cities in highland areas. The extent of this disease was significantly influenced by anti-malaria insecticide spraying over the past decades. *Ph. sergenti* is the primary sandfly vector of ACL transmitting the organism in the endemic foci of Iran (25, 47–49).

4.6. Demographic and clinical features

We collected the CL new cases data (n = 695,541) between April 1983 and March 2020. Subsequently, a subsample (n = 129,009) from April 2013 to April 2020 was used by advanced statistical analyses. The CL patients consisted of males (58%) and females (41%) aged 1–83 years old who were interviewed, medically examined, and diagnosed for the presence of active skin lesions or scars compatible with CL by the health networks in 31 provinces of the Islamic Republic of Iran. There was a significant difference between males and females ($p < 0.001$). All age groups were affected, but children <10 years old performed the highest (23%), and > 60 years the minor level of infection (8%) ($p < 0.001$). Most of the patients lived in urban areas (52%), the majority were Iranian (93%), and the remaining (7%) Afghan migrants. Most of the lesions were single (47%), 1 cm in diameter (46%), and frequently on hands (57%).

Figure 1 exhibits the nationwide CL prevalence and incidence of cases during 1983–2020. Over this period, the trend was somewhat sluggish, but more or less of fluctuating nature and steadily declined. This variation was mainly due to multiple risk factors, especially earthquakes, natural and anthropogenic environmental changes, population displacement, and drug unresponsiveness (50–52). The five provinces with the highest incidence were Ilam 143, Fars 75.3, Semnan 60.2, Isfahan 48.8, and Golestan 46 per 100,000 people. The average incidence rate was 20.7 per 100,000 persons nationally (Table 1).

Over the last decade, CL has expanded considerably, and numerous outbreaks and emerging epidemics of variable magnitudes occurred, attributed to the spread and resurgence of the disease and the provision of many confounding determinants (12, 14, 24, 53–56). Despite a significant reduction in VL cases, the geographical range of CL has remarkably enlarged. The policymakers, health authorities, and clinical practitioners have seriously overlooked the disease as they do not consider CL a severe threat and urgent public health concern in Iran and EMR. This is mainly due to the perceived no serious and under-reporting disease, other precipitation determinants, and many underlying chronic diseases (57). Iran is among the eight most affected and frontline countries where 15,000–20,000 cases of CL have

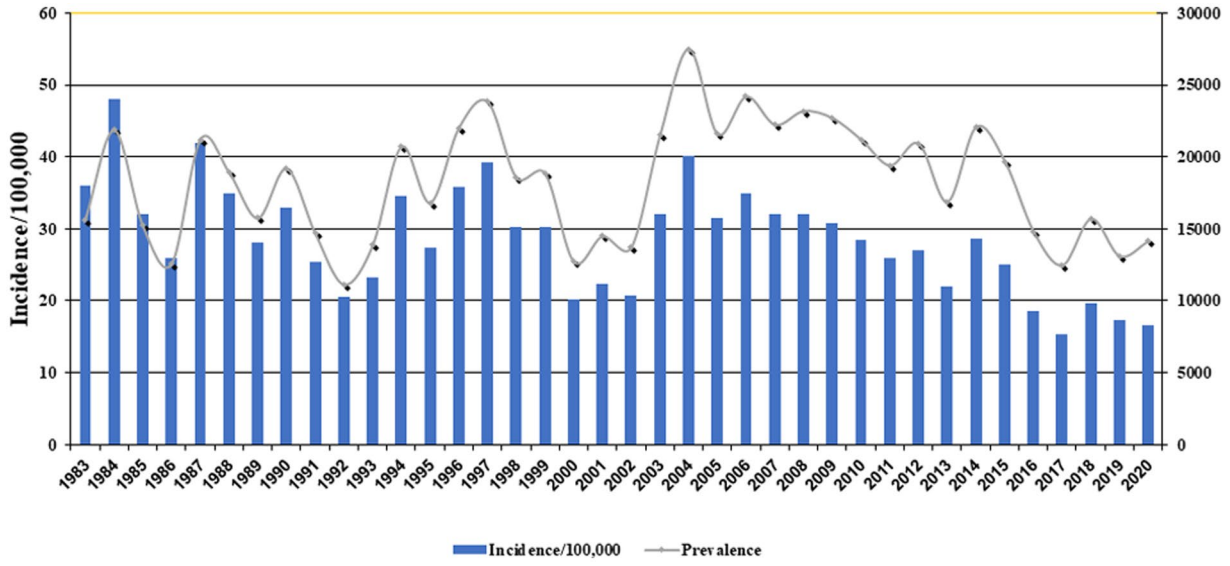

FIGURE 1
Incidence and prevalence rates of cutaneous leishmaniasis in Iran, 1983–2020.

officially been reported, although the actual number of new cases is reported to be in the order of 2-5-fold higher (20).

4.7. Analysis of national incidence trends

A non-linear mixed model (random effect model) was used to explore the incidence trend in different provinces and the effect of demographic data, including age and gender, on the incidence rate over time. To be precise, we employed 'random intercept' and 'random slope' in the above model. To assess the incidence trend based on different months and the nature of the non-linearity of the data, we used two models, including the 'generalized additive model' (GAM) and 'generalized additive mixed model' (GAMM). Finally, to predict the incidence trend for the following months and years, we used the 'seasonal autoregressive integrated moving average (SARIMA) approach by $\text{ARIMA}(2,0,0)\times(2,1,0)_{12}$. To analyze the various data, we performed R4.1.1 by using nlme, smooth, forecast, gam, gamm4, simts, and sarima packages.

The outcome of both effects, 'fix' and 'random' trends in the incidence of CL in different provinces, were significant ($p < 0.001$) at the national level (Table 2). This trend has decreased with time (estimate: −0.88, CI: −1.70, −0.05, and covariance between intercept and year, −0.48). We concluded that the provinces with a higher incidence rate, such as Ilam, Fars, Semnan, Isfahan, and Golestan, showed a faster-declining trend than the other provinces. Therefore, implementing the interventional measures recommended by WHO during 2014–2018 has effectively reduced the disease burden in high-risk provinces (58).

Then we assessed the main effect of age and gender interactions with year on the incidence rate of CL (Table 3). There was a significant difference ($p < 0.001$) among <10 years old children and 10–25 or > 60 years old individuals. Similarly, there was a significant difference in the incidence of CL in males and females ($p < 0.001$) (Table 3). The annual trend of new cases by different age groups is presented in Figure 2. At the same time, if the interaction of age with year is being considered, there was only a significant difference ($p < 0.03$) between <10 and > 60 years older. No significant differences among <10 and other groups were observed (Figure 2A). Figure 2B displays the significance of the difference in incidence trend among males and females by time. There was a relatively constant trend in the incidence of CL in females and males from 2015 to 2020.

An equivalent number of affected males and females were described in public-based studies; however, investigations based on surveillance systems exposed a deceptive upsurge of males (51), possibly described by diverse clinical-seeking behaviors for both genders. A study in Iran confirmed that unresponsiveness to CL therapy is meaningfully linked with increasing age. The impairment of innate and adaptive immune systems may account for amplified drug unresponsiveness in older age groups (12). Male patients frequently do not complete the assigned medication, mainly because of insignificant treatment adherence. This partial adherence to treatment might play a critical role in treatment failure.

Then we explored the monthly trend in the incidence of the disease by the time of occurrence. Figure 2C exhibits seasonal trends reflecting non-linearity modeling tendencies. Incidence rates gradually declined during the spring and early summer from April to July, and a sharp upsurge from August to November during the cold months of the year was observed, where again, sharply decreased until March. It seems that the sandfly activities start sometime in the early months of spring (59) when the *Leishmania* agents undergo the incubation period. The CL lesions gradually appear in late summer, when a peak of CL occurs in August to November during the monsoon period. Meanwhile, the caseloads sharply dropped from January to March compared to the remaining months.

Next, we closely evaluated the incidence trends by the year of occurrence. We found that the highest number of reported morbidities appeared in 2014 and 2015 (Figure 2C). It was

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TABLE 1 Annual incidence (per 100,000 population) trends of cutaneous leishmaniasis in various provinces of Iran, 2013–2020.

Provinces	2013	2014	2015	2016	2017	2018	2019	2020	Average
Ilam	102.6	271.5	224.1	180.6	135.0	77.0	78.7	74.6	143.0
Fars	86.7	112.7	123.6	74.1	57.0	59.7	40.0	48.8	75.3
Semnan	28.7	30.4	29.7	48.5	30.9	41.4	95.9	175.8	60.2
Isfahan	60.3	59.7	38.1	28.1	22.9	68.3	59.1	53.6	48.8
Golestan	32.9	36.2	43.6	51.4	33.3	75.7	60.9	33.9	46.0
Khuzestan	19.2	80.5	60.4	34.9	31.0	29.7	20.7	36.1	39.1
Khorasan Razavi	63.9	53.3	42.7	40.5	34.5	26.4	23.4	15.4	37.5
Khorasan Shomali	50.7	55.5	31.1	22.6	24.8	35.5	24.0	20.0	33.0
Yazd	27.3	33.2	41.8	26.9	16.4	36.6	21.4	32.3	29.5
Kerman	41.6	32.4	25.9	25.8	23.8	22.2	17.1	13.6	25.3
Qom	18.8	15.3	13.4	1.8	11.8	25.7	21.3	19.6	16.0
Sistan and Baluchestan	13.6	15.2	9.5	6.9	5.2	6.7	12.0	18.6	11.0
Kermanshah	3.5	15.5	2.4	13.2	17.9	10.3	5.9	17.9	10.8
Lorestan	5.9	13.3	17.8	9.2	7.7	5.5	3.8	6.7	8.8
Bushehr	11.1	12.9	12.1	7.8	5.1	9.3	2.3	3.8	8.1
Kohkooliye &Boyerahmad	7.8	12.0	9.8	9.8	7.2	6.1	6.7	4.9	8.0
Hamedan	2.8	9.3	12.2	8.1	9.5	6.9	4.5	5.5	7.3
Khorasan Jonoobi	6.9	8.7	13.5	8.1	3.5	9.0	4.8	3.8	7.3
Chahar Mahal and Bakhtiari	7.4	9.5	9.1	4.7	3.6	7.6	6.1	7.7	7.0
Hormozgan	6.4	4.7	4.9	5.7	9.1	7.6	8.4	4.3	6.4
Tehran	1.9	2.2	2.2	2.8	2.3	3.9	3.1	3.3	2.7
Markazi	1.5	3.0	1.8	2.6	2.8	4.1	2.2	3.7	2.7
Alborz	1.1	2.1	2.0	1.9	1.7	2.0	2.4	2.5	2.0
Mazandaran	1.5	1.4	1.2	1.8	1.4	1.4	1.6	2.2	1.6
Ardabil	1.4	2.0	1.2	1.6	2.0	1.9	0.6	1.5	1.5
Ghazvin	1.1	0.7	0.5	0.5	2.9	2.1	1.3	1.4	1.3
Kordestan	0.5	1.6	2.4	1.5	2.4	0.7	0.4	0.6	1.3
East Azerbaijan	0.8	1.3	1.4	1.3	1.1	1.2	1.3	1.4	1.2
West Azerbaijan	0.4	0.9	1.1	0.6	1.1	0.8	1.0	1.0	0.9
Zanjan	0.5	0.5	0.5	1.1	1.0	1.2	0.5	0.7	0.7
Gilan	0.1	0.6	0.3	0.2	0.4	0.2	0.8	0.1	0.3
Average	19.6	28.9	25.1	20.1	16.4	18.9	17.1	19.8	20.7

interesting to know the nature of non-linearity trends of cases in different provinces during the study. We found a robust non-linearity model analyzed by the GAMM model as the actual degrees of freedom (EDF) was 2.79 ($p < 0.001$) (Table 4). Further, we wanted to be sure of the non-linearity of the incidence trend; the outcome confirmed a similar distribution pattern of a non-linearity model (Figure 3A).

Figure 3B illustrates a contour analysis image based on the monthly and seasonal cycle of CL incidence by the time of occurrence. We found that in early spring, the incidence rate was static (blue color), and in early summer (July), the patients rapidly increased where they reached a peak in December (brown color) and decreased after that. This image also shows that the highest number of new cases appeared in 2014 and 2015. Considering the yellow and brown colors in the upper part of Figure 3B, we expect a peak incidence of new CL cases from 2022 to 2023. The emergence of cases shifted from August to September in the current date and the cessation of cases is delayed from January to December. Figure 3C displays a 3-dimensional image of monthly and annual incidence peaks. One of the peaks occurred in 2014 and 2015, and the other is expected to occur in 2022 or 2023. In addition, this image (Figure 3C) shows a non-linear association of the new CL cases.

To forecast the incidence of CL in Iran, we used the Seasonal Autoregressive Integrated Moving Average (SARIMA) model. This model can be a valuable tool to predict the trend of CL in planning future public health programs. Therefore, based on the previous data, we developed a SARIMA time series model from 2013 to 2020 to predict the monthly incidence of CL for 2021 and 2022. Cutaneous leishmaniasis incidence in a given month can be assessed by the number of cases occurring in 2021 and 2022. The prediction

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TABLE 2 The incidence trend of cutaneous leishmaniasis in various provinces of Iran by the fix and random effect models, 2013–2020.

Fix effect				
Parameters	Estimate	SE	value of *p*	%95 CI for estimate
Intercept	27.39	6.54	<0.001	(14.02, 40.77)
Year	-0.88	0.40	0.04	(−1.70, −0.05)
Random effect				
Parameters	Variance	value of *p*		Covariance between Intercept and Year
Intercept	42.86	<0.001		−0.48
Year	0.16	0.05		

TABLE 3 Age and gender interactions of cutaneous leishmaniasis incidence trend in various provinces of Iran, 2013–2020.

Variable		Estimate	SE	Value of *p*
Age	<10	0		
	10–25	−3.10	0.69	<0.001
	25–60	1.27	0.69	0.07
	≥60	−5.27	0.69	<0.001
Sex	Male	0		
	Female	−3.44	0.97	<0.001
Year		−0.57	0.21	0.008
	(<10) with year	0		
Interactions of age with year	(10–25) with year	0.20	0.30	0.52
	(25–59) with year	0.16	0.30	0.59
	(≥60) with year	0.63	0.30	0.03

TABLE 4 The generalized additive mixed model (GAMM) represents the strong non-linearity of cutaneous leishmaniasis in different provinces of Iran, 2013–2020.

Smooth term	EDF	RDF	*F*	Value of *p*
S (months)	2.79	3	57.51	<0.001

EDF, Effective degrees of freedom; RDF, Reference degrees of freedom; < 1 linear, 1–2 weak non-linear, and > 2 strong non-linear.

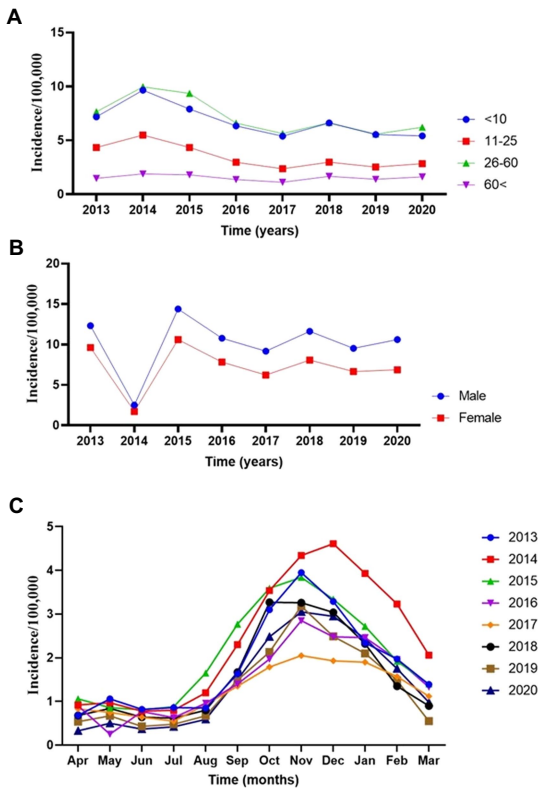

FIGURE 2
The annual incidence trend of cutaneous leishmaniasis in various provinces of Iran, 2013–2020. **(A)** Age-groups. **(B)** Sex. **(C)** Monthly.

data illustrates a non-linear seasonal incidence trend similar to 2020 (Figure 3D). More supplementary data to confirm the prediction assessment are shown in Supplementary Figure 1. We presented the trend values, including months, and point forecast at 95% confidence interval (CI) (Table 5) to closely evaluate the incidence. During September and March 2022, the incidence rate coinciding with autumn and winter (cold months) was statistically significant while not substantial in other months of the year. The plan requires a deep commitment to initiate interventional measures, implement appropriate control strategies, and highlight temporal and spatial hotspots. Identification of the persistent and high-incidence areas could help the program prepare and initiate an elimination plan for future control programs, as WHO requested (58).

Furthermore, in the present study, we utilized the R 4.1.1 software and spatstat, shapefiles, maptools, rgdal, tmap, raster, sp., and spdep packages to identify hot and cold spots in the data, as well as spatial outliers. We used both the Global and Local Moran's I to demonstrate the dimension of spatial autocorrelation statistics to detect clusters or local outliers, understand their contribution, and provide a decomposition to the Moran's I global clustering statistic. The spatial Autocorrelation (Global Moran's I) tool simultaneously measures spatial autocorrelation based on feature locations and feature values. The tool calculated Moran's Index value and both a Z-score and *p*-value to evaluate the significance of that index.

Tabular numbers and maps illustrate that high or low values tend to concentrate in some provinces more than others. These geographical patterns reflect variability in the socioeconomic and environments of the studied population and species differences of

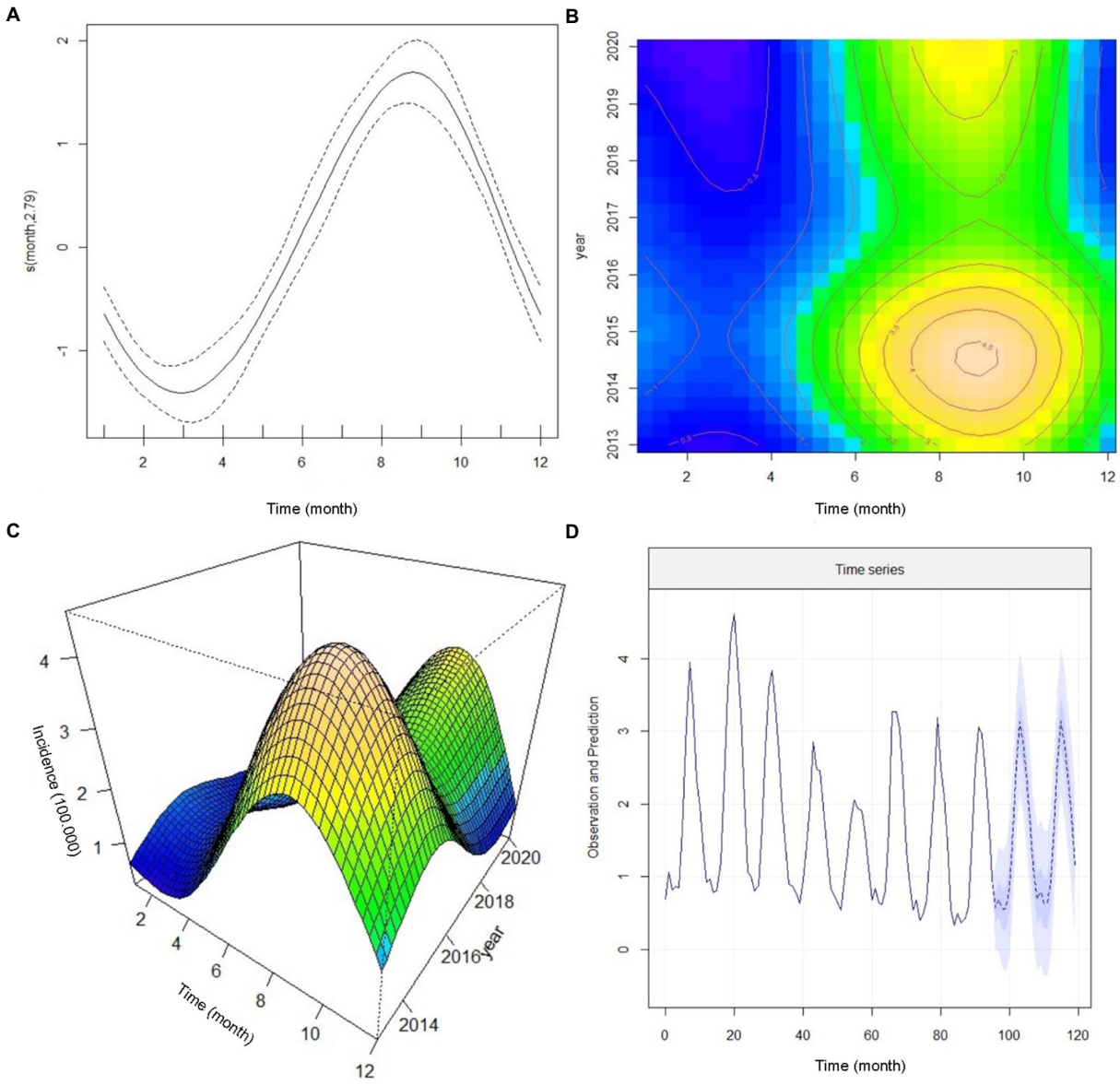

FIGURE 3
The annual incidence trend of cutaneous leishmaniasis in various provinces of Iran by the generalized additive mixed model (GAMM), 2013–2020. **(A)** Confirmation of a non-linearity model of incidence trends **(B)** a Contour analysis-based seasonal cycle displaying the incidence trends, **(C)** A 3-dimensional monthly-annual image of the incidence trends, and **(D)** Predicting the trend in the number of new cutaneous leishmaniasis cases from 2013 to 2022.

the causative *Leishmania* parasite and the host's immune response. We specifically examined the nature of the sample to distinguish the "fitted (smooth) from the "residuals" to follow descriptive data analysis terminology. This correlation may be different as low, high, or non-existent based on the variables used. Therefore, four categories were illustrated based on Moran's values related to the locations and surrounding provinces. We highlighted the connection between the Moran scatter plot and the cluster map. The Moran clustering map provides a classification of spatial association into four categories corresponding to the location of the points in the four quadrants of the plot. Locations with high values and similar neighbors are evaluated as high-high ("hotspot cluster"). Locations with low values and similar neighbors are referred to as low-low ("cold spot cluster"). Low-high locations have low-risk but high-value neighbors (potential geographical outliers or "cold outlier areas"). A high-low ("hot outlier area") is a region with high values surrounded by provinces with low risk. The local Moran statistic is constructed from the average incidence of the neighbors, which is sensitive to the effect of outliers.

These groupings can directly interpret provincial incidence over time in the nationwide areas. Whole data were used to construct maps to interpret disease behavior and endemic provinces of the country and demonstrate the temporal expansion of disease incidence over the years and geographical areas. In the following maps (Figures 4, 5), green, yellow, brown, and red reflect Moran's I incidence rates representing a specific topographical pattern. As

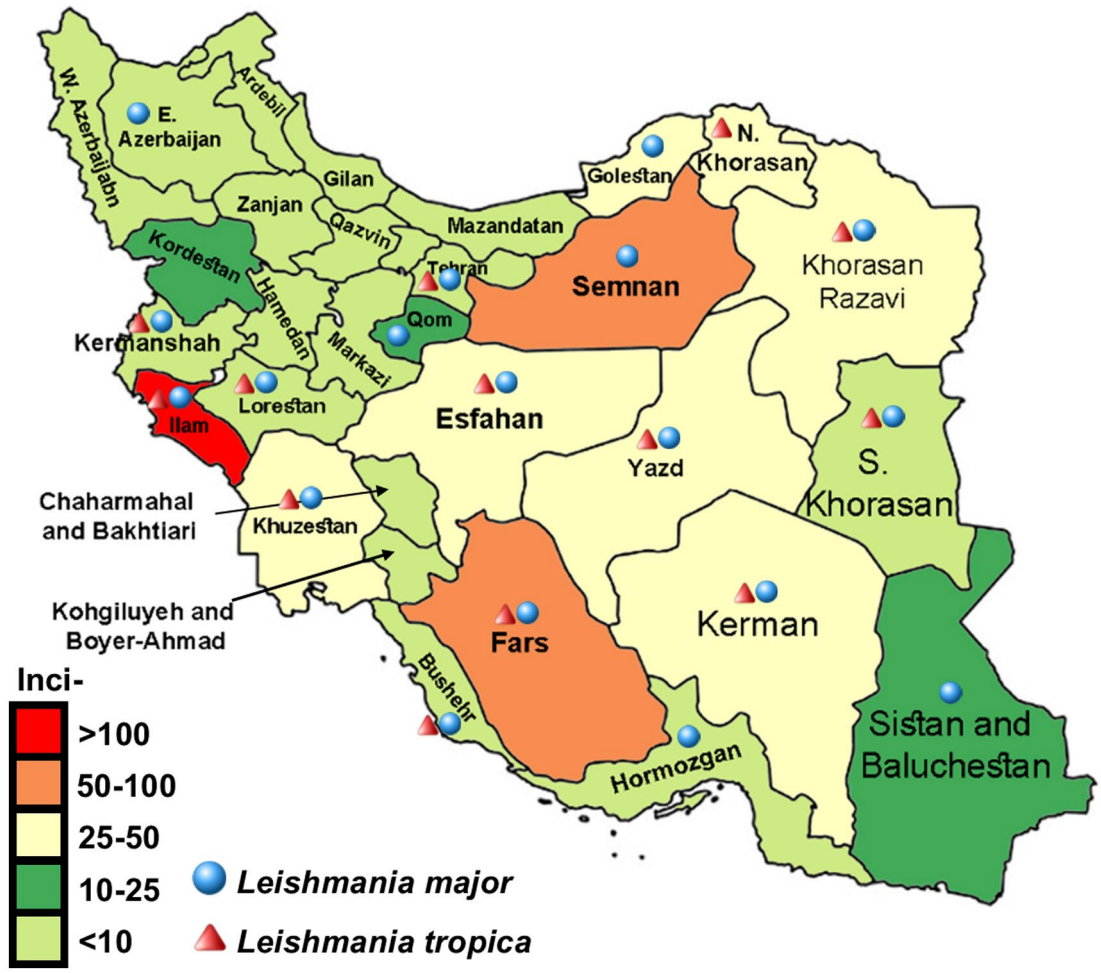

FIGURE 4
Displaying distribution and average incidence rate (2013–2020) of zoonotic cutaneous leishmaniasis and anthroponotic CL caused by *Leishmania major* and *L. tropica,* respectively. Infections with both diseases are detected in most provinces of Iran.

TABLE 5 Prediction of cutaneous leishmaniasis incidence based on 95% CI for point forecast, 2022.

The month of 2022	Point forecast	%95 CI for point forecast
Apr	0.33	(−0.84, 1.50)
May	0.50	(−0.67, 1.66)
Jun	0.37	(−0.79, 1.54)
Jul	0.42	(−0.74, 1.59)
Aug	0.59	(−0.58, 1.75)
Sep	1.50	(0.33, 2.67)
Oct	2.48	(1.31, 3.65)
Nov	3.05	(1.89, 4.22)
Dec	2.95	(1.79, 4.12)
Jan	2.44	(1.28, 3.61)
Feb	1.85	(0.68, 3.02)
Mar	0.98	(−0.18, 2.15)

CI: Confidence intervals are zero non-significant, while the positive values are significant.

the trends shift to darker green, the spatial autocorrelation becomes positive (high-high or low-low cluster). While the colors turn to brown or red, the status of provinces shows high-low or low-high. For example, in 2013, the spatial autocorrelation demonstrated a high cluster; the incidence in Yazd province was high, and the value was also significantly high in those spatially autocorrelated provinces. In contrast, Zanjan province revealed a low-low situation simultaneously, and the incidence rate was significantly low in neighboring provinces.

In 2017 like in 2014, the spatial autocorrelation signified a low-high outlier value in the cluster map for Lorestan province. As a result, the average incidence of the neighbors turned out to be much higher than would be the case under spatial randomness. Conversely, in 2018 the situation was similar to 2013; that is, the spatial autocorrelation presented low-low cluster locations in the map exhibiting similar clusters of low incidence rates in the adjacent provinces. Also, in 2018, several provinces, including Yazd, Semnan, and Khorasan Shomali, characterized high-high cluster locations confirming both high incidence rate cluster centers (with blue outlines) as well as their neighbors. The spatial autocorrelation

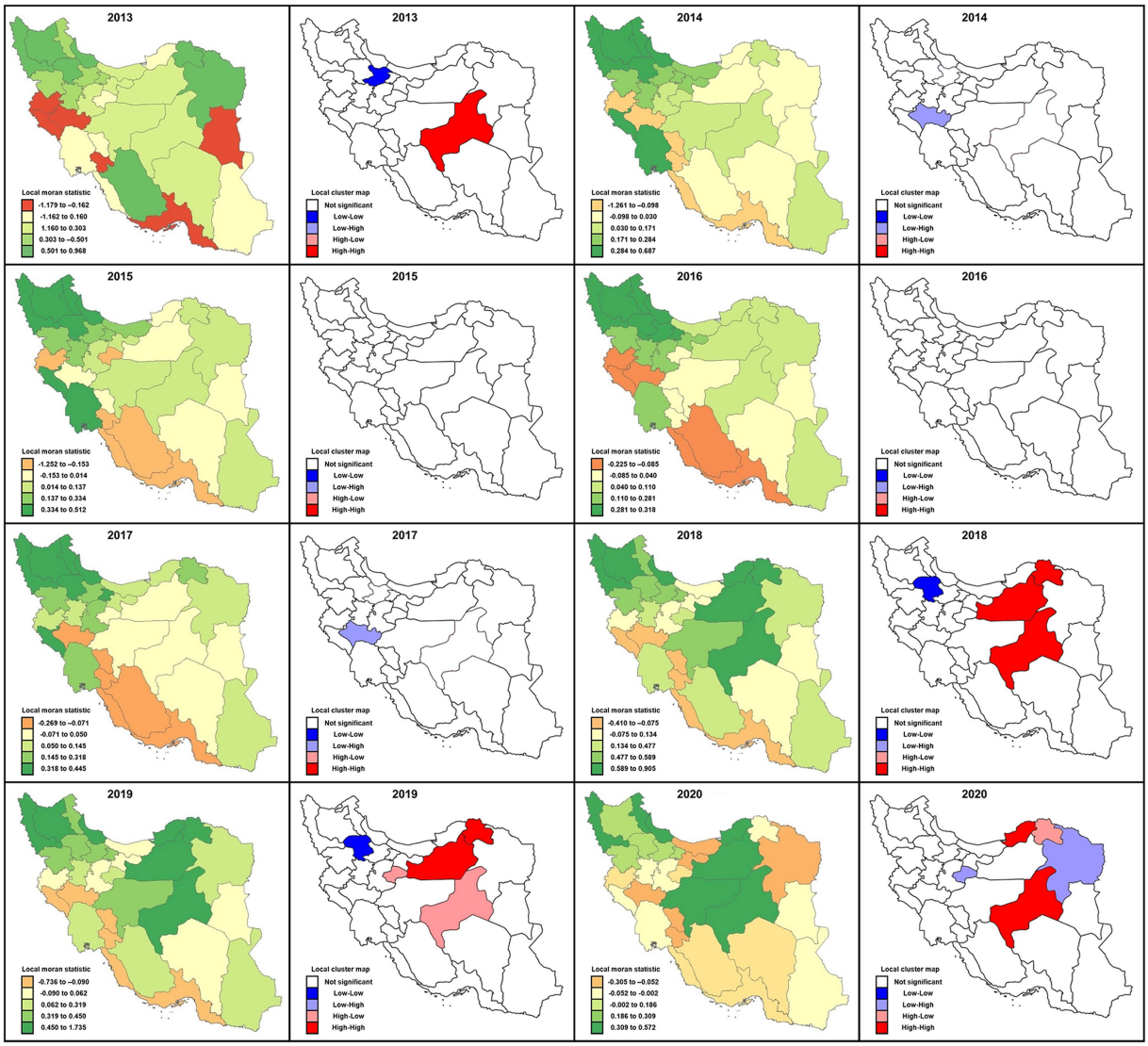

FIGURE 5
Local Moran's I statistics and local cluster map for spatial autocorrelations illustrating clustering locations of cutaneous leishmaniasis distribution in different provinces of Iran, 2013–2020. Moran's value has been mapped to highlight the incidence rates according to relative importance and surrounding neighbors' behavioral relationships possessing four different categories: high-high, low-low, low-high, and high-low.

picture was substantially different in 2019 relative to 2018. The status was the same for Semnan, Khorasan Shomali, and Zanjan; (low-low), while high-low for Yazd and Qom provinces, and the incidence rates changed to a high-low value. Unlike in 2018, these latter provinces indicated high incidence rates surrounded by low-value neighbors. In 2020 the circumstance was noticeably mixed. Yazd and Golestan provinces were high-high, but Qom and Khorasan Razavi were in high-low positions.

Based on the results of this study, the average incidence rate significantly reduced during 2013–2020, and the overall spatial autocorrelation was significant ($p < 0.05$). However, an exception was for 2015 and 2016, which showed no significant level ($p = 0.06$ and $p = 0.07$, respectively) when the average incidence rates were evaluated (Table 6). The highest spatial autocorrelation belonged to 2018 (Moran's index = 0.25) and the least related to 2016 (Moran's index = 0.08).

5. Prevention and control measures

Prevention and control of CL require an integrated effort entailing a combination of intervention strategies as transmission occurs in a complex biological-environmental scheme involving the parasite, host (human and/or animal), and vector. Control measures vary from region to region and are prioritized based on an allocated budget, although the approach mainly depends on costly, ineffective, and challenging chemotherapies. Key preventive and therapeutic measures are listed below:

5.1. Personal protection

Protection of the population by repellents such as diethyltoluamide (DEET), insecticide-impregnated nets (pyrethroids), windows,

TABLE 6 Annual nationwide cutaneous leishmaniasis average incidence rates/100,000 and investigation of spatial autocorrelation using the Global Moran'I statistic, 2013–2020.

Year	Average incidence rate	Moran's I statistic	Moran's I standard deviate	Value of *p*
2013	19.60	0.17	1.93	0.02
2014	28.90	0.11	1.84	0.03
2015	25.10	0.10	1.55	0.06
2016	20.10	0.08	1.45	0.07
2017	16.40	0.11	1.82	0.03
2018	18.90	0.25	2.59	0.004
2019	17.10	0.21	2.38	0.008
2020	19.80	0.11	1.75	0.04

clothing, fabrics, curtains, and door fences was frequently used in different endemic foci within the country. However, their beneficial aspects have not been well evaluated in different localities (60). Dressing the skin lesions dramatically reduced contact between humans and sandflies (61).

5.2. Chemical control

The essential methods for controlling sandfly vectors with chemical insecticides are indoor residual spraying, spraying of resting sites of wild species, use of insecticide-impregnated resources including bednets and curtains, and pyrethroid-impregnated dog collars. Data indicated that insecticide-impregnated bed nets, indoor residual spraying with insecticide (IRS), and long-lasting insecticide-treated nets (LLITNs) were highly effective against ZCL and ACL primary vectors; however, they are not well accepted behaviorally by the residents (61, 62).

5.3. Environmental modification

Management of the environmental alterations has had a drastic effect on the frequency of vectors, resulting in a reduction in phlebotomine-human contact, or sandfly population, and transmission levels. This strategy may include the replacement of inhabitants away from sandfly habitations and physical modification of their environments. Elimination of propagating multiple risk factors such as solid wastes, garbage, maneuvers, leaves, organic remnants, and rubbles had a crucial role in peri-urban and high-risk areas in controlling vector densities and transmission profiles (25, 63). The destruction of the habitat followed by the exploitation of the land by settlements and farming is the only permanent way of controlling sandflies. This approach is successful but at a great expense in a highly endemic ZCL focus transmitted by *Ph. papatasi* in central Iran (64).

5.4. Gerbils and dogs' control

Control of competent reservoir hosts is fundamental in ZCL and ACL, as control strategies can be directed at these hosts. There are numerous experiences gained by investigators in central Iran, where zinc phosphide poison mixed with wheat grains and vegetable oil (2.5%) were used in a radius of 500 m from houses, once a month during May, June, July, and September in the first year and repeated once every two years in the coming years for ZCL (49, 65). They suggested that gerbil control actions employing zinc phosphide within a 500-meter radius of houses once every two years in late April before the commencement of the active season of sandflies. In foci where dogs are reservoir hosts of *L. infantum* and probable hosts of *L. tropica,* the control of all dogs by sheltering, as practiced in a few places in Iran, is an efficient complementary means of ZVL and ACL control (66–68). As implemented in some localities in Iran and abroad, the selected culling of seropositive dogs was followed by a drop in canine infection while it is costly (69). A cheaper and more likely economic means of fitting dogs with deltamethrin-impregnated collars act as a long-term depot releasing the insecticide into the skin's lipid (64).

In Iran, guard dogs were protected against 80% of bites of *Ph. papatasi* eight days after collars were attached (70). However, the effectiveness of dog collars will depend on the importance of domestic and wild canids as reservoir hosts of *L. infantum* or probable secondary hosts of *L. tropica.*

In areas where *L. major* induces CL, reducing rodent populations by physical destruction of burrows, followed by planting, has proved to be an effective and sustainable method of controlling CL where *Psammomys obesus* is the known reservoir. Poisoning with wheat grains and vegetable oil is effective for other migrant rodents such as *Meriones* (71).

5.5. Health education

Training programs for health care providers on various aspects of leishmaniasis, including rapid detection, case management, different control modalities, and rapid response to epidemics, have continuously been carried out in provinces where CL control units or centers were functioning as Kerman, Isfahan, and Bam. Education is undoubtedly a very cost-effective preventive measure (72–74). Notifying populations in endemic areas leads to appropriate case detection, a better acceptance of preventive and therapeutic measures, and lower behavioral risk. Fortunately, significant systematic health education has been implemented to evaluate different aspects of effectiveness and health impacts at the district and provincial levels. A cost-effectiveness education in Iran and elsewhere assessed the advantage of combined prevention strategies for CL-endemic areas, including applying insecticide-impregnated clothing and curtains plus premature CL diagnosis training plans for health care personnel (75, 76).

5.6. Treatment of patients

Based on the WHO guidelines, cases of ZCL do not need to be treated unless vital organs are affected. In contrast, patients with ACL must be treated since humans are the only reservoir host (77, 78). The type of treatment is based on five criteria (79), including the size of the most extensive lesion, the number of lesions, the location of lesions, the causative agent (type of *Leishmania* species), and in some

instances, immunologic status. Meglumine antimoniate (Glucantime®) is the drug of choice, administered intralesionally once a week for 8–12 weeks along with biweekly cryotherapy or intramuscular injection alone for 3–4 weeks according to the national guideline consistent with that of WHO (20, 79). Another second-line medication, mainly in combination with MA, was used on a special occasion. The overall efficacy of MA against ACL was 89% in a cohort study with a significant focus in southeastern Iran (51); however, in routine practice, variable ranges of treatment failures have been reported for ACL (80–82) and ZCL throughout the country (82, 83). In a contraindication for conventional therapy of CL, other treatment choices, particularly multiple therapies, were used (78).

6. Major challenges

6.1. Associated-risk determinants

Cutaneous leishmaniasis is linked with numerous confounding factors. The following risks are the significant determinants associated with the perpetuation of the organism, which is directly involved with the outbreaks caused by ZCL or ACL in Iran.

6.2. Agricultural developments

Launching agricultural projects has increased the risk of ZCL in rural areas. Under such conditions, anthropogenic ecological disturbances occur, and simultaneously, many non-immune immigrants intrude into the area where the sylvatic ZCL cycle is present (84, 85). Transmission to humans is promoted by people sleeping outdoors without bed nets throughout the transmission months. There are many examples of these epidemics where new agricultural developments in Iran (86, 87). One example was the epidemic of ZCL in the southern villages of Baft district with high severity in all age and sex groups in a new agricultural region.

6.3. Socioeconomic factors

Primitive and poor housing conditions and the resulting low living standards increase the risk of transmission in peri-domestic areas. These are organic remnants, manures, piles of bricks, debris, rotten leaves, and stones, which constitute potential breeding areas and resting sites for sandflies. Such places in the vicinity of poor households, and low-income individuals such as laborers, favor CL transmission. Also, many people sleeping in the shared room attract peri-domestic anthropophilic sandflies; therefore, poverty increases the disease risk. Housing sanitary conditions, including the lack of solid waste management and sewage treatment plants, could provide vectors' resting sites and enhance sandflies' proliferation potential, facilitating contact with humans. Sandflies are frequently attracted to crowded housing, which provides a good source of blood meals. The human behavioral attitude of sleeping outside and on the ground could also increase the risk of infection. Such risk-associated determinants are frequently observed as the cause of epidemics/outbreaks in Iran (88–90).

6.4. Climate changes

Leishmaniasis is a highly climate-sensitive disease, and epidemiological characteristics can be changed in several ways: physical factors include temperature, humidity, precipitation fluctuations, influence vectors, and reservoir populations (91). Even slight fluctuations in temperature can substantially affect the biological development of the promastigote stage in the gut of sandflies contributing to the transmission of the organism in the previously non-endemic areas (18, 92). Drought, flood, starvation, and disaster contribute to enormous migration and population displacement to new endemic foci, creating an epidemic situation of variable magnitude. Such conditions provide new risk factors that favor the parasite, vector, and reservoir, leading to the disease's propagation. Extreme examples have previously been documented in the CL epidemic foci following the presentation of risk factors in favor of illness (14).

6.5. Population movement

Outbreaks of CL are often linked with population displacement from non-endemic areas to new places where the CL infection is already present. The movement of inhabitants and the creation of new settlements in suburban areas in close vicinity with endemic foci are major confounding factors and are directly connected to the incidence of CL, either leading to exposure of the susceptible and at-risk population to new hazards or contributing to the introduction of the causative parasite into new areas. The estimate of such outbreaks is related to the accessibility of ecological and epidemiological information. After the Bam earthquake in 2003, approximately 60,000 newcomers arrived to provide services to the survivors. The city was divided into 12 zones, and all zones were given to health personnel from different provinces of Iran. A massive outbreak of ACL occurred two years after the earthquake and was sustained for nearly four years. In this outbreak, the number of cases increased from 800 to 5,000. In addition to providing numerous risk factors, the new arrival population was vulnerable to CL causing such a continued and enormous epidemic with variable proportions (14, 93).

The spread of ACL in southeastern Iran, particularly in Kerman province, has resulted from population movement and the provision of many risk factors for the proliferation of vectors and transmission of the disease (14). Several emerging epidemics of ACL occurred in rural areas within and outside the County of Bam including, Dahbakri, Nazamshar, Ghal-e-shahid, and Mohammadabad (51, 52, 55, 93–97). The epidemic of CL is frequently connected with the movement of non-immune people into foci where active transmission is already present. The epidemics of varying scales have been reported due to population movements for economic reasons and agricultural development in Iran and other countries such as Afghanistan, Brazil, Libya, Iraq, Sudan, Syria, Morocco, Peru, Saudi Arabia, and Yemen (1, 24, 98–104).

6.6. Environmental changes

Ecological changes in the landscape in rural and urban areas where the disease is endemic contribute to the outbreak. As a result,

many risk factors favoring sandflies' breeding potentials will be provided, leading to a sudden or gradual increase in cases. Garbage left around houses permits some localities to store grain outside houses attacks rodents, serving as the reservoir for the propagating disease. One of the reasons for the low number of CL in the past decades was the implementation of mass insecticide spraying in high-risk areas around human and livestock dwellings (105).

7. Other challenges and gaps

7.1. Health care system

In 1985, relevant health fields and medical education were initially combined within the Ministry of Health and later on in 1993, renamed MOHME. Therefore, both sections, medical education, and health services were integrated and formed Universities of Medical Sciences and Health Services throughout the country's provinces. At present, MOHME is in charge of both tasks; providing health care services for the communities and training human resources (106, 107).

Although some posts may be subject to change over time. The healthcare system in Iran is based on three pillars including the public-governmental system, which provides most services, the private sector, and NGOs.

There are numerous health system barriers potentially affecting various segments of control programs. The government and the patients, including the heavy economic burden, sometimes logistic problems in accessing primary healthcare centers, and treatment costs, although offered free, contribute to poor adherence to treatment. Since the burden of CL has diverse dimensions, this requires sustained and tremendous efforts and actions from the government such as policy-makers and healthcare providers, private sectors, and national and international agencies, notably WHO. Other health system challenges entail insufficient capacity for surveillance, especially at the peripheral level. The number of health facilities is not adequate in some areas, health personnel often have scant experience, supplies are not adequate, and the procurement supply chain ensures that medicines available at request are not sometimes enough. Lack of sufficient budget and inter-sectoral coordination, fast turnover of health personnel, lack of district and provincial attention to control strategies, and many other related issues are often unsatisfactory.

7.2. Health services

Health services are provided at primary, secondary, and tertiary levels, including health houses, and health centers (urban and rural, hospitals at district and provincial levels). Most of the primary health needs are provided by PHCs in health houses, followed by higher professional personnel and physicians in rural and urban health centers. Hospitals are responsible for providing secondary health care services for the residents. Health expenditure has increased rapidly in the last decade, but there is still a shortage of healthcare financing. The out-of-pocket expenditure share used to be around 50%, although this ratio has significantly changed to a lower proportion during the past seven years, mainly provided by several public and non-public insurance schemes. Human resources have increased to approximately 300,000 employees, including health, clinical, and educational services at different levels. Leading health indicators have improved mainly over the past three decades, owing to the government's strong commitment to the effective delivery of PHC and inter-sectoral development (108, 109).

The decision-making body is centralized, directly influencing on-time decision-making, particularly in emergency events and responses. Decentralization of leishmaniasis control is planned, implemented, reported, monitored/evaluated locally, and supervised centrally by the health authorities at provincial and CDC members at the national levels. Retirement of staff and health personnel with no substitution retards the whole process of the control activities. Government is not entirely able to substitute retired personnel officially.

7.3. Poor follow-up assessments

There is inadequate follow-up examination of patients afflicted with CL following treatment regimens. There seem to be some patients having absent from the treatment follow-up assessment. Such underprivileged follow-up examinations contribute to treatment failure and intensification of cases, notably in ACL endemic areas. The primary barriers are as follows (51):

i. Non-availability of medicine
ii. Long-distance to healthcare centers
iii. Remote areas of living conditions and scattered residents in the affected areas
iv. Receiving incomplete treatment adherence
v. Work constraints

7.4. Suboptimal reporting

In addition to the insufficiencies above, which influence the control measures, there are also essential obstacles that could play a precipitating role in the optimal provision of healthcare services (110, 111).

The majority of official data are passive case detection, which is reported via the PHC surveillance system. Many cases are unreported, undiagnosed, or misdiagnosed, especially in remote areas and poor endemic villages where the availability and accessibility of diagnostic methods are scant (77, 112), while the actual burden might be worse, and the reports from the PHC surveillance system reveal only a fraction of the actual picture (7, 113).

7.5. Cross-border movements

Cross-border movement and tourism from neighboring endemic countries for ZCL and ACL are major challenges for a comprehensive control program. It is essential that some types of cooperation with the health authorities of these countries should be set up to combat, control, and settle such challenges threatening the program. Migration of non-immune populations from non-endemic areas to endemic regions and *vice-versa*, population movement, and migration from endemic areas to non-endemic localities where the conditions for propagating the parasite, vector, and reservoir are provided could

gradually or promptly abrupt into epidemic conditions (52, 114, 115). Iran is connected to Turkey, Azerbaijan, Armenia to the northwest, Afghanistan, and Pakistan to the east, Turkmenistan to the north, and Iraq to the west. The country border around 499 km with Turkey, 432 km with Azerbaijan, 35 km with Armenia, 909 km with Pakistan, 936 km with Afghanistan, 992 km with Turkmenistan, and 1,599 km with Iraq. Iran borders 650 km also, along the Caspian Sea. The above boundaries are aside from 2,440 km coastline sea borders with six other Arabian countries, including Kuwait, Oman, Qatar, Bahrain, the United Arab Emirates, and Saudi Arabia.

7.6. Lack of side-benefit of previous anti-malarial activities

Although it is controversial, marked changes as a side benefit of anti-malarial activity and a reduction in the number of sandflies have previously been reported. Following the cessation of spraying houses and dwelling areas, a resurgence of CL cases was observed. Discontinuation of spraying actions against malaria could be a protective factor for leishmaniasis in malaria areas where the incidence of the disease has significantly increased (49, 116). Also, house spraying with insecticides to control malaria in the 1950s and 1960s was experienced by reducing the kala-azar (VL) in India caused by *L. donovani* and transmitted by *Ph. argentipes* (116).

7.7. Demographic transition and fast urbanization

There is one-third of the population between 15–30 years old. The vulnerability of the three age groups to social and behavioral problems makes them at risk of suffering from major health problems like opium and drug addiction, violence, traffic injuries, and psychological complications, contributing to economic burden and other financial crises (117). One of the most outstanding landscapes of recent decades has been the wild urbanization rate. The urbanization pattern and socioeconomic status can promote disease transmission concentration, contributing to injustice in allocating medical and healthcare facilities and creating hotspots of diseases within communities, towns, and cities for the rapid spread of emerging diseases (118).

In Iran and most developing countries, mass human migration has lately been directed to unpredicted enlargement challenges of diverse 'megacities,' where living standards for sanitary conditions are unsatisfactory. Hence, such situations create appropriate breeding environments and augment human contact for spreading vector-borne diseases (VBDs), such as leishmaniasis (119, 120).

7.8. Cutaneous leishmaniasis outbreaks

In recent years Iran has experienced several outbreaks of CL due to natural events, man-made environmental alterations such as fast urbanization, deforestation, and population displacement for economic reasons, including agro-industrial projects, road constructions, and widespread migration more frequently from rural to urban areas in small towns and large cities (e.g., current droughts and earthquakes) (55, 56, 93, 94, 97, 121).

7.9. Natural events

Iran is highly vulnerable to natural and man-made (anthropogenic) disasters, particularly earthquakes, flash floods, and droughts (14, 86). Iran is ranked first regarding the frequency of earthquakes *per annum*, with approximately 5.0 to 7.0 on the Richter scale. This country is also ranked first in relative vulnerability and survivors each year due to earthquakes. Approximately 75% of the country's major cities are located in potential earthquake zones. Seasonal flash floods have drastically increased, whereby many local people in many provinces died, and their houses, fields, and livestock were devastated in May 2019. In Iran, it is estimated that 5 to 10% of the annual GDP budget has been allocated to manage disasters (122). Following disasters so many risk factors in favor of the propagation of vectors and multiplication of reservoirs will be created, and as a result, epidemics of varying degrees of CL will occur (96, 123–125).

7.10. Clinical forms, diagnosis, and treatment

Clinical assessment of CL lesion combined with identifying its causative *Leishmania* agents is crucial for selecting proper therapeutic modality and designing appropriate strategic approaches, especially in ACL endemic foci caused by *L. tropica* where control measures are limited to early case detection and prompt treatment of patients. Since CL lesions, acute or chronic mimic a variety of disease conditions, notably a broad range of viral (zoster, herpes-like, and wart viruses), fungal (lupus vulgaris, and sporotrichosis), bacterial infections (tuberculosis, and mycobacterial ulcers), skin diseases, tropical ulcers, myiasis, acute furunculosis, ecthyma, foreign-body granuloma, sarcoidosis as well as some parasitic infections and cancers (carcinoma of the skin); knowledge of such disease presentations and confirmation of the complication based on demonstration of the parasite is essential for any dermatologic practice in endemic areas (51, 77, 78, 126, 127).

Both CLs; ZCL, and ACL, generate a broad spectrum of disease manifestations in Iran and abroad. In addition to "dry type" and "wet type" lesions, typically represented by ACL and ZCL, respectively, there are also several atypical clinical forms that are presented depending on the condition; the causative *Leishmania* species or variant and state of the host's immune response, typical stages of skin lesion including papule, plaque, ulcerated nodule, and ulcerated plaque (52).

7.11. Drug unresponsiveness

Leishmaniasis is treated with pentavalent antimonial agents such as MA. Meglumine antimoniate (Glucantime®) and sodium stibogluconate (Pentostam®) have been a mainstay of treatment in the past 80 years, but resistance to these drugs has significantly increased in the endemic foci throughout the provinces of Iran and worldwide as well (78, 128). Failure is a frequent phenomenon reported for many years in treating CL in Iran and around the globe. Antimonial compounds have been used for many years in Iran and many countries despite their parenteral administration, toxicity, resistance, and long duration of applications. There are reasons for the emergence of resistance in Iran. Several determinants have facilitated this

phenomenon, including the high level of endemicity of the *Leishmania* species, the high proportion of routinely treated patients, and the most exclusive human-to-human transmission. Various reports have demonstrated such treatment failure in several localities within the provinces (12, 51, 129, 130).

Meglumine antimonite has extensively been used against all forms of leishmaniasis in Iran. Variable ranges of treatment failure against CL have previously been reported from endemic areas; thereby, many patients with ACL have developed unresponsiveness to Glucantime®; the rate of clinical failure of ACL patients in experimental models was reported to be approximately 12 to 15% in Mashhad, Kerman, and Bam (130, 131). *L. tropica* causing ACL is restricted to humans as anthroponotic species drug-refractory is a significant challenge in properly treating CL cases. Humans are the sole source of reservoir infection. The patient should be treated to prevent further dissemination of the organism to sandflies and, in turn, susceptible individuals. Also, some drug failures against ZCL have been reported from endemic foci since the disease undertakes an acute course and humans are incidental hosts. Presumably, non-treated cases have no role in transmitting the disease; although, the patients are often treated with proper compounds, accordingly. Suboptimal doses of the drug and poor treatment adherence could be predisposing factors for developing drug unresponsiveness (50, 81, 130, 132, 133).

Control of ACL caused by *L. tropica* is primarily based on early detection of the CL cases, diagnosis, identification of the causative agent, and prompt treatment via an effective surveillance system (51). Given that humans are the only reservoir host, untreated chronic cases such as leishmaniasis recidivans (lupoid leishmaniasis) remain the infective reservoir for disseminating the organism (95, 134). In general, treatment failure has been somewhat neglected in the conventional delivery of PHC services due to non-compliance with treatment protocol by patients. A robust assurance of a multidisciplinary method is necessary to make advancements in this part. Therefore, coordinated action from health experts, investigators, health providers, and strategy–makers is required. Treatment consequence is a multifactorial issue determined by the interaction of multiple factors (50, 51, 88, 135, 136).

Poor adherence to the treatment occurs for various reasons, including doubt about the expected benefits, the efficacy of treatment, unpleasant side effects, work constraints or economic situations, traveling away from home, feeling sick or depressed, and simple forgetfulness. In the evaluation of unresponsive cases, a considerable number of patients show difficulty in adhering to their recommended therapeutic regimens. The facts above represent Glucantime® mismanagement as a critical contributor to failure in the country's endemic areas. Treatment failure should be monitored to maintain the life span of existing antileishmanial drugs, delivery, and clinical response. Healthcare providers should pay special attention to CL patients who receive partial treatment regimens and closely monitor such patients to reduce the chance of drug failures (111, 137–139).

7.12. Vaccines development

Vaccination against CL has been used in humans for over 70 years. In protozoal diseases of the world, inoculating infectious organisms from the infection of a sore in naked parts of the body is an antique exercise. Iran has taken the lead in vaccine development for leishmaniasis since the 1960s. *Leishmania major* skin test antigen (LST) was first produced by Alimohammadian and colleagues at Pasteur Institute of Iran (140), and an experimental killed autoclaved *L. major* (ALM) vaccine (141) by Dr. Fesharaki in Razi Institute (Karaj, Iran). The products have been given to different countries for conducting vaccine trials against CL or VL (142). Trials of single and multiple doses of the first-generation vaccine have been used in Isfahan and Kerman and supported by TDR/WHO and MOHME in Iran (95, 143–146). At present, there is no efficacious human vaccine available against any form of leishmaniasis (147).

The only available non-approved vaccine which can be used in certain circumstances is a live virulent strain of *Leishmania major*. Leishmanization inoculates live *Leishmania major* metacyclic promastigotes from a purified culture medium to produce a self-healing lesion. It was an occasional practice against CL in Middle East countries, mainly used in Iran, Uzbekistan, and Israel to protect against future lesion development (148). Historically, CL was found to produce lifelong immunity to reinfection. Moreover, only a tiny proportion of individuals infected with *Leishmania* agents develop full clinical symptoms of the disease, while most are either asymptomatic or self-curing. Leishmanization programs were initiated in a hyperendemic area (Isfahan), and a high-risk group participated in the Iran-Iraq war, where a massive epidemic of ZCL was sustained. Over two million people undertook leishmanization, and it was reported to reduce the incidence of ZCL significantly; however, it induces lesions lasting several months or years (149). The biggest problem was the development of non-healing forms at the site of inoculation (1%–3%). However, with the onset of HIV epidemics and low production quality, leishmanization has been abandoned, but it remains an option when people are at high risk of afflicting CL (150).

Currently, vaccine development against humans and even canines is under critical investigation in Iran and globally. Although some vaccines have been developed for vaccination against the disease in murine and canine models, in reality, they could not help eliminate leishmaniasis in either case (humans and dogs). During the past three decades, tremendous efforts and significant studies have been carried out in vaccine development, notably in Iran. However, due to the complexity of adaptive and innate immunity, the search for a vaccine against leishmaniasis gains an extensive opportunity to give rise to a long list of potential vaccine candidates against leishmaniasis. This also remains unsolved until more profound knowledge and tools become available.

The main challenges and limitations for the progress of an efficacious vaccine against various forms of leishmaniasis are briefly listed as follows (151):

i. One of the main challenges in vaccine development against leishmaniasis is the poorly understood mode of host–parasite interaction and the complexity of the immune response associated with *Leishmania* species.
ii. Another main challenge is the proper immunobiological requirements for vaccine development against all types of leishmaniasis (CL, MCL, and VL).
iii. Lack of understanding of the many components that might cause such reactions.
iv. Establish trustworthy methodologies and approaches for assessing vaccination effectiveness.

v. Develop acceptable animal models for preclinical vaccination effectiveness testing.
vi. Discovery of suitable adjuvants and delivery systems to elicit a protective immune response.
vii. High cost of vaccine production.

Finally, long-term memory cells are generated for protection.

8. Health facilities situation

8.1. Health system

In 1985, relevant health fields and medical education were initially combined within the Ministry of Health and later on in 1993, renamed MOHME. Therefore, both sections, medical education, and health services were integrated and formed Universities of Medical Sciences and Health Services throughout the country's provinces. At present, MOHME is in charge of both tasks; providing health care services for the communities and training human resources. The health care system in Iran is based on three pillars: The public-governmental system, which provides most services, the private sector, and non-governmental organizations (NGOs).

9. Capacity and capability

9.1. Human resources

i. There are various human resource development plans in various fields relevant to leishmaniasis, including physicians, laboratory technicians, environmental health and entomology bachelors, and masters of medical sciences. Most human resources should officially be the Iran Health Care Reform Plan initiated on May 5th, 2014. Such resources provide rural and urban health clinics, hospitals, and environmental health units for PHC and leishmaniasis management.
ii. In addition to their educational background, human resources gain skills through periodically planned programs during the recruitment period.
iii. There is a description of the job instructions to indicate their path, experiences, and skills, and there are opportunities for career progression at each health or clinical level. Their career pathways are also defined.

9.2. Personnel/staff/physicians at the national level

The nature of human resources is integrated, and the only exception is in the centers located in high endemic foci where the main tasks are restricted to leishmaniasis works. Presenting a precise list of human resources at various levels is impossible due to the vast variability of jobs and the integration nature of leishmaniasis in the country, where multiple CL foci in 60% of the country's provinces are present.

9.3. Training

The personnel, staff, and physicians have to be trained periodically in health and related units, but it is not complete, and there are gaps in their training careers. The training system for new personnel in health units is located at the district level, where educational and training services are available. Professional health forces residents are trained in Bahvarzi schools, where these people learn professional units relevant to PHC. Other training experiences and skills are mediated through health managers or different faculties of the related universities.

9.4. Financial resources

i. According to the CDC authorities, an annual budget is allocated for leishmaniasis control at various levels. The amount that the government is allocated for different activities such as planning,
ii. Implementation, surveillance, monitoring, evaluation, and reporting are not adequate to carry out routine tasks, but depending on the province, the expenses are variable.
iii. The budget for leishmaniasis is negligible and is not adequate for most activities as this disease is neglected. Therefore, there are major constraints and gaps in providing duties, particularly at a peripheral level where an at-risk population is present. The area in which the budget is allocated include:
iv. Disease surveillance, case detection and management, program management, reservoir host control, integrated-vector control, capacity building, and health education.
v. It is of note that the PHC surveillance system, as previously mentioned, has its budget administered by the University of Medical Sciences (UMS) in the country, and they have their budget both for salaries and plans. For leishmaniasis, a sum of 10,400,000,000 Rials was initially allocated through CDC in 2020; but for some reason, nearly one-third of the budget was not allocated. Hence, the budget is not adequate to cover various management chains and interventions.
vi. Furthermore, there are still other sources of budget allocation where the authorities in UMS, sometimes through the governorate budget from public sources or proprietary revenues, shift the money for specific tasks. Nevertheless, the amount precisely allocated for leishmaniasis activities due to the complex nature of health issues is not well known.
vii. According to an inquiry from a responsible authority, the amount of money allocated annually for leishmaniasis activities is roughly around 30%–40% of the expenses required to manage the program.
viii. There are no other sources of funds to perform the tasks, and also there is a lack of global NGOs in support of plans for leishmaniasis activities.
ix. The post-elimination budget is another problem that the units are faced. It is supposed that the personnel should be substituted, annually regularly while it never occurs and therefore a significant amount of such deficient budget has been reduced.
x. Approximately 10%–12% of CL patients are referred to higher hospitals or higher health facility levels for advanced therapy.

Although these cases receive thoughtful attention and proper treatment modality, they often remain untreated, and the cases become chronic or transform into resistant clinical forms, which is one of the main problems notably in ACL foci where *L. tropica* is predominant.

9.5. Intra-sectoral collaboration

There is a reasonably good collaboration between various health sections within the MOHME. This is more likely prominent when a public health campaign at national, provincial, and district levels is conducted in the health system plans such as polio or malaria. Although leishmaniasis is generally well-focused and defined at any level, intra-sectoral collaboration needs more support.

9.6. Inter-sectoral collaboration

Since most health issues extend beyond the provision of health care services, a harmonized inter-sectoral collaboration between ministries, different governmental levels, NGOs, and stakeholders is crucial to address health issues and guide leishmaniasis actions at various levels. Basically, besides the University of Medical Sciences authorities, the health council at the provincial level strengthens and promotes such inter-sectoral cooperation, and coordination between all organizations within the ministry of health needs further attention. The council also works at the district level between the health authorities, the governor, and the representative of different administrations. Since the nature of health issues are multifactorial and multidimensional, the efficiency of such collaboration is variable.

9.7. Engaging and mobilizing communities

There is a national plan for community mobilization and participation in various health activities coordinated by the MOHME. As coordinated by health authorities and community engagement, many activities are carried out, especially in public education, awareness, and advocacy. There is a good experience with health volunteer forces participating actively in pubic-related health issues at different levels. However, since there is no defined monitoring and evaluation system, their effectiveness is not well apparent, although their works are well appreciated.

9.8. Surveillance system

At present, the most case detection system is based on passive case-finding approaches. This is why a substantial number of cases (2 to 5 times the detected cases) are not found (20). This under-reporting phenomenon further complicates detecting cases of ACL in endemic areas where humans serve as the primary source of infection and dissemination of the disease through the bite of the female *Ph. sergenti* at large in the area.

9.9. Case-management

In ZCL foci, since small gerbils are the primary reservoir hosts and the course of the disease is acute, there is no obligation to treat the patients unless a vital organ (e.g., face, nose, joints, and ears) is involved. However, early case detection, diagnosis, and prompt and effective treatment in ACL endemic areas are essential to reducing the principal source of infection (humans). Unfortunately, since most cases are passively detected, managing the diseases takes longer than 2–3 months. Therefore, this late treatment schedule contributes to drug unresponsiveness and leaves more complications and prominent scars. Many cases remain chronic, and some cases develop lupoid leishmaniasis, providing a source of infection for vulnerable populations in endemic areas. In addition to passive case finding, active case detection is highly recommended to decrease the disease burden. Improving public awareness and knowledge among vulnerable people to encourage early diagnosis and treatment-seeking behavior.

9.10. Basic and applied research agenda

Most basic and applied research needs and investigations, including the health research system, are centered in the UMS and research institutions. If there is a need, the above research bodies should be ordered and carried out. There is no agenda held for basic and applied research, but sometimes depending upon the priority of the needs, a research agenda will be established at the district or provincial level.

A search in Scopus reveals over 1,231 (approximately 317 articles on treatment and171 articles on epidemiology) articles of various types have been published from 2014 to 2020, and a PubMed search returns 1981 articles (including 925 articles about the treatment and 438 articles about the epidemiology of leishmaniasis) published during the same period. Most of such research is in epidemiology, treatment, and to a lesser degree, vaccine, and drug developments.

As leishmaniasis is a common disease in the country, almost all the UMS, Medical Schools, Faculty of Health, and Paramedical Schools are involved in research activities. The exceptions are leishmaniasis research centers and institutions such as the Dermatology Research Center in Isfahan, Tehran Center for Research and Training in Skin Diseases and Leprosy, Kerman Leishmaniasis Research Center, Pasteur Institute and the School of Public Health (and Parasitology Research Center), Tehran University of Medical Sciences, which are directly involved, and therefore, are responsible for most publications in the field.

Whether the research findings are reviewed, utilized, or implemented by the program is not well known, but the CDC is involved in applied research and vaccine development. Examples of this involvement are the Ampholeish project (liposomal formulation of amphotericin B) and the development of an attenuated *L. major* vaccine candidate is directly supported and assisted by the CDC.

9.11. Preventive measures

Since there are no efficacious vaccines available, prophylactic measures should be used systematically in endemic areas, but at

present, these measures are used sporadically and only in a selective manner. ITNs and LLITNs are not behaviorally well accepted (20). Most people in endemic areas sleep late at night they become infected by the bite of female sandflies early at night. The screens are not affordable, and the local villagers do not use them at peripheral levels. Furthermore, most people avoid using impregnated curtains and clothing (62). Due to budget restrictions, there is no regular use of insecticide in endemic areas. Previously, insecticide spraying was used two times *per annum* in malarious areas, but currently, the number of malaria cases (incidence) in different rounds of elimination programs, as supported partially by WHO, has decreased to less than 200 cases. As a result, the cases have become limited to remote and scattered areas where spraying has most likely not been practiced. Therefore, the exact frequency of insecticide resistance is not well-known. Some findings showed that *Ph. papatasi* was resistant to DDT, deltamethrin, permethrin, bendiocarb, and susceptible to cyfluthrin. On the other hand, the *Ph. sergenti* collected from indoor and outdoor sites were susceptible to all insecticides (152, 153).

9.12. Data system

At present, portable data collection and reporting systems are imperative in endemic areas. Data are reported through districts to provincial levels and then to national headquarters (CDC).

9.13. Monitoring and evaluation

Monitoring is the routine follow-up examination of the leishmaniasis program, including data collection, analysis, recording, reporting, documentation, development of actions planning, and evaluation of public health practice. The main objective of monitoring is to evaluate progress and implementation status, detect challenges and limitations, and ensure accountability and decision-making based on evidence. Generally, the used indicators measure follow-up, processes, outcomes, or impact.

The monitoring of the leishmaniasis control plan is based on the list of indicators used to evaluate the program's performance. Such indicators will provide evidence-based information on different phases of the control program at various levels: village, district, province, and national. All indicators, including epidemiological and operational indicators, are expected to be monitored and evaluated during the action plan.

10. Present status of the strategic position of leishmaniasis

10.1. Internal and external environmental analysis

A leishmaniasis expert team for assessing the gap analysis was established to find the leishmaniasis control program's strengths, weaknesses, opportunities, and threats (SWOT) toward an elimination plan. The SWOT analysis was intended to facilitate a data-driven look and fact-based at the ongoing position of the leishmaniasis organization within the country. SWOT analysis is illustrated as a square part into four quadrants, each devoted to a component of SWOT. This visual overview provided a rapid indication of the leishmaniasis position. A complete list of issues was compiled to collect data related to each component of the SWOT. Several meetings were held to screen, categorize and weigh each component. The most related information and desired data about the disease management and control program were selected to analyze the internal and external factors presented in Supplementary Table 1. Hence, a scoring matrix of the internal and external factors directly and/or indirectly associated with the application of the control program was established, and subsequently, a final score of the internal and external factors was obtained (Figure 6). Based on the scoring matrix SWOT of Bryson (154), the overall position of the national leishmaniasis situation was calculated to be in the Strength/Opportunity (SO) position. This score indicates that the national leishmaniasis strengths and opportunities are already reasonably moderate. This situation allows us to firmly step towards its activities and targets in the future elimination program.

11. Discussion and conclusions

In the last decade, CL has considerably been expanded spatially, and numerous outbreaks and emerging epidemics of variable magnitudes occurred, attributed to the dynamic of the disease and provision of many confounding determinants such as environmental changes, climatic conditions, disasters (earthquakes and floods), migration, and population movement among many others. Despite a significant reduction in VL cases, the geographical range of CL has remarkably enlarged (12, 14, 55, 56, 86, 155, 156). The policy-makers have seriously overlooked the disease, and health authorities do not consider CL a severe threat and urgent public health concern in Iran and abroad. This is mainly due to the disease's apparent non-serious and under-reporting nature, other confounding factors, and numerous chronic diseases. Iran is among the frontline-affected countries where

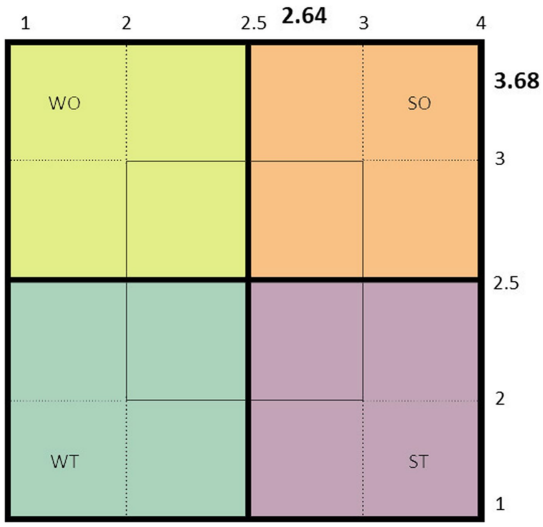

FIGURE 6
The present status of the strategic position of cutaneous leishmaniasis is based on the scoring scale of the strength, weakness, opportunities, and threats analysis (SWOT matrix).

many cases have officially been reported, although the actual number of new cases is assumed to be far beyond the reported statistics.

An important event in efforts against leishmaniasis was attained following the meeting held by WHO in 2007 (157); however, it underlined gains and challenges, eliciting further pledges from member states and private partners. Additional commitment is highly required to achieve the goals to control this neglected disease. Of the strategies recommended by the WHO, intensified CL management where effective treatment and confirmed vaccines are not yet available should be firmly targeted through better access to resources, organized cases detection, and decentralized clinical management to reduce morbidity and interrupt transmission (58).

Control of vector and reservoir hosts in an integrated manner combines multiple inter-sectoral interventions that enhance various measures, including efficacy, ecosystem approaches, and sustainability of disease control measures against sandflies and small gerbils. Lastly, improving socio-economic factors plays a critical role in achieving the goals. Documents compiled by the United Nations indicated that not only in Iran but also in the world as well, there are still 2.0 billion people who do not have basic sanitation facilities such as toilets or latrines (158). CL and all infectious diseases and several NTDs will not be eliminated and not eradicated until this situation improves. It is worth mentioning exploring and getting lessons from the VL regional leishmaniasis control programs in the Indian Subcontinent (159). During the past years, substantial efforts have been made, including capacity-building, strengthening improvement, accessibility to medicines and diagnostic tools, enhanced surveillance, monitoring, and evaluation.

In conclusion, analyses of the current epidemiological data indicate that Iran has made steady progress and remarkable advancement over the past decade, yet persistent challenges exist to reduce the CL burden in the country. Well-trained staff and experienced clinical practitioners must strengthen country-level capacity-building to sustain efficient control programs through the healthcare system (160). Cutaneous leishmaniasis is rooted in poverty; therefore, improving socioeconomic necessities toward basic living standards will ensure long-term control strategies and foster the elimination of the disease. A robust commitment to multidimensional approaches is critical to making advancements nationwide. This will require coordinated challenging activities through all governmental sections, including policy-makers, health professionals, clinical practitioners, senior researchers, private parties, and NGOs. Promotion of early case detection, prompt diagnosis, intensified effective treatment, increased access to available medicines, and capacity for environmental interventions on a long-term basis could hopefully facilitate the strategies and improve the prevention and control of not only CL but also neglected zoonotic diseases.

Furthermore, setting up early warning systems for CL can support forecast CL outbreaks. Hence, operational readiness and immediate response mechanisms should be in place in high-risk areas within endemic countries. However, the most decisive evidence gaps persist, and more innovative tools are critical before CL can eventually be controlled.

Author contributions

IS and MG designed research and generated the study plan. FG, BA, MZ, SD, FS, AKh, MB, and AA performed the data collection in the field. IS, MG, AKh, MB, MN, OZ, and MS conducted the analysis and drafted the manuscript. IS, MG, MRF, AKa, ES, SA, and MB analyzed the data. IS, MG, OZ, AKh, MB, and MRS revised the manuscript. MG and IS had primary responsibility for final content. All authors contributed to the article and approved the submitted version.

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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/fpubh.2023.1091709/full#supplementary-material

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Epidemiological analysis and risk prediction of scrub typhus from 2006 to 2021 in Sichuan, China

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Background: In the past decade, the number of reported cases of scrub typhus (ST) has increased dramatically in Sichuan Province. We aimed to overview the epidemiological characteristics of ST, identify the variables contributing to the spatial distribution, and estimate the risk areas of ST occurrence.

Methods: Daily ST cases reported at the county level from 2006 to 2021 and datasets on environmental and socioeconomic variables were obtained. Joinpoint regression model was utilized to examine the incidence trends and to calculate the annual percentage change. Global spatial autocorrelation analysis was employed to explore the spatial temporal patterns. Then BRT model was employed to identify variables that make sense and predict the risk areas of ST occurrence.

Result: It has been reported that there were 6,338 ST cases in Sichuan Province from 2006 to 2021, and the incidence rates continued to rise. Most cases were distributed between June and October each year, peaking in August. During the study period, the cases showed spatial clustering at the county level, mainly in the Panxi area, and then slowly spread to the northwest and northeast. Shrubs, precipitation, farmland and maximum temperature were the primary variables that affected the spatial distribution of this disease. It was estimated that the areas including Liangshan, Panzhihua, Bazhong, and Guangyuan were most at risk of transmission. and there were approximately 32.315 million people living in the areas with potential risk of infection throughout Sichuan.

Conclusion: Many counties in Sichuan Province were estimated to be susceptible to ST. Our found in this data-driven study could be used to guide the implementation of targeted prevention and control measures in high-risk areas.

KEYWORDS

scrub typhus (tsutsugamushi disease), BRT model, epidemiological feature, contributing variables, risk areas

1. Introduction

Scrub typhus (ST) is a vector-borne disease transmitted through the bites of chigger mites carrying *Orientia tsutsugamushi* (1–3). The primary manifestations of the disease are eschar, fever, headache, systemic lymphadenopathy, rash, etc. (4–6). Delayed treatment may result in pneumonia, myocarditis and even death (7, 8). Generally, chiggers are only active within a certain area of their breeding site. However, alterations in human behavior

and social environment lead to migration of vectors and hosts from one location to another, which may spread pathogens to more distant areas and cause disease transmission (9–11). ST is widespread in the "tsutsugamushi triangle" area ranging from Afghanistan in the west to the east coast of Russia in the north to northern Australia in the south (12). Approximately 1 billion people worldwide are at risk of infection, leading to at least 1 million ST cases each years (13, 14). Recently, the incidence rates of ST have begun to rise in known endemic areas such as India and Korea (15, 16), while cases have been widely reported in tropical and subtropical regions far from the "tsutsugamushi triangle" (17–19), which may be an indication for the resuscitation of this unheeded disease (13, 20).

In China, since the first ST case was detected in southern Guangdong in 1948, the natural focus of ST has been limited to the area south of the Yangtze River (21, 22). Until the 1980s, the endemic area began to expand slowly northward and westward, and multiple local outbreaks occurred (23, 24). Currently, ST cases have spread across China, and cases have been discovered in both urban and rural areas in all provinces (25). Recently, the incidence rates of the disease in our country have risen rapidly with the acceleration of urbanization, tourism exploitation of natural environment, climate alteration, and migration movement, which has become an important health problem (26–28). Sichuan Province is the focus of ST in southern China, faced with the dilemma of rapid increase in the incidence of ST (29). However, research on ST in this area is still limited.

Based on available genomic and genotyping studies (30), the antigenic and genetic diversity of *Orientia tsutsugamushi* has become a primary impediment to the development of effective vaccines, making the prediction of risk regions even more important at this stage. In recent years, spatial analysis and ecological modeling have been utilized to investigate the influencing factors associated with ST and to predict epidemic risk (28, 31). Previous studies have successfully estimated the distribution of ST in southern China (32), but studies on the distribution of the disease in Sichuan Province have still not been conducted. In this research, we analyzed the epidemiological characteristics of ST in Sichuan Province from 2006 to 2021. Transmission patterns of the disease were modeled to identify environmental and socioeconomic variables affecting distribution, and to forecast the areas at risk in Sichuan Province.

2. Materials and methods

2.1. Study area

Sichuan Province is located in southwest China, having jurisdiction over 21 prefectures and 183 counties. By the end of 2020, the resident population was 83.71 million. Sichuan has complex landforms, rich soil types and obvious regional climate differences. It could be categorized into three major climatic zones in accordance with the differences in water, heat and light conditions. Briefly, the basin is a subtropical humid climate zone, warm and humid throughout the year; the southwest mountainous area is a subtropical and semi-humid climate zone, with a higher average annual temperature; and the northwest plateau is an alpine climate zone, with large altitude differences and obvious climate alterations (33).

2.2. Data collection and management

All the data of scrub typhus cases from January 2006 to December 2021 were obtained from China's National Statutory Infections Disease Reporting Information System. The data covered all reported probable cases (clinically diagnosed) and confirmed cases (laboratory diagnosed), incorporating information including gender, year, occupation, time of onset, time of reporting, and place of residence. Case definition was based on the guidelines issued by the Chinese Center for Disease Control and Prevention[1] (details was displayed in Supplementary material). Probable cases were diagnosed by physicians based on epidemiological exposure (field activities in endemic areas 1–3 weeks before illness onset) and clinical manifestations (fever, specific eschar, or ulcers). A confirmed case was defined as a probable case meeting any of the following criteria: (1) a positive Weill-Felix test; (2) a positive indirect immunofluorescence antibody assay; (3) positive PCR; (4) the pathogen was isolated (34).

All the cases data in the current research were anonymized. And the cases data were not subject to institutional review board assessment.

Environmental variables were collected to investigate the potential contributing variables on the spatial distribution of ST. We extracted the monthly cumulative sunshine hours, monthly cumulative precipitation, monthly maximum temperature, and monthly minimum temperature between January 2006 and December 2020 from the National Earth System Science Data Center, National Science & Technology Infrastructure of China.[2] Simultaneously, the percentage of scrub, farmland, grassland, forest and wetland area in each county of Sichuan Province in 2010 was manually extracted from the land cover dataset of the same data center. Furthermore, the altitude data of each county were obtained from the Global Change Research Data Publishing and Repository.[3] Besides, to assess the contribution of the variables to ST occurrence, socioeconomic variables were also collected. In this study, the 1 km grid GDP datasets of Sichuan Province in 2019 were obtained from the Resource and Environmental Science and Data Center[4] to describe economic differences. Moreover, a gridded urban accessibility dataset of approximately 1 km by 1 km that were designed to estimated travel time to the nearest city of 50,000 or more people were obtained from the Joint Research Centre of the European Commission[5] to estimate the impact of trade and travel on disease transmission. The database of contributing variables of ST was established by corresponding the variables collected above with the maps of administrative divisions at county level. The base map comes from the National Catalog Service for Geographic Information.[6]

1 https://www.chinacdc.cn/tzgg/200901/t20090105_40316.htm
2 http://www.geodata.cn
3 http://www.geodoi.ac.cn/WebCn/doi.aspx?Id=887
4 https://www.resdc.cn/DOI/DOI.aspx?DOIID=33
5 https://forobs.jrc.ec.europa.eu/products/gam/
6 https://www.webmap.cn/main.do?method=index

2.3. Analysis of epidemiological characteristics of ST

The Joinpoint regression model is mainly used to analyze the trend change characteristics of the time series data. Its dependent variables for applicable data mainly include the number of cases, incidence rate, or composition ratio, etc. It can be selected when the type of data distribution conforms to a normal, exponential or Poisson distribution (35). This method has been widely used since its introduction (25, 36). In this study, Joinpoint regression model was employed to examine the incidence trends from 2006 to 2021 and to calculate the annual percentage change. The annual incidence rates were calculated by dividing the number of ST cases by the gross population of a given year. The population data were obtained from the Sichuan Provincial Bureau of Statistics (37).

As a spatial statistical method, global spatial autocorrelation has been used to describe the relationship between study regions, measuring the degree of aggregation or dispersion (38, 39). In brief, if the Moran index is greater than 0, the distribution is spatially aggregated. If the Moran's I index is less than 0, the distribution is spatially dispersing. If the Moran's I Index is equal to 0, the distribution is spatially random. In this study, the global spatial autocorrelation method was used to investigate the spatial correlation of scrub typhus at county level in Sichuan Province each year.

Joinpoint version 4.9.1.0 was used to examine the incidence trends, ArcGIS version 10.3 was used for spatial analysis, data drawing and model output.

2.4. Assessment of the risk factors of ST occurrence

The boosting regression tree (BRT) model integrates the advantages of both regression tree model and boosting tree model. Through continuous self-learning and optimization, prediction models are built to evaluate the characteristics and outcomes of predicted unknown data (40, 41). Such models are increasingly being used to predict disease risk (32, 42). In this study, the BRT model was employed to assess the risk factors of ST occurrence. The database of contributing variables of ST were employed as the predictors for ST occurrence. The 64 counties that reported ST cases were defined as the case group, while the 119 counties that did not report ST cases were considered as the control group. The control group were randomly sampled for 300 times at a case–control ratio of 1:1 to construct 300 datasets together with the case group. For each dataset, training samples and verification samples accounted for 75 and 25%, respectively. We fitted a BRT model to each dataset and calculated the average contribution and response curves of each contributing variables and the average of the possible prevalence probability of ST in each county. The modeling process was performed using the "dismo" and "gbm" packages in the R statistical programming environment. The primary parameters were set as follows: tree complexity = 4, learning rate = 0.005, bag fraction = 0.75, the distribution type is "Bernoulli," with others kept as default values (40). The area under the curve (AUC) was used to judge the accuracy and robustness of the model. R statistical software version 4.2.2 was used for model construction.

3. Results

3.1. Epidemiological features

It has been reported that there were 6,338 ST cases in Sichuan Province from 2006 to 2021, including 304 (4.80%) laboratory confirmed cases and 6,034 (95.20%) clinically diagnosed cases. The incidence rates increased over time, from 0.03 per 100,000 in 2006 to 1.12 per 100,000 in 2021 (Table 1). In accordance with the results of Joinpoint regression model, the annual percentage change in incidence was 15.67% (95% CI 10.78–20.76%, $p < 0.001$) (Figure 1).

The median age of the patients was 40 years (interquartile range [IQR], 15–54), increasing from 28 years (IQR, 9–41) in 2006 to 44 years (IQR, 17–56) in 2021. Among them, the median age of clinically diagnosed cases was 40 years (IQR, 13–55), and that of confirmed cases was 38 years (IQR, 13–55). Moreover, the 40–59 age group accounted for the largest number of cases (34.22%), The incidence rates were highest in 0–9 age group, followed by 50–59 age group, which were 0.77/100,000 and 0.63/100,000, respectively. During the study period, there were more cases reported in females. The ratio of male to female of the patients was 0.88, dropping from 1.2 in 2006 to 0.91 in 2021. Among them, this ratio of clinically diagnosed cases was 0.89, and that of confirmed cases was 0.83. Furthermore, the mean incidence rates were also higher in females (0.52/100,000) than in males (0.45/100,000). Among all ages, the incidence rates were higher in females vs. in males, except for the 0–9 and 10–19 age groups. The epidemiological surveillance of different occupations revealed that the number of cases was the largest in farmers, accounting for 67.61%. Among them, the proportion of farmers among clinically diagnosed cases and confirmed cases was 67.6 and 67.4%, respectively, both accounting for the highest percentage. Similar to the previous results of the age-specific incidence, the number of cases was also large among scattered and preschool children (14.01%) and students (13.00%). The majority of cases (75.91%) were diagnosed 3 days after the onset of illness, and approximately half of these cases were diagnosed 7 days after the onset of illness (Table 1).

3.2. Spatial and temporal distribution

Most ST cases were distributed from June to October each year, peaking in August. A total of 95.38% of the cases occurred between June and October from 2006 to 2021. During the study period, almost all cases (97.27%) occurred in the Panxi Region, including Panzhihua and Liangshan Prefecture. The epidemic has slowly expanded to the northwest and northeast since 2015, and the number of affected counties increased from 13 in 2006 to 43 in 2021. Until 2021, a total of 19 cities (90.48%) and 64 counties (34.97%) had reported ST cases in Sichuan Province (Details were displayed in Figure 2). The global spatial autocorrelation analysis revealed that the incidence rates exhibited characteristics of clustering in each year, indicating a positive spatial correlation (Table 2).

TABLE 1 Demographic characteristics of ST cases in Sichuan (37).

Features	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	Total
No. confirmed cases	2	0	11	4	13	6	5	13	28	29	25	18	16	12	79	43	304
No. probable cases	20	15	131	178	331	207	204	277	283	207	472	557	918	592	744	898	6,034
Incidence (1/100,000)	0.03	0.02	0.17	0.22	0.42	0.26	0.26	0.36	0.38	0.29	0.61	0.70	1.13	0.72	0.98	1.12	0.48
Sex																	
Male	12	6	71	95	173	98	106	116	157	109	237	265	432	277	369	449	2,972
Female	10	9	71	87	171	115	103	174	154	127	260	310	502	327	454	492	3,366
Age																	
0–9	6	3	33	45	67	38	44	53	62	58	89	92	130	116	137	150	1,123
10–19	3	2	14	17	43	24	22	34	33	18	48	67	112	66	90	104	697
20–29	2	0	20	7	27	18	13	22	15	14	39	49	93	59	78	79	535
30–39	5	2	20	35	45	28	26	29	36	29	58	58	105	75	96	94	741
40–49	1	1	13	33	66	45	36	70	67	42	113	113	158	89	134	139	1,120
50–59	3	4	26	24	41	23	31	43	45	37	74	100	147	104	144	203	1,049
60–69	2	2	12	11	40	28	26	21	31	26	53	60	123	61	94	101	691
70–79	0	1	3	10	14	7	9	15	18	10	17	29	56	31	42	64	326
≥80	0	0	1	0	1	2	2	3	4	2	6	7	10	3	8	7	56
Occupation																	
Farmer	8	11	95	111	221	140	137	191	204	150	342	412	682	408	572	601	4,285
Scattered and preschool children	2	2	25	35	56	32	34	48	55	45	73	70	100	84	113	114	888
Student	6	2	19	24	49	28	30	35	36	25	54	73	123	89	104	127	824
Others	6	0	3	12	18	13	8	16	16	16	28	20	29	23	34	99	341
Time from illness onset to diagnosis, d																	
<3	2	6	31	45	82	55	45	51	56	47	123	173	234	150	199	228	1,527
3–7	13	5	66	85	160	87	92	128	150	124	243	197	367	247	283	332	2,579
≥7	7	4	45	52	102	71	72	111	105	65	131	205	333	207	341	381	2,232
No. in endemic county	13	5	14	19	22	17	13	19	19	14	23	24	32	35	34	43	64

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3.3. Risk assessment of the spatial distribution

The validation statistics showed that The AUC value of the training data was 0.990 ± 0.011 s.e. and the validation data were 0.916 ± 0. 019s.e., suggesting that the BRT model in the current analysis can provide a good predictive accuracy. Variables including the scrub, precipitation, farmland and maximum temperature were considered as important predictors with a total contribution of 57.05% to the BRT model (Relative contribution for each variable: scrub, 20.84% ± 2.27%s.e.; precipitation, 12.52% ± 1.94%s.e.; farmland, 12.10% ± 3.01%s.e.; maximum temperature, 11.59% ± 2.90%s.e.) As the most contributing variable, the risk of ST increased sharply in areas with scrub cover, while there was no further increase in risk when the proportion of scrub exceeded 2%. Furthermore, the farmland and maximum temperature were both positively correlated with the risk of the disease, a rapid rise in the risk was noted when the area of farmland exceeded 20% or the temperature was higher than 17°C. Conversely, a negative correlation was detected between the precipitation and the risk of morbidity. When the precipitation was 70–80 mm, the risk of the disease was the highest. In addition, other variables that contributed to this model were grassland, forest, minimum temperature, wetland, and GDP (Relative contribution for each variable: grassland, 6.74 ± 2.47%s.e.; forest, 6.41% ± 2.91%s.e.; minimum temperature, 5.83% ± 2.49%s.e.; wetland, 5.60% ± 2.84%s.e.; GDP, 5.10% ± 2.41%s.e.). On the contrary, variables including sunshine, urban accessibility, and elevation did not contribute significantly to this model (Relative contribution for each variable: sunshine, 4.92% ± 2.47%s.e.; urban accessibility, 4.22% ± 1.96%s.e.; elevation, 4.13% ± 2.03%s.e.) (Details were displayed in Figure 3).

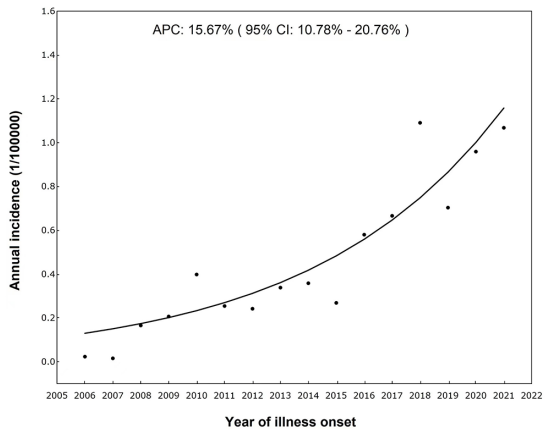

FIGURE 1
Temporal trends of incidence rates of ST in Sichuan (37).

3.4. Infection risk areas

Infection risk areas in Sichuan Province were mapped on the basis of the correlation between variables and ST in the BRT model. The areas with the highest risk of ST were concentrated in Liangshan, Panzhihua, Bazhong, and northern Guangyuan, of which Liangshan and Panzhihua were the traditional endemic areas. A small number of cases have been recorded in Bazhong and Guangyuan, however, a higher transmission risk was predicated in these zones. Moreover, our study found that some areas without case report detected a high risk of transmission, including Chengdu, Deyang, Mianyang, Ya 'an in the central plain, and parts of Neijiang and Luzhou counties in southern Sichuan (Details were displayed in Figure 4).

The population size of the potential risk areas was computed by combining the predicated map with the population data of the seventh census. First, the areas with risk level above the threshold value of 0.5 were defined as the potential risk areas for ST occurrence. Then, the population data was overlaid on the potential risk areas to compute the population at risk. There were approximately 32.315 million people in the potential risk areas in Sichuan Province, accounting for 38.62% of the total population. All the people in Liangshan, Panzhihua, Bazhong and Guangyuan were at risk of transmission, and higher proportion of people living in Neijiang, Luzhou, Ziyang and Deyang were also at risk (Population proportion: 69.05% in Neijiang, 44.52% in Luzhou, 41.19% in Ziyang, 40.10% in Deyang).

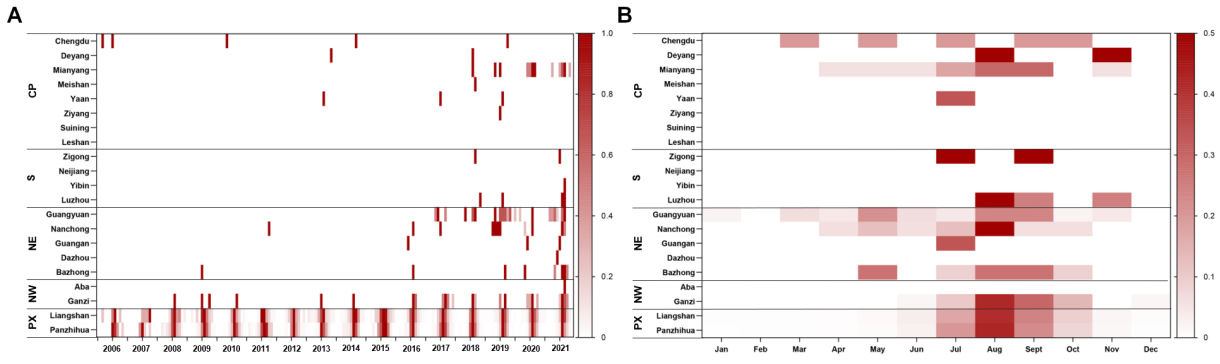

FIGURE 2
Heat map of ST for each city by region. **(A)** Time series of monthly cases during 2006–2021, standardized by the annual number of cases reported in each city and standardized to a range of 0–1. **(B)** Seasonal distribution of ST in each city, plotted as the average proportion of cases in each month from 2006 to 2021. CP, Chengdu Plain; S, Southern Sichuan; NE, Northeastern Sichuan; NW, Northwestern Sichuan; PX, Panxi region.

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TABLE 2 Global spatial autocorrelation analysis of ST in Sichuan (37).

Year	Moran's I	Z-score	*p*-value
2006	0.48	12.24	<0.001
2007	0.08	7.00	<0.001
2008	0.32	9.21	<0.001
2009	0.09	7.14	<0.001
2010	0.25	6.57	<0.001
2011	0.25	8.14	<0.001
2012	0.26	8.47	<0.001
2013	0.21	9.42	<0.001
2014	0.32	9.17	<0.001
2015	0.21	6.02	<0.001
2016	0.26	6.99	<0.001
2017	0.19	5.07	<0.001
2018	0.31	8.28	<0.001
2019	0.30	7.81	<0.001
2020	0.30	7.71	<0.001
2021	0.27	6.97	<0.001

4. Discussion

Based on the long-term surveillance data of ST in Sichuan Province, we comprehensively overviewed the epidemiological characteristics of the disease in Sichuan from 2006 to 2021. Then the BRT model was employed to identify variables that make sense and estimate the risk areas of ST occurrence.

ST is a mite-borne disease caused by *Orientia tsutsugamushi* (43). Generally, ST is transmitted by chigger (larval mites), which feed on rodents, such as rats, rabbits, and birds. The chiggers are both the vector and the natural reservoir. ST is spread to human through bites of infected chiggers. After infection, pathogens spread along the blood stream, invading vascular endothelial cells and macrophages (Figure 5). Similar to the symptoms and complications reported in other countries (44, 45), symptoms of ST usually appear within 10 days of being bitten, may include: fever, headache, muscle pain, a dark eschar at the bite site, enlarged lymph nodes, and rash (46, 47). Furthermore, complications usually involve the lungs, liver and cardiovascular system, causing dysfunction and even life-threatening in severe cases (48). For people at risk, prevention and control of ST is essential, and for infected patients, early diagnosis and treatment will determine the prognosis of the disease.

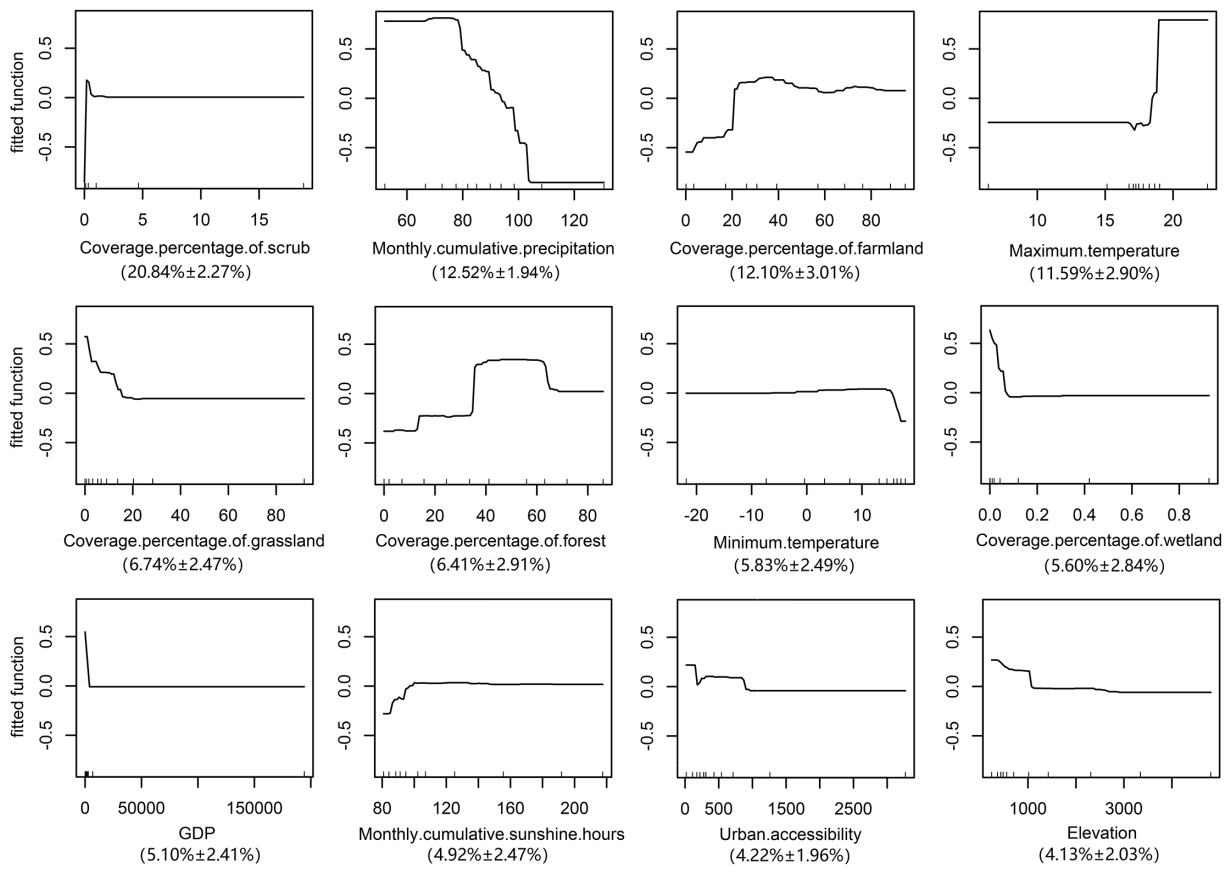

FIGURE 3
Response curves of each variable.

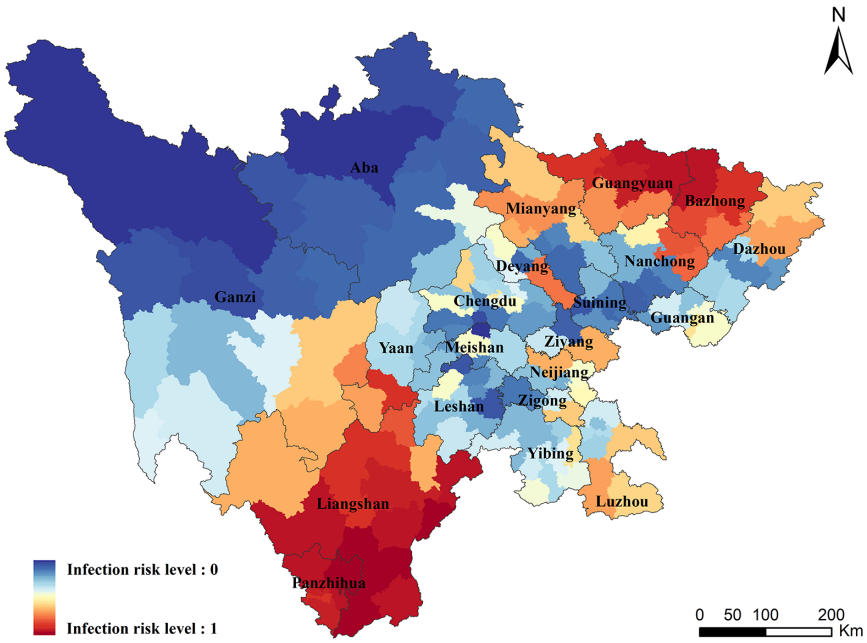

FIGURE 4
Geographic distribution of the predicted risk areas of ST in Sichuan Province.

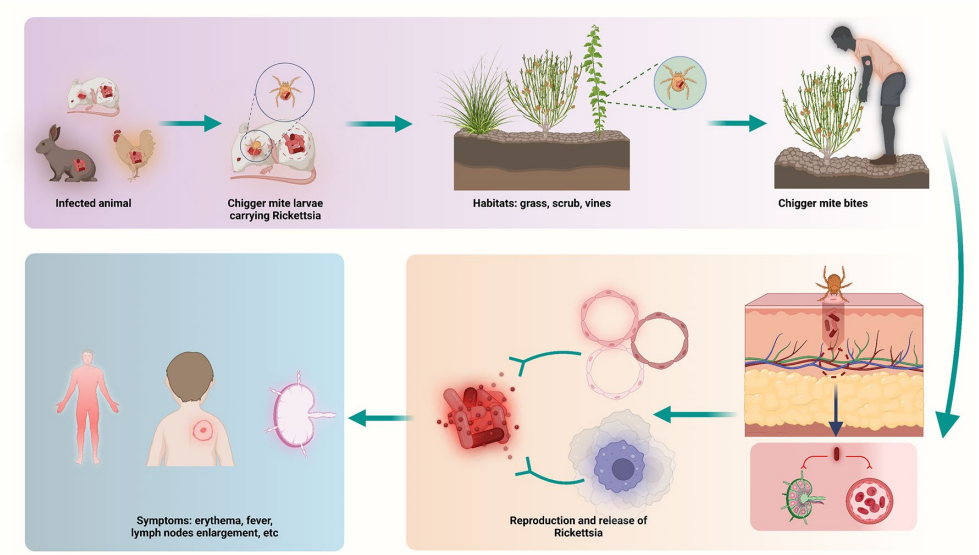

FIGURE 5
Schematic illustration on the process of ST infection.

Since ST was listed as a reported disease by the National Notifiable Disease Reporting Information System in 2006, the reported incidence rates of ST in Sichuan Province have increased dramatically. In 2009, the Chinese Center for Disease Control and Prevention issued the technical guidelines for the prevention and control of this disease, and more cases were discovered. With the in-depth understanding of the disease, scholars gradually realized the impact of natural environment and socioeconomic variables on the spread and transmission of the disease, and managed to estimate the risk of disease through scientific methods and provide targeted prevention and control measures (25, 32, 42, 49, 50).

In this study, we noted that the incidence rates were relatively higher in preschoolers and boys were more susceptible than girls, which are supposed to be related to the fact that boys are more

interested in outdoor play and sports. However, the majority of adult cases among adults were the older adult engaged in agriculture, and the incidence rates were higher in females. Commonly, youth and older adult male population in rural areas choose to work in cities for higher incomes, while elder women remain in the countryside, doing most of the agricultural work and taking care of the children. As everyone knows, the transmission of ST depends on the hosts and vectors. Staying in the grasslands and fields can increase the exposure to rodent hosts and chigger mites (32), leading to an increase in risk of ST. These findings are partially consistent with the study conducted by Yu et al. (31), who found that the number of ST cases in Jiangsu Province presented an inverted-U relation with age, moreover, the susceptible population of ST were farmers. Therefore, personal protection and health education to farmers and child guardians is recommended in risk areas to reduce the risk of infection.

The current study found that ST has obvious seasonality and clustering properties, and outbreaks are more likely to occur in the Panxi area in summer and autumn each year, which could be due to the differences in the distribution of chigger mites. According to the record, Panzhihua and Liangshan prefecture were the foci of ST. A variety of chigger mites were widely distributed in local areas, of which Leptotrombidium tsutsugamushi was the dominant species, and its activity peaked in the hot season ranging from June to September each year (51), which is consistent with our findings. Furthermore, the current study found that the risk areas increased rapidly and gradually spread to the economically developed urban plain areas, which may be associated with the growing demand for people to choose to go to the wild or parks for outdoor exercise. Another possible explanation is the increased number of the chigger mites and rodent hosts in cities, after all, it has been confirmed that both hosts and vectors can inhabit in urban environment (11). Taken together, it is recommended that necessary steps were supposed to be adopt to reduce the densities of rodents and chigger mites in endemic areas during the epidemic season, and personal protection and health education should be strengthened for travelers entering endemic areas.

The BRT model was employed to examine the correlation between variables and ST occurrence. The relative contribution of the scrub was observed to be high in the BRT model, suggesting the crucial role of land type in ST occurrence. Similar results were also reported by Hongwu Yao et al. (50), who found that scrub and grass land were the primary factors affecting the epidemic of scrub typhus in South natural foci in southern China. In general, scrub can be used as a platform for the activity of chigger mite larvae after hatching, and provide a suitable habitat for rodent hosts. In accordance with the land cover dataset, the scrub is primarily concentrated in Panxi area, which may partially explain the high incidence rates of ST in Panxi area. Moreover, previous research has demonstrated that the secondary vegetation types with changes in ecological environment provided ideal conditions for the survival and reproduction of tsutsugamushi (52). Hence, densely vegetated places near residences, villages, and ridges and ditches are considered to be the main places of tsutsugamushi infection (42). These are the regions we need to focus on to eliminate hosts and cut off transmission routes.

Previous study reported that ST occurrence is related to meteorological variables (34). The alterations of the meteorological variables such as temperature, precipitation and sunshine can not only alter chigger mite abundance, but also affect human behavior directly (35, 36). In the current analysis, a positive correlation was noted between maximum temperature and ST occurrence. Usually, rising temperatures provide a suitable living environment for hosts and vectors, and human activities increase as well. The main epidemic seasons for ST in Sichuan are summer and autumn (32). As temperatures rise, people spend more time in the field and their clothing becomes thinner, which increases the potential for human contact with chiggers or rats. In the current study, we noted that the Panxi region had the highest incidence of ST. The climate is primarily subtropical, with an average temperature above 20°C, which is suitable for the survival and reproduction of both host animals and chiggers. This is also relatively close to the prevailing suitable temperature proved by this study. In addition, a negative correlation was detected between precipitation and ST occurrence, which contradicts the understanding that humid environment is more conducive to chigger mite reproduction and thus increasing the risk of transmission. One possible explanation is that high humidity is detrimental to the life cycle of mites (50). In addition, several previous studies explained this phenomenon in terms of the interaction between human behavior and surrounding environment (32), namely, people were more inclined to be outside in the dry season vs. in the rainy season. Therefore, personal protection to reduce or avoid exposure to chigger mites is an effective measure for the prevention of ST.

There were more than 30 million people living in the potential risk areas in Sichuan Province. Especially those living in Liangshan, Panzhihua, Bazhong and Guangyuan. Moreover, some areas without case report detected a high risk of transmission, suggesting that surveillance in these places should be strengthened immediately.

Two limitations were noted in this research. First, our data come from a passive monitoring system, and there may be under-reporting and mis-reporting that affect the accuracy of the data. Second, we constructed our model without the use of vectors and hosts data, which are thought to be important variables affecting disease transmission. The next step is to collect more information on cases, pathogens, hosts, and vectors, including data on *O. tsutsugamushi* genotypes and the accurate distribution of Leptotrombidium mite species, and to conduct more detailed studies to formulate prevention and control.

In conclusion, we comprehensively evaluate the epidemiological characteristics of ST in Sichuan. For the first time, the BRT model was employed to investigate the underlying relationship of ST with meteorological, environmental and social variables in Sichuan Province. Many counties in Sichuan Province were estimated to be susceptible to ST. Our found can determine the priorities of disease control to achieve precise and effective allocation of resources.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: The data of scrub typhus cases were available from China's National Statutory Infections Disease Reporting Information System. Demographic data was obtained from the seventh population census of the National Bureau of Statistics of China (http://www.stats.gov.cn/). The meteorological data was obtained from the National Earth System Science Data Center, National Science and Technology Infrastructure of China (http://www.geodata.cn). the altitude data of each county were obtained from

the Global Change Research Data Publishing and Repository (http://www.geodoi.ac.cn). The 1 km grid GDP datasets of Sichuan Province in 2019 were obtained from the Resource and Environmental Science and Data Center (https://www.resdc.cn). The gridded urban accessibility dataset was obtained from the Joint Research Centre of the European Commission (https://forobs.jrc.ec.europa.eu/products/gam/). The maps of administrative divisions at county level comes from the National Catalog Service for Geographic Information (https://www.webmap.cn/main.do?method=index).

Author contributions

CY, RW, and YZ had the idea for the article. Data analysis was performed by YZ, MZ, and YQ. The first draft of the manuscript was written by YZ and MZ. YZ, MZ, YQ, LZ, DK, RW, and CY commented on previous versions of the manuscript. All authors contributed to the article and approved the submitted version.

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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/fpubh.2023.1177578/full#supplementary-material

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A rare sugar, allose, inhibits the development of *Plasmodium* parasites in the *Anopheles* mosquito independently of midgut microbiota

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A rare sugar, allose, was reported to inhibit the development of *Plasmodium* parasites in *Anopheles* mosquitoes; however, the mechanism remains unknown. The present study addressed the inhibitory mechanism of allose on the development of the *Plasmodium* parasite by connecting it with bacteria involvement in the midgut. In addition, further inhibitory sugars against *Plasmodium* infection in mosquitoes were explored. Antibiotic-treated and antibiotic-untreated *Anopheles stephensi* were fed fructose with or without allose. The mosquitoes were infected with luciferase-expressing *Plasmodium berghei*, and parasite development was evaluated by luciferase activity. Bacterial composition analysis in gut of their mosquitoes was performed with comprehensive *16S ribosomal RNA* sequencing. As the result, allose inhibited the development of oocysts in mosquitoes regardless of prior antibiotic treatment. Microbiome analysis showed that the midgut bacterial composition in mosquitoes before and after blood feeding was not affected by allose. Although allose inhibited transient growth of the midgut microbiota of mosquitoes after blood feeding, neither toxic nor inhibitory effects of allose on the dominant midgut bacteria were observed. Ookinete development in the mosquito midgut was also not affected by allose feeding. Additional 15 sugars including six monosaccharides, four polyols, and five polysaccharides were tested; however, no inhibitory effect against *Plasmodium* development in mosquitoes was observed. These results indicated that allose inhibits parasite development in midgut stage of the mosquito independently of midgut microbiota. Although further studies are needed, our results suggest that allose may be a useful material for the vector control of malaria as a "transmission-blocking sugar."

KEYWORDS

allose, microbiota, midgut, *Anopheles*, *Plasmodium*, luciferase assay, next-generation sequencing

1 Introduction

Malaria is a life-threatening disease caused by *Plasmodium* parasites, with the estimated number of deaths reaching 627,000 per year in 2020 (World Health Organization (WHO), 2021). *Plasmodium* parasites are transmitted by *Anopheles* mosquitoes, ubiquitously distributing in endemic areas (Moffett et al., 2007; Autino et al., 2012). Vector control is a cornerstone in malaria control, owing to the lack of reliable vaccines, the emergence of drug resistance, and unaffordable potent antimalarials. Studies on transmission-blocking strategies, which aim to interrupt the life cycle of the *Plasmodium* species within mosquitoes, are progressing, and elucidation of more detailed transmission mechanisms including interaction between *Anopheles* mosquitoes and *Plasmodium* is expected.

The midgut of the mosquito is important not only for digestion and uptake of nutrients but also as the entry site of pathogens including *Plasmodium* parasites. When *Anopheles* mosquitoes ingest *Plasmodium*-infected blood from the host, parasites mate and develop into motile ookinetes within 16–20 h (Blandin et al., 2004). Ookinetes traverse across the midgut epithelium within 24 h after the feeding and transform into oocysts on the basal side of the midgut (Baton and Ranford-Cartwright, 2004; Blandin et al., 2004). Sporozoites develop and proliferate within oocysts over about the next 10 days. The sporozoites released from oocysts invade the salivary glands, in which they wait for transmission to the next host via a mosquito bite (Blandin et al., 2004; Frischknecht et al., 2004). The early phase of the parasite infection, from ingestion to invasion into midgut epithelium, is the bottleneck for the parasite in the mosquito cycle and, therefore, is considered to be a suitable target for a transmission-blocking strategy.

Mosquitoes develop protective mechanisms against infectious agents in the midgut, which include the immune system and biological barriers. Parasite invasion and development in the midgut are affected by immune-related factors such as thioester-containing protein 1 (TEP1) (Blandin et al., 2004; Osta, 2004; Dong et al., 2006; Baxter et al., 2010; Povelones et al., 2011) and antimicrobial peptides such as gambicin regulated by the immune deficiency (IMD) pathway through transcription factor Rel2 (Vizioli et al., 2001; Dong et al., 2006), as well as nitric oxide and reactive oxygen species (ROS) induced by invasion of gut epithelium (Luckhart et al., 1998; Molina-Cruz et al., 2008). In addition, malaria parasites are continuously exposed to microbes in the mosquito gut. This naturally acquired microbial flora can modulate the mosquito's vectorial capacity. Comprehensive functional approaches have been undertaken to elucidate the interplay between commensal bacteria and the development of the malaria parasite in its natural vector. Mosquitoes treated with antibiotics, which diminish bacteria quantity, are known to be more susceptible to *Plasmodium* parasite infection (Dong et al., 2009). Administration of some characterized bacteria, such as *Enterobacter* sp. and *Serratia marcescens*, reduced the development of *Plasmodium* oocysts by eliciting anti-*Plasmodium* immunity in mosquitoes (Cirimotich et al., 2011; Bando et al., 2013; Bahia et al., 2014).

In nature, both male and female *Anopheles* mosquitoes ordinarily ingest sugars from floral nectar, but only females suck blood at a time of reproduction. Sugars function not only as an energy source but also as prebiotics for gut microbiota. As examples, oligosaccharides, which are composed of two to 10 monosaccharides, improve the host gastrointestinal condition by promoting beneficial microbiota (Mano et al., 2018), and polyols and hydrogenated carbohydrates are known to affect the ecology of oral microflora and exhibit a cariostatic effect in mammals (Msomi et al., 2021). Sucrose, an oligosaccharide, maintains antiviral immunity in the gut of *Aedes aegypti* (Almire et al., 2021). A recent study reported that glucose induces the expansion of *Asaia bogorensis*, a commensal bacterium in the mosquito gut, that remodels glucose metabolism, resulting in the promotion of *Plasmodium* gametogenesis and parasite infection by increasing the pH of the mosquito midgut (Wang et al., 2021). However, a rare sugar, allose, which has been shown to influence ROS generation in mammalian and plant cells (Murata et al., 2003; Kimura et al., 2005; Kano et al., 2013), was shown to inhibit *Plasmodium* infection in mosquitoes (Tokuda et al., 2015). Currently, it remains unknown how allose inhibits malarial parasite development and whether it affects commensal bacterium in the mosquito gut.

The present study addressed the inhibitory mechanism of allose on the development of the *Plasmodium* parasite and the involvement of the microbiota in midgut fed allose in its mechanism. In addition, other inhibitory sugars potentially affecting the development of *Plasmodium* were explored.

2 Materials and methods

2.1 Mosquito and malaria parasite maintenance

Anopheles stephensi SDA-500 were maintained at 26°C with 60%–80% relative humidity under the condition of 13-h light/11-h dark at the Jichi Medical University. Larvae were fed with fish food, Hikari (KYORIN Co., Ltd., Hyogo, Japan). Adults were fed on filter paper soaked with 8% fructose (FUJIFILM Wako, Osaka, Japan). Luciferase-expressing *P. berghei* ANKA234 under the control of elongation factor 1-α promoter (*Pb*-luc) was injected into an Institute of Cancer Research (ICR) mouse intraperitoneally (Matsuoka et al., 2015). The mouse was intraperitoneally administered 100 μL of pyrimethamine (1 mg/mL) daily. Infected blood was stained with Giemsa's stain solution (Merck KGaA, Darmstadt, Germany) to determine parasitemia. All procedures were carried out in accordance with the ethical guidelines, and this study was approved by the Institutional Animal Experiment Committee of the Jichi Medical University (approval numbers: 20003-01).

2.2 Sugar feeding, antibiotic treatment, and infection of *An stephensi* with *P berghei*

Sixteen sugars—seven monosaccharides (D-glucose, D-fructose, D-galactose, L-sorbose, D-mannose, D-xylose, and D-allose), four polyols (D-sorbitol, xylitol, erythritol, and maltitol),

and five oligosaccharides (sucrose, D-lactose, lactulose, D-maltose, and D-raffinose) (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan)—were used in this study to evaluate their inhibitory effects on the development of *Plasmodium*. Of these, D-allose, erythritol, L-sorbose, and xylitol are categorized as rare sugars as they are rarely present in nature. Mosquitoes were fed 8% fructose with 100 mM of each abovementioned sugar for 24 h *ad libitum* and fasted for the following 24 h. The mosquitoes were allowed to feed on *Pb*-luc–infected mice showing 1.5%–2.0% parasitemia for 30 min, and unfed and partially blood-fed mosquitoes were removed from cages. Fully engorged mosquitoes were maintained on 8% (444 mM) fructose containing 100 mM of each sugar for an additional 10 days at 21°C. The concentration of additional sugar was matched with the effective concentration of allose (100 mM) (Tokuda et al., 2015). For the antibiotic treatment, mosquitoes were fed 8% fructose with 100 mM allose containing penicillin (200 U/mL) and streptomycin (200 μg/mL; Thermo Fisher Scientific Inc., Waltham, MA, USA) for 24 h *ad libitum*. After starvation for the following 24 h, the mosquitoes were allowed to feed on *Pb*-luc–infected mice showing 10% parasitemia for 30 min. The mosquitoes were maintained as mentioned above. Infection of Pb-luc in mosquitoes fed with the infected blood after 10 days was determined by measuring luciferase activity and by counting oocysts in the midgut (Matsuoka et al., 2015).

2.3 Luciferase assay

Luciferase activity was measured using a Luciferase assay kit according to the manufacturer's protocol (Promega Inc., Madison, WI, USA). Briefly, the whole body of mosquitoes was individually homogenized with disposable homogenizer in a 1.5-mL tube containing cell lysis buffer. The homogenized samples were centrifuged at 15,000 × *g* for 1 min, and 10 μL of each supernatant was mixed with 50 μL of substrate in a 96-well black microplate. Luminescence was measured by a SpectraMax® M5 microplate reader (Molecular Devices, LLC., San Jose, CA, USA).

2.4 Effect of allose on *Plasmodium* parasite *ex vivo*

To evaluate the effect of allose on ookinete development, mosquitoes were dissected at 24 h after infection, and midguts were collected. The midgut containing ookinetes was suspended individually in Roswell Park Memorial Institute (RPMI) 1640 medium (Nakarai Tesque Inc., Kyoto, Japan) supplemented with 20% heat-inactivated fetal calf serum, penicillin (100 U/mL), streptomycin (100 μg/mL; Thermo Fisher Scientific Inc.), and 25 mM Hydroxyethylpiperazine ethane sulfonic acid (HEPES) (pH 7.4; Tokyo Chemical Industry Co., Ltd.). The suspension was fixed and stained with Giemsa's stain solution, and, then, ookinetes were counted under a microscope. The number of red blood cells (RBCs) in each sample was also counted to compensate the amount of ingested blood in each mosquito, and the ookinete count per 10^6 RBCs was determined.

2.5 Midgut sample collection and DNA extraction for microbiota analysis

Mosquitoes were collected on days 1, 3, and 7 post-infection with *Pb*-luc at 21°C and prior to the infection as a control. They were anesthetized on ice, sterilized with 70% ethanol containing 1% hypochlorite, and then rinsed with phosphate-buffered saline (PBS). Midguts were taken out and washed with sterilized PBS (−), and 10 midguts were pooled in a 2-mL tube containing 0.5 mL of PBS with 0.1-mm Zirconia/Silica beads (Bio Spec Products Inc., Bartlesville, OK, USA). The midgut samples containing microbiota in the gut were mechanically disrupted by a bead beater (TAITEC Co., Saitama, Japan) at 3,500 rpm for 1 min, and DNA was purified using the ReliaPre DNA Clean-Up and Concentration System (Promega Inc.).

2.6 *16S ribosomal RNA*–based metagenomic analysis of mosquito midgut microbiota

Bacterial *16S ribosomal RNA* (*16S rRNA*) fragments containing hypervariable V3–V4 region were amplified, and index sequences were added by PCR with Tks Gflex DNA polymerase (Takara Bio Inc., Shiga, Japan) using primer sets according to the manufacturer's protocol (https://jp.support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html ; accessed on 1 February, 2022). The amplified fragments were purified using a FastGene PCR/Gel extraction kit (Nippon Genetics Co., Ltd., Tokyo, Japan), and the concentration was quantified using a Qbit3 Fluorometer with Qbit dsDNA HS assay kit (Thermo Fisher Scientific Inc.). Paired-end sequencing analysis was conducted on the MiSeq® platform with MiSeq Reagent Kit v3 for 600 cycles (Illumina, Inc., San Diego, CA, USA). Sequence data were trimmed and merged by DADA2 plugin in QIIME2 program version 2020.6 (Callahan et al., 2016; Bolyen et al., 2019). The sequences were clustered in amplicon sequence variants (ASVs) by feature-classifier plugin with the dataset of SILVA version 138 with ≥ 99% sequence identity (Quast et al., 2013; Yilmaz et al., 2014; Bokulich et al., 2018). To compare the diversities between groups, beta-diversity analyses with the Bray–Curtis dissimilarity index were performed with the q2-diversity plugin in QIIME2 at a sampling depth of 1,500 (Anderson, 2001).

2.7 Quantification of midgut bacteria in mosquitoes

Midgut bacteria in mosquitoes were quantified by quantitative PCR (qPCR) with KOD -SYBR- (TOYOBO Co., Ltd., Osaka, Japan) using Thermal Cycler Dice Real Time System Lite (Takara Bio Inc.). The bacterial *16S rRNA* was used as the target, and the *An. stephensi glyceraldehyde 3-phosphate dehydrogenase* (*Asgapdh*) was used as the reference. The primer sequences were 5′-ACHCCTACGGGDGGCWGCAG-3′ (16S-q-337F) and 5′-GTDTYACCGCGGYTGCTGGCAC-3′ (16S-q-514R) for amplification of the bacterial 16S rRNA gene and 5′-

GCCGTCGGCAAGGTCATCCC-3′ (Asgapdh-q-F) and 5′-TTCATCGGTCCGTTGGCGGC-3′ (Asgapdh-q-R) for that of the *Asgapdh* (Yamamoto et al., 2016). Relative quantities of *16S rRNA* were determined by the comparative cycle threshold (ΔΔCt) method using the *Asgapdh* as the reference.

2.8 Isolation and identification of mosquito midgut bacteria

Mosquitoes were anesthetized on ice and washed with 70% ethanol containing 1% hypochlorite followed by sterilized PBS (−). The midgut was removed, rinsed with sterilized PBS (−), and homogenized in 20 μL of sterilized PBS (−). Ten microliters of the homogenate was spread on brain-heart infusion agar plate (Sigma Aldrich Inc., St. Louis, MO, USA), and the plate was incubated at 28°C for 3 days under aerobic and anaerobic conditions. For the identification of bacteria, the bacterial *16S rRNA* was amplified with KOD -One- (TOYOBO Co., Ltd.) using a pair of primers: 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Delong, 1992). The amplicon was purified using a FastGene PCR/Gel extraction Kit (Nippon Genetics Co., Ltd.), and the sequence was determined by the dideoxy chain termination method with a specific primer, 519F (5′-CAGCMGCCGCGGTAAT-3′), using a BigDye Terminator v.3.1 Cycle Sequencing Kit (Thermo Fisher Scientific Inc.). Homology was performed with the nucleotide BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and a search was conducted of the taxonomic database (https://www.ncbi.nlm.nih.gov/taxonomy) of the National Center for Biotechnology Information (NCBI) database.

2.9 Effect of allose on bacterial growth *in vitro*

A single colony of bacteria isolated from mosquito midgut was cultured in Luria-Bertani (LB) liquid medium at 28°C overnight. A part of the bacterial culture was transferred into LB liquid medium containing 8% fructose with 100 mM glucose or allose, and the optical density at a wavelength of 600 nm (OD_{600}) of the suspensions was adjusted to 0.02 by spectrophotometer. The bacteria were further cultured at 28°C, and the growth was monitored for 36 h.

2.10 Sequence data availability

The microbiome sequencing data obtained in this study were registered in the NCBI/GenBank/ the DNA data bank of Japan (DDBJ) database under the accession numbers DRR337101-DRR337141. The *16S rRNA* sequences of *Leucobacter* sp. and *Phyllobacterium* sp. isolated from mosquito midgut are available under the accession numbers LC669551 and LC669552, respectively.

2.11 Statistical analysis

Statistical analyses were performed using R version 4.0.3. Welch's *t*-test with Bonferroni's modification was applied to ookinete counting, quantification of bacteria by qPCR, and evaluation of bacterial growth. Wilcoxon rank sum test with Bonferroni's modification was conducted for the development of *Plasmodium* parasites within mosquitoes given different sugars. One-way analysis of variance with Holm's modification was applied to assess the quantification of midgut bacterial amount the time-dependent changes of the relative abundance of the six dominant midgut bacteria genera.

3 Results

3.1 Allose inhibits development of *Plasmodium* parasite in the antibiotic-treated mosquito

Allose inhibits the development of *P. berghei* within *An. stephensi* by assessing the development of oocysts (Tokuda et al., 2015). To reconfirm this finding, luciferase activity was investigated in allose-fed mosquitoes infected with *Pb*-luc. As shown in Figure 1; Supplementary Figure 4, luciferase activities in the infected mosquitoes were consistent with oocyst counts at 10 days after *Pb*-luc infection. The inhibitory effect of allose was evaluated in mosquitoes treated with antibiotics, which have diminished microbiota in the midgut. Similar to untreated mosquitoes, the development of *Plasmodium* was inhibited by allose feeding in the antibiotic-treated mosquitoes (Figure 2). This result suggested that allose inhibits development of the *Plasmodium* parasite in the mosquito through a midgut microbiota-independent mechanism.

3.2 Allose affects the proliferation of midgut microbiota but not bacterial composition

As ingested sugars can affect midgut microbiota that may impact the development of parasites directly or indirectly, quantitative and qualitative changes of bacterial communities in the midgut of allose-fed mosquitoes were assessed. Midgut microbiota was quantified by qPCR. The relative quantity tended to be suppressed in allose-fed mosquitoes at 3 days after infection with no significant difference compared to allose-unfed mosquitoes and was comparable between groups on day 7 (Figure 3A). The decrease of bacterial quantities in the allose-fed group seemed to be attributed to inhibition of the rapid proliferation of gut bacteria initiated by blood feeding (Figure 3A). The composition of midgut bacteria was identified by *16S rRNA*–based metagenomic analysis and compared between allose-fed and allose-unfed mosquitoes. In total, 2,706,788 reads were obtained from 40 pooled samples, and *de novo* assembly of the reads generated 427 ASV. Of these, 113 ASVs consisting of 1,146,318 reads (42.4% of total reads) were not

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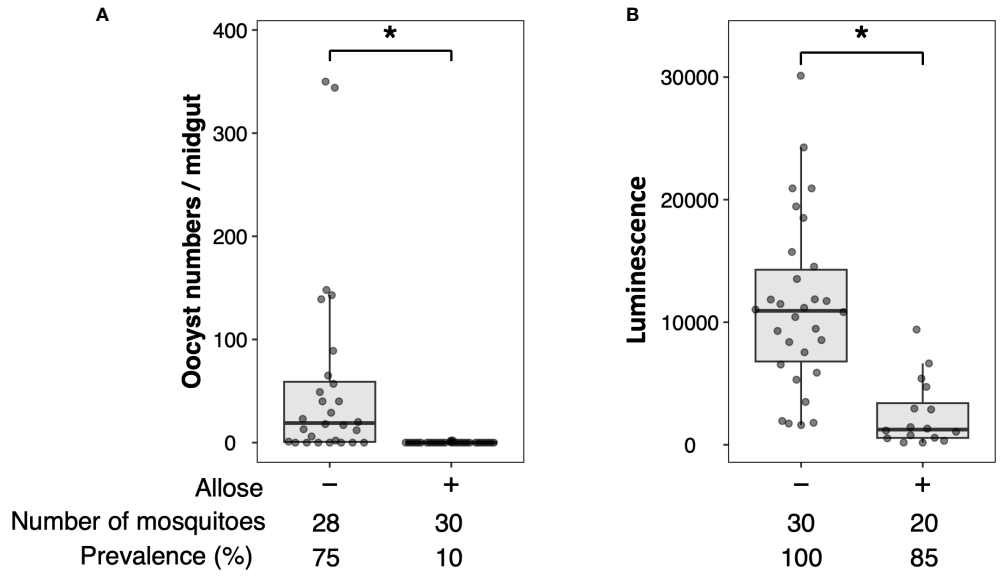

FIGURE 1
Allose inhibits oocyst development in the mosquito midgut. Allose-fed and allose-unfed mosquitoes were infected by luciferase-expressing *Plasmodium berghei*, and infectivity was determined 10 days post-infection by counting of the number of oocysts **(A)** and by measuring the luciferase activity **(B)**. The number of mosquitoes is indicated under each group. The prevalence of infection was calculated number of detected oocyst or luminescence sample divided by number of total sample. The results represent the standard deviation of the mean (SD) of biological replicates. Statistical significance was determined by Wilcoxon's rank sum test with Bonferroni's modification. *$P < 0.01$.

categorized in bacteria at the taxonomic level in the SILVA classifier, and, finally, 314 ASVs consisting of 1,497,066 reads (57.6%) were classified into families and genera of bacteria (Supplementary Table S1). The most abundant bacteria belong to the genus *Phyllobacterium* in the family *Rhizobiaceae* (51.4% of bacterial reads, 769,144 reads, 25 ASVs), followed by the genus *Leucobacter* in the family *Microbacteriaceae* (19.9% of bacterial reads, 297,535 reads, 12 ASVs), the genus *Staphylococcus* in the family *Staphylococcaceae* (3.5% of bacterial reads, 51,813 reads, 34 ASVs), the family *Peptococcaceae* (2.7% of bacterial reads, 40,358 reads, 23 ASVs), the genus *Proteus* in the family *Morganellaceae* (2.4% of bacterial reads, 35,621 reads, 7 ASVs), and the genus *Pseudomonas* in the family *Pseudomonadaceae* (2.3% of bacterial reads, 33,916 reads, 8 ASVs) (Supplementary Table S1). Other bacteria families consisting of less than 2% of reads were categorized as "others" in this study (17.9% of bacterial reads, 268,679 reads, 205 ASVs). More detailed information obtained by the metagenomic analysis is provided in Supplementary Table S1; Figure 3B shows the time-dependent changes of the relative abundance of the six dominant midgut bacteria genera. The most abundant genus, *Phyllobacterium*, consisted of 35.6%–57.0%, on average, in the allose-unfed group, and the abundance did not change dramatically before (day 0) or after the infection (days 1–7). In the allose-fed group, *Phyllobacterium* consisted of 19.3%–73.1% on average during the course before (day 0) and after post infection (days 0–7), and a slight decrease of the composition ratio (19.3%) was observed on day 1 post-infection with no significant difference compared to the allose-unfed group. The composition ratio of the second most abundant genus, *Leucobacter*, was slightly increased in the allose-unfed group during the course before (day 0) and after post infection (days 0–3), whereas the ratio of the allose-fed group was slightly decreased on day 1 post-infection with no significant difference compared to the allose-unfed group, similar to that of *Phyllobacterium*. Similarly, although the composition ratio of the other four genera—*Staphylococcus*, *Peptococcaceae*, *Proteus*, and *Pseudomonas*—were slightly changed during the course before (day 0) and after infection (days 0–7), the differences between two groups were not significant (Figure 3B). In addition, heatmap clustering analysis based on the relative abundance of each genus and beta-diversity analysis showed an allose-independent cluster formation (Supplementary Figure S1) and no significant diversity between the allose-fed and allose-unfed groups during the course (Supplementary Table S1; PERMANOVA, pseudo-$F = 1.03$, $P = 0.371$), respectively. These results strongly suggested that allose affects the transient growth of midgut microbiota initiated by blood feeding but not their diversity.

3.3 Allose did not inhibit growth of bacteria isolated from mosquito midgut

A bacterial growth assay *in vitro* was performed to assess whether allose inhibits the growth of bacteria in the midgut. Two bacteria in the mosquito midgut were successfully isolated and identified as *Leucobacter* sp. and *Phyllobacterium* sp., both of which are dominant in the gut, based on the sequencing analysis of *16S rRNA* fragments with 96.8%–99.7% and 96.4%–99.2% identities, respectively, in the BLASTn analysis. The effect of allose on the growth of these bacteria was assessed *in vitro*. The growth of *Leucobacter* sp. in LB medium containing 100 mM allose and 8% fructose was comparable with that containing 100 mM glucose and 8% fructose as a control (Table 1). In addition to the growth rate,

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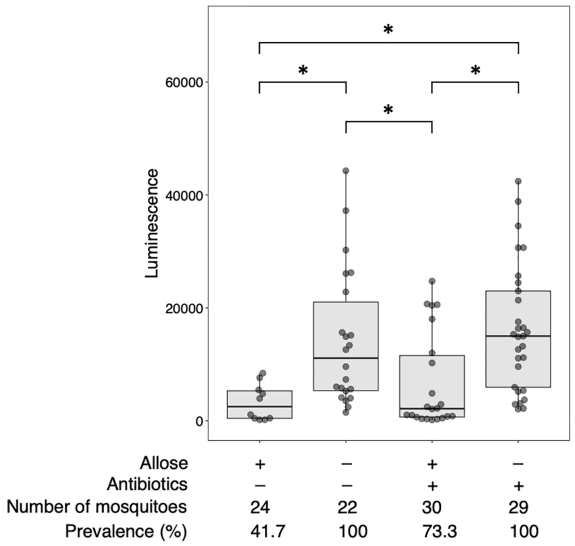

FIGURE 2
Allose inhibits the development of *Plasmodium* parasite in the mosquito by a midgut microbiota-independent mechanism. Antibiotic-treated and antibiotic-untreated mosquitoes were fed fructose with or without allose and then infected by luciferase-expressing *Plasmodium berghei*. Infectivity was determined by a luciferase assay. The number of mosquitoes is indicated under each group. The prevalence of infection was calculated number of detected oocyst or luminescence sample divided by number of total sample. The results represent the standard deviation of the mean (SD) of biological replicates. Statistical significance was determined by Wilcoxon's rank sum test with Bonferroni's modification. *$P < 0.01$.

the maximum of the growth (stationary phase) in LB medium containing 100 mM allose and 8% fructose was comparable with that containing 8% fructose with 100 mM glucose as a control (Supplementary Figure S3A). Similarly, the growth of *Phyllobacterium* sp. in LB medium containing 100 mM allose was comparable with that containing 100 mM glucose as a control (Table 1; Supplementary Figure S3B) although the growth was inhibited by the presence of 8% fructose in the medium. These results indicated that allose does not have a bactericidal or growth inhibitory effect on these bacteria.

3.4 Effect of allose on ookinete development in the mosquito gut

Allose might impact the development of *Plasmodium* parasites rather than midgut microbiota. The effect of allose on ookinete development within infected mosquitoes was evaluated *ex vivo*. The number of ookinetes in the midgut of allose-fed mosquitoes was comparable to that observed in mosquitoes fed fructose alone at 24 h after infection (Figure 4). In addition, ookinetes showing abnormal morphology were not observed in the midgut of allose-fed mosquitoes compared with that in the midgut of allose-unfed mosquitoes (Supplementary Figure S2). These results indicated that allose feeding did not inhibit ookinete development of *Plasmodium* parasites at the midgut stage of the infected mosquito.

3.5 Exploration of sugars that impact *Plasmodium* development

Additional sugars that impact the development of *Plasmodium* in mosquitoes were explored by targeting the 16 sugars listed above in the Materials and Methods section. Their sugars about the ability to block *Plasmodium* parasites have not been evaluated, except for glucose (Wang et al., 2021). As shown in Figure 5, no apparent inhibitory effects on the development of *Plasmodium* in the mosquito were observed with most of the sugars tested. Although fructose, sorbitol, and xylitol slightly inhibited the development of the *Plasmodium* parasite, the inhibition was not significant. These results indicated that these sugars do not appear to have inhibitory effects on the development of *Plasmodium* in mosquitoes.

4 Discussion

The midgut stage in the mosquito is one of the bottlenecks in the life cycle of *Plasmodium* parasites, and it can be a target of a transmission-blocking strategy. A previous study showed that allose inhibits oocyst development of *Plasmodium* parasites in the mosquito. However, the underlying mechanism remains unknown (Tokuda et al., 2015). The present study showed that inhibition by allose is effective even when mosquitoes were treated with antibiotics, strongly suggesting that the primary inhibitory mechanism is independent of the midgut microbiota of the mosquito. In addition, ookinete development in the mosquito was not inhibited by allose feeding. These results suggested that allose mainly inhibits development of the parasite after or possibly during its traverse across the midgut epithelium, e.g., ookinete migration across the midgut epithelium, ookinete-to-oocyst developmental transition, and the early phase of oocyst formation.

The interaction of ookinetes with the midgut surface via specific molecules is crucial for the invasion into midgut epithelium (Osta, 2004). Multiple pathways associated with protein–protein interactions between ookinete proteins [enolase, von Willebrand factor A domain–related protein (WARP), membrane-attack ookinete protein (MAOP), *Plasmodium* perforin-like proteins (PPLP5), subtilisin-like protease (SUB2), cell-traversal protein for ookinetes and sporozoites (CelTOS), secreted ookinete adhesive protein (SOAP), and ookinete surface P28 and P25 proteins] and mosquito midgut surface molecules [enolase-binding protein (EBP), aminopeptidase 1 (APN1), annexin-like proteins, carboxypeptidase B, croquemort scavenger receptor homolog, and calreticulin] were suggested to be involved in the invasion process (Siden-Kiamos et al., 2000; Soo Han et al., 2001; Tomas et al., 2001; Yuda et al., 2001; Dessens et al., 2003; Kadota et al., 2004; Kotsyfakis et al., 2005; Kariu et al., 2006; Dinglasan et al., 2007; Ecker et al., 2007; Lavazec et al., 2007; Rodríguez M del et al., 2007; González-Lázaro et al., 2009; Ghosh et al., 2011). In addition, several carbohydrate-binding proteins, i.e., lectins, bind specifically to the midgut epithelial cells of mosquitoes, and carbohydrate moieties are suggested to play a part in the binding of ookinetes to midgut epithelium of the mosquito (Rudin and Hecker, 1989; Zieler et al.,

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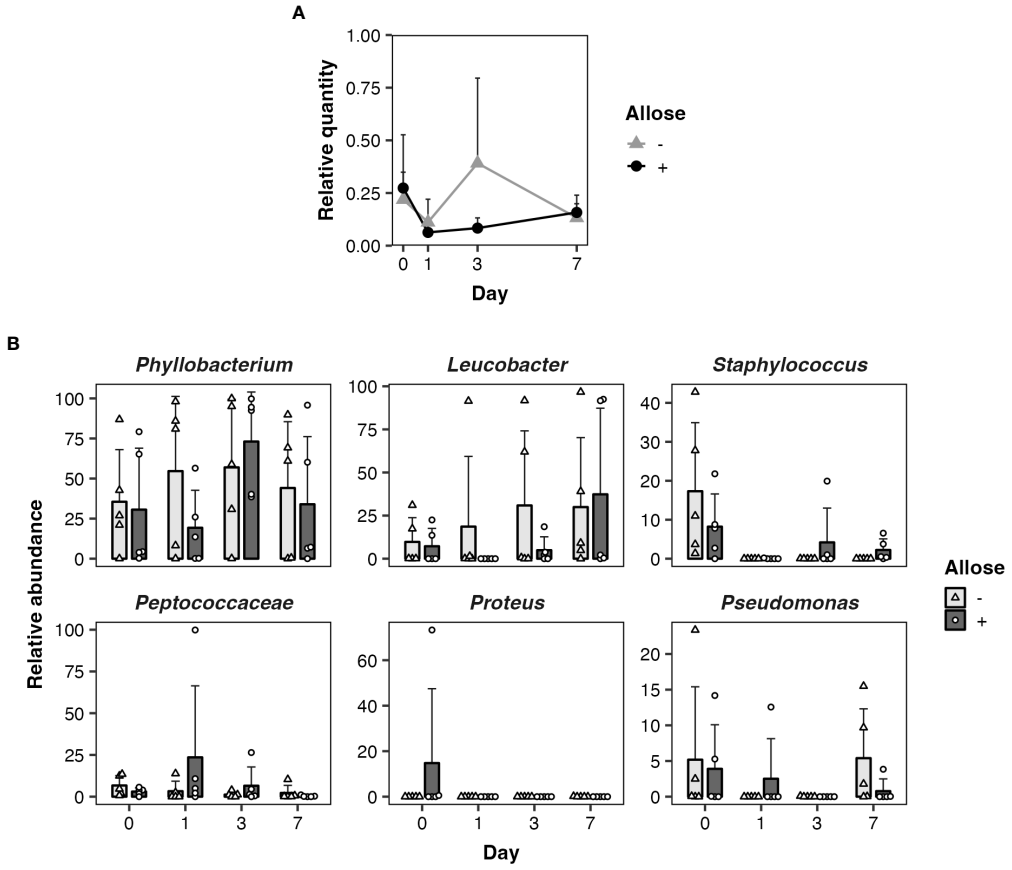

FIGURE 3
Allose affects the midgut quantity of bacteria but not their composition. Quantitative and qualitative changes of bacterial communities in the midgut were determined on days 0, 1, 3, and 7 after allose feeding. **(A)** Midgut bacteria of allose-fed (black circle) and allose-unfed (gray triangle) mosquitoes were quantified by qPCR. Relative quantities of the bacterial *16S rRNA* were normalized to the *An. stephensi glyceraldehyde 3-phosphate dehydrogenase* as the reference. Error bars indicate standard deviation (n = 5). **(B)** Midgut bacterial composition of allose-fed (open circle) and allose-unfed (open triangle) mosquitoes was determined by *16S rRNA*–based metagenomic analysis. The relative abundance of the six dominant genera—*Phyllobacterium*, *Leucobacter*, *Staphylococcus*, *Peptococcaceae*, *Proteus*, and *Pseudomonas*—are shown. The results are expressed as the mean titer and standard deviation for five biological experiments in each group.

1999; Osta, 2004). Interestingly, *Plasmodium gallinaceum* ookinetes were reported to specifically interact with a carbohydrate ligand on the midgut surface of *Aedes aegypti*, and the specific interaction was competitively blocked by the monosaccharide N-acetylneuraminic acid (Zieler et al., 1999). Therefore, allose may inhibit specific binding of *P. berghei* to midgut epithelium of *Anopheles* mosquitoes. Another possible inhibitory mechanism of allose is related to mosquito immunity against *Plasmodium* parasites. Parasite invasion and development in the midgut is affected by host immunity, including immune-related factors (Blandin et al.,

TABLE 1 In vitro growth of Phyllobacterium sp. and Leucobacter sp.

Bacteria	Cultures	Growth rate (OD_{600}/h)
Leucobacter sp.	LB	0.3459 ± 0.0187
	LB + 8% fructose	0.0507 ± 0.0006
	LB + 8% fructose + 100 mM allose	0.0330 ± 0.0013 a[b]
	LB + 8% fructose + 100 mM glucose	0.0337 ± 0.0013 a[b]
Phyllobacterium sp.	LB	0.0515 ± 0.0013
	LB + 100 mM allose	0.0352 ± 0.0013 [c]
	LB + 100 mM glucose	0.0315 ± 0.0017 [c]

[a]P < 0.01, compared with LB culture in Leucobacter sp.
[b]P < 0.01, compared with LB + 8% fructose culture in Leucobacter sp.
[c]P < 0.01, compared with LB culture in Phyllobacterium sp.

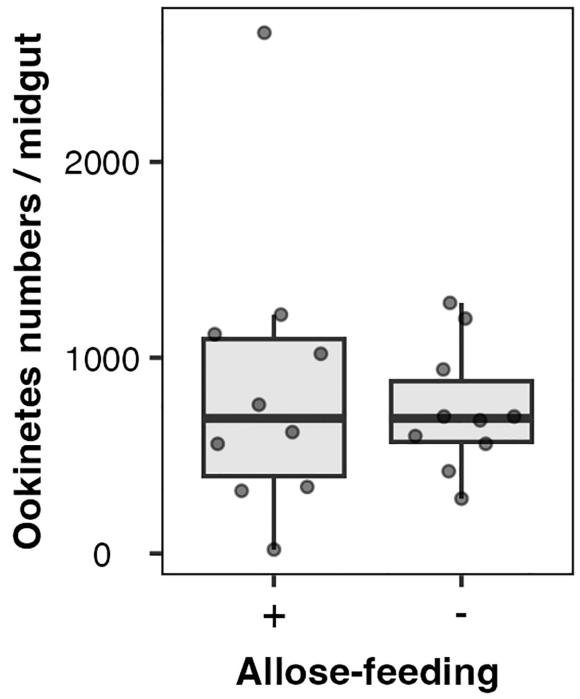

FIGURE 4
Allose does not inhibit ookinete development in the mosquito midgut. Ten mosquitoes were dissected 24 h after infection, and ookinete count per a midgut was determined. The experiment was repeated three times, and the results are represented as the standard deviation of the mean (SD) of biological replicates.

2004; Osta, 2004; Dong et al., 2006; Baxter et al., 2010; Povelones et al., 2011), antimicrobial peptides (Vizioli et al., 2001; Dong et al., 2006), and nitric oxide and ROS (Luckhart et al., 1998; Molina-Cruz et al., 2008). ROS have been shown to have a role in insect innate immune responses as a potent pathogen-killing agent, and allose is reported to induce ROS generation by activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase through the activity of glucose 6-phosphate dehydrogenase (Kano et al., 2013). RNA interference (RNAi)-mediated silencing of catalase, a ROS detoxification enzyme, was reported to result in decreased *P. berghei* infection (Molina-Cruz et al., 2008) and decreased bacterial load in the mosquito midgut (Bahia et al., 2013). These findings suggest that allose might inhibit the development of *Plasmodium* parasites and affect midgut bacteria via ROS generation in the mosquito midgut.

Several gut symbiotic bacteria, such as *Serratia* spp., *Enterobacter* sp., *Chromobacterium* sp., and *Asaia* sp., are reported to confer resistance to *Plasmodium* infection through activation of mosquito immunity (Cirimotich et al., 2011; Bando et al., 2013; Bahia et al., 2014; Ramirez et al., 2014; Bai et al., 2019; Wang et al., 2021). Initially, we expected that allose may work as a prebiotic for such symbiotic bacteria, resulting in the inhibition of *Plasmodium* development in the mosquito. However, the composition of midgut microbiota in the mosquito was not affected by allose. It seems that allose does not promote the growth of specific bacteria in the gut. In addition, none of the abovementioned bacteria were detected as a major midgut microbiota in our colonized mosquitoes. The microbial community of mosquitoes varies among populations and is associated with locality, climate, and their nutrition (Boissière et al., 2012; Muturi et al., 2018; Sandeu et al., 2022). Because the principal inhibitory mechanism of allose to *Plasmodium* is independent from commensal microbes, allose is expected to inhibit *Plasmodium* development in any population of mosquitoes.

Allose inhibited transient growth of midgut microbiota of mosquitoes initiated by blood feeding without toxic and inhibitory effects on the dominant bacteria. Several probable reasons may explain this observation: 1) allose is not suitable nutrition for midgut microbiota, 2) allose inhibits the bacterial metabolic system necessary for growth, and 3) allose enhances innate immune responses, resulting in the inhibition of bacterial

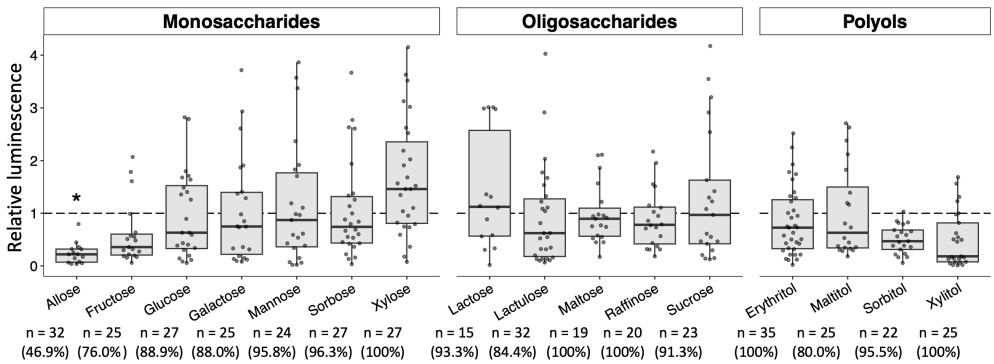

FIGURE 5
Effect of various sugars on oocyst development in the mosquito midgut. Mosquitoes were fed 8% fructose with 100 mM of each sugar and infected by luciferase-expressing *Plasmodium berghei*. Infectivity was determined by a luciferase assay. Infection of Pb-luc in mosquitoes fed the infected blood after 10 days was determined by measuring luciferase assay. Relative luminescence indicates the luminescence of each explored sugar-fed group normalized by mean luminescence of control group not added their explored sugar in 8% fructose. The number of mosquitoes is indicated under each group. The prevalence of infection was calculated number of detected oocyst or luminescence sample divided by number of total sample. The results represent the standard deviation of the mean (SD) of biological replicates. Statistical significance was determined by Wilcoxon's rank sum test with Bonferroni's modification. *$P < 0.01$.

growth. In this study, an equal amount of fructose was given to allose-fed mosquitoes as the control group, and allose was shown to be catabolized in several bacteria such as *Aerobacter aerogenes*, *Escherichia coli*, and *Listeria monocytogenes* (Gibbins and Simpson, 1964; Poulsen et al., 1999; Zhang et al., 2018). In addition, allose did not inhibit the *in vitro* growth of *Leucobacter* sp. and *Phyllobacterium* sp., the dominant bacteria isolated from the mosquito midgut. Recently, modulation of the pH of the mosquito midgut by changing its bacterial composition and metabolites was reported to influence *Plasmodium* gametogenesis (Wang et al., 2021). Our preliminary study showed that allose did not change the pH of the mosquito gut (data not shown). These findings suggested that allose does not directly affect the growth of microbiota in the mosquito gut; rather, the event might be caused by host immunity such as ROS generation.

This study explored 15 additional sugars that might impact the development of *Plasmodium* in mosquitoes. These sugars having various properties were screened, but none of the sugars tested inhibited parasite development, suggesting that allose has unique characteristics. Although pure allose is costly, an affordable and commercially available sweetener, Rare Sugar Sweet (https://www.matsutani.co.jp/english/products/raresugar.html), which contains D-allose in addition to D-glucose, D-fructose, D-mannose, and another rare sugar, D-psicose, inhibits the development of *Plasmodium* in mosquitoes (Tokuda et al., 2015). Recently, toxic sugar baits were reported to be used as a mosquito control strategy (Fiorenzano et al., 2017); the principle is using fruits and plant as an attractant to attract mosquitoes, but it has some risks for other organisms. Because allose is an eco-friendly natural product, there are fewer limitations to using the attractive toxic sugar bait applications: mixing this sugar instead of an insecticide in a field study to control transmission of *Plasmodium* parasites by mosquitoes.

The present study showed that allose inhibited the development of *Plasmodium* parasites in the mosquito independently of midgut microbiota. Such unique activity appears to be specific to allose. Further study will be needed to disclose the underlying mechanisms, especially focusing on the mosquito immunity elicited by allose. This sugar may be a useful material for vector control of malaria as a "transmission-blocking sugar" in the future (Ferreira et al., 2018).

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The animal study was reviewed and approved by Institutional Animal Experiment Committee of Jichi Medical University (approval numbers: 22002-01).

Author contributions

Conceptualization: DM, MA, and HK. Methodology: DM, DY, AT, MA, and HK. Software: DM. Validation: DM, AT, MA, and HK. Formal analysis: DM. Investigation: DM and MA. Resources: DM, DY, AT, MA, and HK. Writing—original draft preparation, DM and HK. Writing—review and editing: DM, DSY, AT, MA, and HK; visualization: DM; supervision: AM and HK; project administration: DM and HK. Funding acquisition: DM and HK. All authors have read and agreed to the published version of the manuscript.

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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/fcimb.2023.1162918/full#supplementary-material

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Case report: First report on human infection by tick-borne *Babesia bigemina* in the Amazon region of Ecuador

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Babesiosis is a protozoan disease acquired by the bite of different species of ticks. More than 100 *Babesia* spp. infect wild and domestic animals worldwide, but only a few have been documented to infect humans. Generally, babesiosis is asymptomatic in immunocompetent persons; however, in immunocompromised can be life-threatening. A 13-year-old boy from the Amazon region presented with a 3-month evolution of fever, chills, general malaise, and arthralgia accompanied by anemia and jaundice. In the last 4 years was diagnosed with chronic kidney failure. By nested-PCR using 18S RNA ribosomal gene as target and DNA sequencing, the phylogenetic analysis showed *Babesia bigemina* as the causative agent in the blood. Treatment with oral quinine plus clindamycin for six continuous weeks was effective with no relapse occurring during 12 months of follow-up. This is the second human case in Ecuador but the first caused by the zoonotic *B. bigemina* which confirms the existence of active transmission that should alert public health decision-making authorities on the emergence of this zoonosis and the need for research to determine strategies to reduce tick exposure.

KEYWORDS

babesiosis, *Babesia bigemina*, case report, zoonosis, tick-borne, Ecuador, Amazon

Introduction

Babesiosis is an emerging tick-borne zoonosis with a worldwide distribution. The number of reported human cases in the literature has increased over recent years, indicating an extension of risk areas (1). Human infections are caused by some species of *Babesia*, that include but are not limited to *B. microti*, *B. divergens*, *B. duncani*, *B. motasi*, *B. crassa*, and two *Babesia* strains, i.e., *Babesia* spp. KO1 and *Babesia* spp. CN1; the latter two may represent a new *Babesia* species (1, 2). The predominant species causing human infections in the North America is *B. microti*,

in Europe it is *B. divergens*, while in Asia several cases with *B. microti, B. venatorum*, and *B. crassa*-like have been reported (3). Other species, such as *B. microti* Kobe-type, *B. microti*-like, and some *B. divergens*–like parasites, have also been implicated in human infections (1, 2, 4). There are rare cases where *Babesia* spp. that normally infect cattle and other animals, cause disease in humans especially if they are asplenic or immunocompromised (5).

Few human cases have been diagnosed from South America. There were two from Brazil without molecular identification of the *Babesia* spp. (6). In Colombia in 2003, the first parasitological confirmed case was reported by *B. bovis*, along with three serological positive cases for *B. bovis* and one for *B. bigemina* (7). In a subsequent study, using PCR, four asymptomatic cases due to *B. bovis* and two of *B. bigemina* were detected (8). However, both reports have been disputed given a lack of more definitive evidence from DNA sequencing (9). In a study in Bolivia, 3.3% of persons were positive for *B. microti* by microscopy and PCR, while seroprevalence was 45.7% (2). A traveler from Uruguay to Spain presented mild symptoms due to *B. microti* infection (10). The only symptomatic case reported to have originated in Ecuador and diagnosed in USA was caused by *B. microti* (11).

Ecuador, located in the northwest of South America, is crossed by the Andes belt that divide into three ecoregions: the Andean temperate region, the Pacific Coast tropical region, and the interior tropical Amazon basin. Non-continental Ecuador includes the Galápagos Islands at 1,369 km from the Pacific coast. Babesiosis in cattle is considered endemic and is a national veterinary public health problem where the main tick vector is the *Rhipicephalus microplus*, a species widely distributed in the tropics, subtropics and Andes, between 0 and 2,600 m altitude (12–14). Furthermore, *Rh. microplus* collected from tropical regions resulted positive for *B. bovis*, *B. bigemina* and co-infections (12, 13). At least 41 Ixodid tick species, 32 species of hard ticks (Ixodidae) and 9 species of soft ticks (Argasidae), belonging to Amblyomma, Dermacentor, Haemaphysalis, Ixodes, and Rhipicephalus genera have been documented in Ecuador, including Galapagos Islands (15, 16), with additional species recorded in the Andes region (17).

The clinical manifestations of babesiosis in immunocompetent persons range from asymptomatic to a mild illness. In contrast, severe illness requiring hospital admission is common in persons who are immunosuppressed or splenectomised; or who have cancer, human immunodeficiency virus infection, haemoglobinopathy, or chronic heart, lung, kidney, or liver disease. The severity of babesiosis depends primarily on the immune status of the patient and may cause death (18). With an infection by *B. divergens, B. duncani* and *B. venatorum*, there seems to be a higher probability of severe disease (19). After a gradual onset of malaise and fatigue, the most common clinical sign is fever, sometimes as high as 40.9°C (105.6°F), with chills and sweats accompanied by headache, myalgia, anorexia, arthralgia, and nausea. In severe infections, fever may be accompanied by splenomegaly, hepatomegaly, jaundice, and acute respiratory failure (18). Babesiosis is a protozoal infection like malaria, confusing both clinical and laboratory diagnosis, and more so in areas where the two diseases are overlapping (11).

The diagnosis of babesiosis is based on epidemiology and clinical presentation (4). Laboratory findings that are consistent with hemolytic anemia include a low hematocrit, hemoglobin, and haptoglobin levels, but elevated reticulocyte count and lactate dehydrogenase level; thrombocytopenia is commonly observed (18). Because of difficulties in the laboratory identification of *Babesia* species that have a similar morphology and because of antigenic cross-reactivity, molecular techniques such as PCR and DNA sequencing are often required for species identification (1). DNA sequencing for the phylogenetic analysis of *Babesia* provides data on nucleotide sequences present in an amplified target gene sequence while PCR generates a large number of copies of a specific DNA fragment, represented as a single band (20). PCR is more sensitive and specific than blood smear (19). Among DNA-based assays, nested-PCR based on 18S RNA small subunit fragment for *Babesia* species have been used extensively for the diagnosis of babesiosis, and is highly sensitive, even at low levels of parasitemia (21).

Patients with mild to moderate babesiosis are treated with combination therapy of atovaquone and azithromycin, and severe cases with clindamycin plus quinine. Based on multiple case reports, a 7-to-10-day course of clindamycin-quinine combination is often used to treat severe babesiosis (3). A minimum of 6 weeks for highly immunocompromised patients is recommended (21).

Here we report a severe case of babesiosis caused by *B. bigemina* in an immunocompromised Ecuadorian child from the Amazon basin region who was treated successfully with the combination of clindamycin plus quinine.

Case report

A 13-year-old boy, Amerindian of the Shuar ethnic group, was born and raised on a farm located in Guayusa (Lat = −0.24837, Long = −77.06041), province of Orellana, northern part of the Ecuadorian Amazon, 8 hours from Quito, the capital of Ecuador. An important antecedent was that he had suffered from stage 3 chronic kidney disease for the previous 4 years (glomerular filtration rate of 49 mL/min), secondary to an anatomical malformation of the urinary tract. He had never received a blood transfusion. Clinical history revealed that for 3 months the child had presented a clinical picture characterized by a fever of 38 to 39.4°C, accompanied by chills, sweating, anorexia, general malaise, and arthralgia. One month prior to hospital admission, the fever was accompanied by jaundice, initially affecting the sclera and later became generalized, with pain of moderate intensity in the right flank. The child had always resided in the Amazon region where the presence of numerous wild animals including deer (*Mazama americana*), rodents, and insects such as mosquitoes, sandflies, and ticks are abundant. He was first medically managed with antipyretics and antibiotics in a local health center and provincial hospital. Since the fever did not subside and the clinical picture of kidney failure worsened, he was transferred to a referral hospital in Quito. Upon admission, he had a fever of 38°C, heart rate of 114 beats/min, respiratory rate of 22/min, oxygen saturation of 97%, blood pressure 129/65 mm Hg, weight 35.4 Kg, height 136.5 cm, abdominal pain, arthralgia, myalgia, and urinary incontinence with dark urine. On physical examination, pale skin and mucous membranes were observed, as were multiple scratching excoriations on the lower extremities.

The blood tests revealed a white blood cell count of 19,500 mm3 with 67% neutrophils, 19% lymphocytes, 11% eosinophils, 2% basophils, and 1% monocytes. Hemoglobin 6.3 mg/dL, hematocrit 19.7%, platelets 139,000 mm^3, urea 145 mg/dL (12–54 mg/dL),

creatinine 3.5 mg/dL (0.7 to 1.2 mg/dL for men), blood urea nitrogen (BUN) 29 mg/dL (7–20 mg/dL), alanine-aminotransferase 64 U/L (4 to 36 U/L) and aspartate aminotransferase 61 U/L (8 to 33 U/L). The direct Coombs test was positive, and lactate dehydrogenase (LDH) was 295 U/L (140 to 280 U/L). Serological studies for human immunodeficiency virus (HIV), viral hepatitis and immunological tests for malaria, Chagas, leptospirosis, and Lyme disease were all negative. Peripheral thick and thin blood smears for *Plasmodium* spp. and *Trypanosoma cruzi* were negative. No intraerythrocytic structures compatible with *Babesia* spp. were observed.

In imaging studies with sonography, hydroureteronephrosis of the right kidney was observed, while the left one was decreased in size with a loss of corticomedullary relationship and ectasia. The right kidney showed the presence of two ureters that ended together ipsilaterally. A bilobed bladder separated by a septum with a paravesical diverticulum was also observed.

Genomic DNA of whole-blood samples was isolated using commercial Qiagen DNA™-blood MiniPrep kit. A nested PCR for the long fragment of 18S rRNA gene for detection of *Babesia* species were performed. The first PCR step was amplified using the primer set PiroF (5'-GCCAGTAGTC ATATGCTTGTGTTA-3′) and Piro6R (5'-CTCCTTCCTYTAAG TGATAAGGTTCAC-3′). Another pair of primers, Piro1F (5′- CCATGCAGTTCTWAGTAYAARCTTTTA-3′) and Piro5.5R (5'-CCTYTAAGTGATAAGGTTCACAAAACTT-3′) were used in the second PCR (22). In a 2% agarose gel, a clear band of approximately 1,670 bp was observed. The amplicon was sequenced using the Sanger sequencing by an external provider (Macrogen-South Korea). The chromatogram sequence of the gene, obtained with forward and reverse primers, were assembled and consensus sequence was edited using MEGA XI software. The Nucleotide Blast, for the final sequence of 1,546 bp, show a query coverage of 100% and identity of 99.87% for *B. bigemina* using the NCBI resources and a GenBank accession number was obtained (OQ607820.1). The sequences were aligned and compared using the MEGA XI. A phylogenetic tree was constructed through Neighbor Joining method with a Bootstrap of 500 replicates, comparing eleven NCBI sequences from GenBank (Figure 1). Purified DNA of *B. bovis* and *B. bigemina*, kindly supplied by Instituto de Genética de la Universidad Nacional de Colombia, were used as positive controls.

After 32 days of clinical management in the nephrology unit, BUN and creatinine values decreased to 118 mg/dL and 2.2 mg/dL, respectively. Whereas hemoglobin and hematocrit rose to 12.8 mg/dL and 38.8%, respectively. The leukocyte count decreased to 13,400 mm^3. Anti-*Babesia* treatment was started with oral quinine 300 mg every 8 hours plus clindamycin 300 mg every 12 h, which was continued as an outpatient treatment for 6 continuous weeks. No adverse effects were reported to any of the drugs. Symptoms subsided after 7 days of treatment. No symptomatology was reported at 3-, 6- and 12-month controls and no parasitic forms compatible with *Babesia* were observed in peripheral blood smears. His mother signed the consent for the publication of the case.

Discussion

Reports of symptomatic cases of human babesiosis worldwide are rare, although in recent years the increased numbers have led experts to consider it as an emerging disease, especially in tropical and subtropical regions (1). The report of this case with severe symptoms in an immunosuppressed boy with the identification of the *Babesia* specie is important in documenting the geographic distribution of human disease in South America and particularly in Ecuador. This is the second symptomatic case in Ecuador, but the first in the Amazon region with *B. bigemina* identified as the causative agent using molecular methods (e.g., n-PCR and DNA sequencing). The first case, which came from the tropical Pacific coastal region and diagnosed in the USA, was caused by *B. microti* (11). This shows that human *Babesia* infection is present in the two tropical ecoregions of the country.

Most human infections are caused by *B. microti* and *B. divergens* and rarely by other species (1, 2, 4, 9). Few human cases by *B. bigemina* have been reported from Colombia and Ecuador in asymptomatic persons residing in the Amazon (7, 8). However, these cases were not confirmed by DNA sequencing: the use of PCR and or serology cannot confirm the *Babesia* species with certainty (1, 9, 20, 23). *Babesia bigemina* is widely distributed geographically and, together with *B. bovis*, is highly infective in livestock (12, 14). In Ecuador, *B. bigemina* has been reported to infect cattle and ticks from the tropical regions, as well as the Andean region (12, 13). Therefore, it is imperative to consider *B. bigemina* as a potential infectious agent which causes severe disease in immunosuppressed patients, as occurred in the present case. We believe that there is underdiagnosis of human babesiosis in Ecuador due to the lack of information among physicians and laboratory technicians, unavailability of sensitive diagnostic tests, and lack of epidemiological studies. Furthermore, there could be a misinterpretation of intra-erythrocytic microorganisms on blood smear with *Plasmodium* spp. (11) since malaria is endemic in Ecuador (24). There is a report of other febrile illnesses transmitted by ticks in the Amazon such as *Rickettsia* (25). Therefore, the presence of *Babesia* infections should be investigated in febrile patients.

The *Babesia* species that infect cattle and livestock in South America are *B. bigemina* and *B. bovis* (2, 26). Using PCR targeting the 18S ribosomal gene in cattle from the Coast and Andes identified both *B. bigemina* and *B. bovis* (12). This information indicates that active transmission in domestic animals is occurring with a permanent risk of infection to humans. The main vectors of *B. bigemina* and *B. bovis* in cattle are ticks of the genus *Rhipicephalus*, *Rh. sanguineus* but mainly *Rh. microplus*, species widely distributed in the tropics and subtropics of South America (13, 26). Although few studies on ticks exist in the country, several tick species of different genera that could act as potential vectors of *B. bigemina* and others, have been documented (12, 14, 15, 17).

The severe clinical symptoms of the present case are a consequence of the child being immunosuppressed due to his chronic kidney failure and delay in the diagnosis (18, 19, 21). In the hematological profile, the observed decrease in hemoglobin and hematocrit was most probably due to the hemolytic anemia. Associated laboratory findings, such as thrombocytopenia, elevation of liver and kidney enzyme levels, jaundice, and dark urine a month before hospitalization, are consistent with a *Babesia* infection confirmed later by nested-PCR and DNA sequencing. In addition, the history of exposure to ticks also supports the diagnosis. No intraerythrocytic forms of *Babesia* spp. were observed, probably due to it being a chronic infection with 3 months of evolution. In chronic cases, parasites may be undetectable

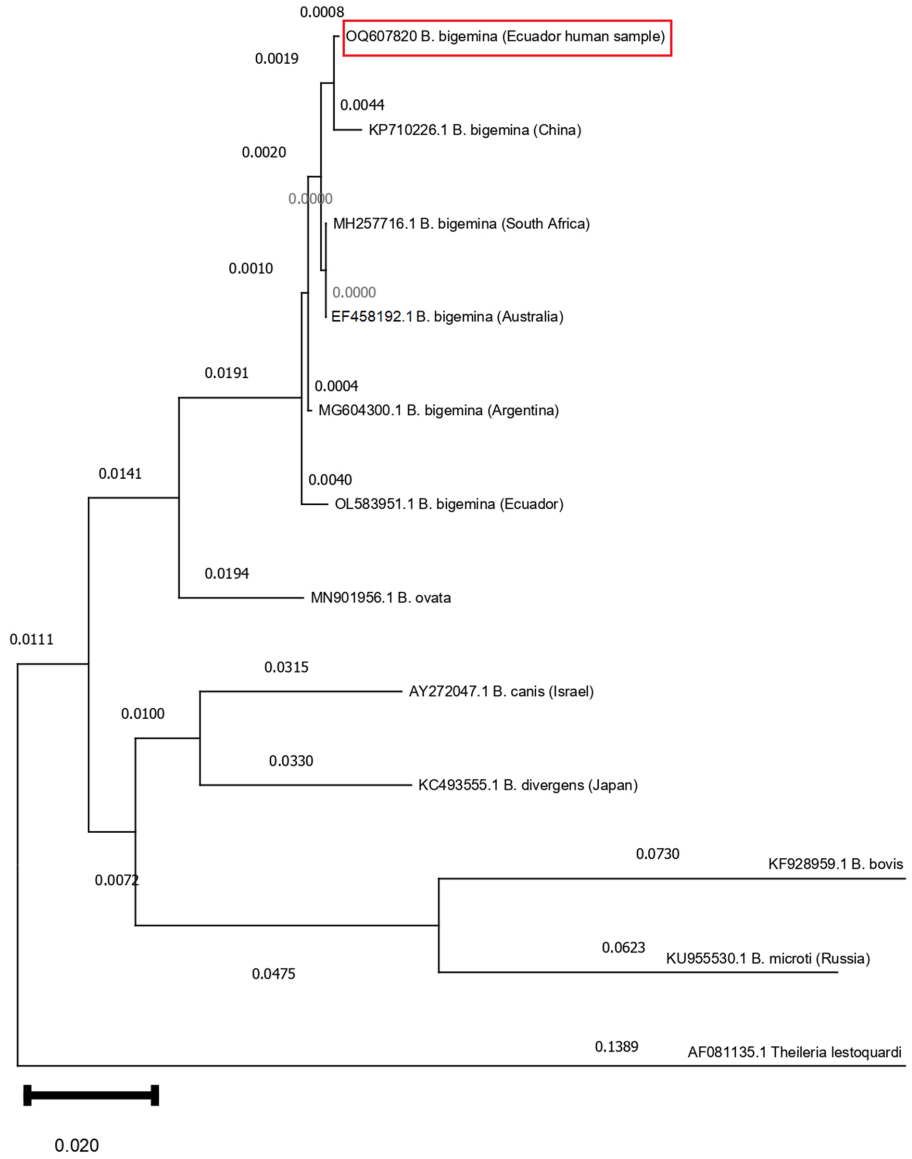

FIGURE 1
Phylogenetic tree based on the 1,670 nucleotides of the 18S rRNA gene of *Babesia bigemina*. The optimal tree was inferred using the Neighbor-Joining method. The confidence probability (multiplied by 500) that the interior branch length is greater than 0, as estimated using the bootstrap test (1,000 replicates is shown next to the branches). The evolutionary distances were computed using the Tamura Nei model (distribution of 48 parameters). Patient (Ecuador human sample, GenBank accession number OQ607820) showed identities of 99.35% when compared with published *B. bigemina* sequences (KP710226.1, MH257716.1, EF458192.1, MG604300.1, OL583951.1). *Theileria lestoquardi* (AF081135.1) compared as outgroup.

by microscopy, so it is important to use the molecular-based techniques, which detects a specific sequence of the nucleic acids of the parasite (20, 23). Along with the progress in molecular techniques, the knowledge of *Babesia* is further expanding and more species will probably be discovered.

Because the boy was with severe and chronic infection was treated using the combination of quinine plus clindamycin for 6 weeks, as recommended by the Infectious Diseases Society of America (IDSA) guidelines (3). Clinical symptoms and parasites may relapse in immunocompromised patients despite 7 to 10 days of antimicrobial therapy and may persist for more than a year if the infection is not adequately treated (18, 19, 21). He recovered completely clinical and did not present any adverse reactions during the 12 months of follow-up.

The report of this human case confirms the existence of the disease and active transmission in Ecuador, as well as its wide geographical distribution, should alert human and veterinary physicians and decision-making authorities of the importance of this emerging zoonosis and the needed research to determine preventive and control strategies. Information on the distribution of *Babesia* species is essential for the diagnosis and prevention of the disease. Unfortunately, surveillance of ticks and tick-borne pathogens do not exist in the country. We recommend strengthening the research capacity in a One Health context in order to develop control strategies

that reduce the direct and indirect health and economic burden caused by ticks and tick-borne diseases.

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

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

MC: overall coordination, writing, editing, and revision of manuscript. MM-A: diagnosis and management of patient, writing, and editing of manuscript. CB-C, RR-H, and SE: parasitological and molecular diagnosis, writing, and editing of manuscript. PC and DA-R: molecular diagnosis and editing of the manuscript. All authors read and approved the final manuscript.

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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

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Malaria in pregnancy in India: a 50-year bird's eye

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Introduction: In 2021, India contributed for ~79% of malaria cases and ~83% of deaths in the South East Asia region. Here, we systematically and critically analyzed data published on malaria in pregnancy (MiP) in India.

Methods: Epidemiological, clinical, parasitological, preventive and therapeutic aspects of MiP and its consequences on both mother and child were reviewed and critically analyzed. Knowledge gaps and solution ways are also presented and discussed. Several electronic databases including Google scholar, Google, PubMed, Scopus, Wiley Online library, the Malaria in Pregnancy Consortium library, the World Malaria Report, The WHO regional websites, and ClinicalTrials.gov were used to identify articles dealing with MiP in India. The archives of local scientific associations/journals and website of national programs were also consulted.

Results: Malaria in pregnancy is mainly due to *Plasmodium falciparum* (*Pf*) and *P. vivax* (*Pv*), and on rare occasions to *P. ovale* spp. and *P. malariae* too. The overall prevalence of MiP is ~0.1–57.7% for peripheral malaria and ~0–29.3% for placental malaria. Peripheral *Pf* infection at antenatal care (ANC) visits decreased from ~13% in 1991 to ~7% in 1995–1996 in Madhya Pradesh, while placental *Pf* infection at delivery unit slightly decreased from ~1.5% in 2006–2007 to ~1% in 2012–2015 in Jharkhand. In contrast, the prevalence of peripheral *Pv* infection at ANC increased from ~1% in 2006–2007 to ~5% in 2015 in Jharkhand, and from ~0.5% in 1984–1985 to ~1.5% in 2007–2008 in Chhattisgarh. Clinical presentation of MiP is diverse ranging from asymptomatic carriage of parasites to severe malaria, and associated with comorbidities and concurrent infections such as malnutrition, COVID-19, dengue, and cardiovascular disorders. Severe anemia, cerebral malaria, severe thrombocytopenia, and hypoglycemia are commonly seen in severe MiP, and are strongly associated with tragic consequences such as abortion and stillbirth. Congenital malaria is seen at prevalence of ~0–12.9%. Infected babies are generally small-for-gestational age, premature with low birthweight, and suffer mainly from anemia, thrombocytopenia, leucopenia and clinical jaundice. Main challenges and knowledge gaps to MiP control included diagnosis, relapsing malaria, mixed *Plasmodium* infection treatment, self-medication, low density infections and utility of artemisinin-based combination therapies.

Conclusion: All taken together, the findings could be immensely helpful to control MiP in malaria endemic areas.

KEYWORDS

malaria, pregnancy, epidemiology, outcomes, prevention, treatment, India

1. Introduction

Globally, an estimated 247 million cases and 619,000 deaths were due to malaria in 2021 (1). This burden has significantly increased compared to previous years, partially due to the current COVID-19 pandemics (2, 3). Malaria is due to five *Plasmodium* species that are transmitted to human through infecting bites of female *Anopheles* mosquitoes (4). *Plasmodium falciparum* (*Pf*) and *Plasmodium vivax* (*Pv*) are the predominant malaria species around the world; *Pf* is the most severe and dangerous species while *Pv* is the most geographically spread but can also induce severe clinical attacks (5, 6).

The sub-Saharan Africa (sSA) and South East Asia (SEA) regions are affected by malaria, especially children under 5 years of age and pregnant women (2). Malaria during pregnancy (MiP) poses an important problem to both; the future mother and unborn child. During pregnancy, malaria infection is associated with several maternal, fetal and birth complications including growth restriction, stillbirth, premature delivery, spontaneous abortions, low birth weight (LBW), and even death of mother and/or child (7, 8). The clinical spectrum and outcomes of MiP are various with distinct features depending on factors such as epidemiological situation of the setting, malaria species, gravidity, and coverage of malaria control measures [e.g., intermittent preventive treatment with sulfadoxine + pyrimethamine – IPTp-SP, indoor residual spraying (IRS), and long lasting insecticide-treated nets (LLINs)] (2).

India accounted for ~79% of malaria cases and ~83% of deaths seen in SEA in 2021 (2). In the present review, we reviewed the situation of MiP in India with emphasis on its epidemiology, clinical presentation, determinants, outcomes, prevention, and treatment. A brief overview of neonatal and congenital malaria (NCM) characteristics is also presented. Finally, we identify knowledge gaps on MiP research and propose solutions for future directions.

2. Materials and methods

2.1. Search strategy

The strategy used to identify relevant studies was inspired from MiP reviews reported previously (7, 9). Briefly, we used Google scholar, Google, PubMed, Scopus, Wiley Online library, the Malaria in Pregnancy Consortium library, the World Malaria Report, The WHO regional websites, and ClinicalTrials.gov to search for articles dealing with MiP in India published in last 50 years. The archives of local scientific associations/journals (e.g., Indian Journal of Medical Research, Indian Journal of Malariology now known as Journal of Vector Borne Diseases) and websites of national programs were also consulted. We used the search terms "malaria," "pregnancy," "pregnant woman," "burden," "prevalence," "epidemiology," "outcome," "placental infection," "congenital malaria," "neonatal malaria," "diagnostic," "prevention," "control," "management," "India," and Indian regions (Tamil Nadu, Chandigarh, Andaman and Nicobar, Assam, Andhra Pradesh, Bihar, Chhattisgarh, Daman and Diu, Goa, Delhi, Gujarat, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Kerala, Kolkata, Karnataka, Lakshadweep, Maharashtra, Manipur, Mizoram, Madhya Pradesh, Meghalaya, Nagaland, Odisha, Pondicherry, Rajasthan, Sikkim, Tripura, Uttarakhand, Uttar Pradesh, Punjab, Haryana, and West Bengal). Boolean operators including "AND" and "OR" were used in combination with the above mentioned search terms to identify relevant papers through databases such as PubMed. The search strategy was tailored to each of the search databases using search terms and Boolean operators (AND, OR). We also included all publications on neonatal and congenital malaria (NCM). To do so, the same search strategy used for MiP papers was used with some differences. For example search terms for NCM papers were "malaria," "placental infection," "congenital malaria," "neonatal malaria," "India," and Indian regions (Tamil Nadu, Chandigarh, Andaman and Nicobar, Assam, Andhra Pradesh, Bihar, Chhattisgarh, Daman and Diu, Goa, Delhi, Gujarat, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Kerala, Kolkata, Karnataka, Lakshadweep, Maharashtra, Manipur, Mizoram, Madhya Pradesh, Meghalaya, Nagaland, Odisha, Pondicherry, Rajasthan, Sikkim, Tripura, Uttarakhand, Uttar Pradesh, Punjab, Haryana, and West Bengal).

2.2. Screening strategy

Titles and abstracts of studies retrieved from databases were independently reviewed by the authors in order to identify those relevant to the study. The full texts were retrieved and scrutinized to extract data of interest. Principal investigators were kindly contacted to request full length paper and/or more details on studies. We also contacted editors-in-chief of national journals to request full length papers in case of a negative reply or no reply at all from principal investigators. Additionally, we reviewed relevant articles cited in references of identified literature and included them as primary sources.

2.3. Eligibility criteria

Only papers published in English and Hindi were included. Publications were considered of interest if they addressed any aspect of MiP and NCM in India including prevalence, clinical presentation, determinants, maternal and fetal/neonatal outcomes, diagnostic, prevention, and treatment. The list of studies is presented in Supplementary Tables 1–5.

2.4. Data extraction

Data of interest were independently extracted from eligible publications, and these consisted of (i) characteristics of studies (first author' name, year of publication, study design, area, state/union territory, urbanization setting, and year of sample collection); (ii) demographical, obstetrical, and gynecological data (age, timing of screening, parity, trimester of gestation, and route of delivery); (iii) clinical characteristics of MiP and NCM (type of malaria, clinical signs/symptoms, presence of comorbidities, and levels of hemoglobin, blood cells, and biochemical markers); (iv) parasitological information (blood source, parasitological screening method, malaria species, total number of individuals included, total number of malaria infected individuals, number of mono-infections for each malaria species, and number of mixed infections), (v) factors associated with malaria infection in pregnant women and babies; (vi) MiP and NCM outcomes, (vii) malaria preventive methods used, and (viii) efficacy of treatments to control MiP. Setting urbanization was categorized as urban,

semi-urban, rural, and not specified. Timing of screening consisted of women screened for malaria parasites in community or at health facility for delivery (DU) and antenatal care visit (ANC). Type of malaria was defined either as asymptomatic malaria, uncomplicated malaria (UM) or severe malaria (SM). In the early 90s, the WHO defined a set of criteria used for diagnosing severe malaria (SM) in children and adults (Table 1) (6, 10, 12–16). The latest WHO guideline lists 12 SM-associated signs/symptoms by malarial species and age group: severe malarial anemia (SMA), severe renal impairment, cerebral malaria (CM; prostration, impaired consciousness/coma, and multiple convulsions), jaundice, hypoglycemia, acidosis/acute respiratory distress syndrome (ARDS), significant bleeding, pulmonary edema, circulatory collapse/shock, and hyperparasitemia (10).

2.5. Data management

Data were keyed into an Excel spreadsheet (Microsoft Office, United States) by reviewer authors and then coded and verified for consistency, and removed any duplicates. Any discrepancies between the authors were resolved through discussion and consensus. Data were analyzed using StatView 5.0 for Windows (SAS Institute, Inc., Chicago, Illinois, United States) and GraphPad v8.01 for Windows (GraphPad, Inc., California, United States), and summarized as percentages and mean in tables and charts where appropriate. Overall aggregation of data using sophisticated approaches such as meta-analysis was not possible due to the heterogeneity of studies related to study design, diagnostic methods, timing of screening, blood origin, analysis conducted, and effect measures presented. Thus, only summaries of study findings stratified by variables such as diagnostic methods, timing of screening and blood origin are presented in this review. In addition, findings from studies with minimum sample size of 30 were extracted to generate charts (17). We think that this approach of analysis is more appropriate to provide reliable results and avoid misleading conclusions on Indian scenario of MiP and NCM.

3. Results

3.1. Burden of malaria in India

Malaria burden profoundly decreased in last two decades in India, and this is due to diverse strategies implemented and/or scaled up over the country (e.g., LLINs, IRS) (6). The recent national data from National Vector Borne Disease Control Program (NVBDCP) indicated that malaria transmission is low with annual parasite incidence (API) < 1 in most of the areas of India (https://nvbdcp.gov.in; Figure 1A). Malaria control needs to be reinforced in some areas including Bihar, Delhi, Uttarakhand, Orissa, Chhattisgarh, and West Bengal where API is >2 (Figure 1A). The risk of malaria infection is highest in two North-Eastern areas of India namely Tripura and Mizoram with API > 10.

The *Pf* and *Pv* species are major species in the country, with ratio close to one at the country level but varied between the different regions (Figure 1B) (18–20). Other species including *P. ovale* (*Po*), *P. malariae* (*Pm*), and *P. knowlesi* (*Pk*) are also found in India but much fewer in extent than that of *Pf* and *Pv* (21–24). The *Pm* species was reported from Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Kerala, Karnataka, and Orissa; while *Po* spp. was mainly seen in Uttar Pradesh, Assam, and Gujarat states (21, 23). Tyagi and colleagues reported the circulation of *Pk* in patients living in the Andaman and Nicobar Islands (24). Recently, *Pk* was reported from Bihar, Uttar Pradesh, and Delhi (22).

3.2. Burden of MiP in India

Data on the epidemiology of MiP in India are largely heterogeneous due to variation in diagnostic methods (LM, PCR, RDT, and histology), timing of screening (ANC, DU, and community), and blood origin (peripheral and placental blood). Again, results are more documented from forested, tribal, and rural areas of three states *viz.* Madhya Pradesh, Rajasthan, and Chhattisgarh (Table 2; Figure 2). Information on MiP epidemiology is lacking in highly malaria prevalent areas such as Delhi, Bihar, and some North Eastern states (e.g., Arunachal Pradesh and Nagaland). On ANC visits, prevalence of MiP in Madhya Pradesh state ranged from 1.9 to 17.9% using LM for detecting malaria parasites in the peripheral blood (Table 2). In Chhattisgarh, peripheral blood-based MiP prevalence ranged from 20.6 and 29.3% using RDT (42, 49). Combining different diagnostic methods, several authors reported placental malaria prevalence at DU of 2.2 and 29.3% in Madhya Pradesh (35, 40), and 21.9% in Uttar Pradesh (26). In Madhya Pradesh and Chhattisgarh, placental malaria was diagnosed at higher rates using PCR compared to impression smear and LM (44, 47), thereby outlining a high proportion of submicroscopic infections during pregnancy. Fewer studies reported MiP burden in community where peripheral malaria prevalence rates of 55.5% using LM and 0.81% using RDT were reported in Madhya Pradesh and Chhattisgarh, respectively (33, 54).

3.3. Plasmodium species involved in MiP

The information on *Plasmodium* species-wise MiP proportion is mainly originated from hospital based studies, in a limited number of states such as Madhya Pradesh, Jharkhand, Maharashtra, Rajasthan, and Chhattisgarh (Figure 2). *Pf* and *Pv* are predominant species involved in MiP irrespective of diagnostic method and timing of screening. In Madhya Pradesh, *Pf* was the main MiP-associated malaria species on ANC visits and DU with overall peripheral *Pf* mono-infections prevalence of 3.4–48.5% based on LM (Figure 3) (28, 30, 31, 33, 36, 43). Likewise, a trend of *Pf* dominance was also seen in Chhattisgarh with LM-based placental prevalence of *Pf* mono-infections ranging from 1.2 to 3.2% (Figure 4), even though the contribution of plasmodial species can vary within the same state (41, 47, 57). Singh and colleagues conducted a study in two districts of Chhattisgarh with different malarial endemicity level (i.e., Rajnandgaon and Bastar), and showed that *Pv* was dominant in Rajnandgaon (low endemic area) both at ANC and DU while *Pf* was dominant in Bastar (high endemic area; Figure 3) (41). The same team reported *Pm* as additional cause of MiP in Chhattisgarh using PCR method (47). Similarly, *Po* was detected as mixed infection with *Pf* in a multicentric study (58). One study from Rajasthan reported the predominance of *Pv* species which accounted for 96.2% of all LM-detected peripheral infections among women attending ANC visits (Figure 3) (50).

TABLE 1 Evolution of WHO definitions of severe malaria clinical and laboratory manifestations due to *Plasmodium falciparum* (1990–2015), *Plasmodium vivax* (2006–2015), and *Plasmodium knowlesi* (2012–2015) in children, non-pregnant adults, and pregnant women (10–16).

Signs/ symptoms	Definitions	1990	2000	2006	2010	2012	2014	2015
Severe anemia*,&	■ Hemoglobin (Hb) < 5 g/dL, or Hematocrit (Hct) < 15%							
	■ Hb < 5 g/dL, or Hct < 15% (Children) ■ Hb < 7 g/dL, or Hct < 20% (Adults)							
	■ Hb < 5 g/dL, or Hct < 15% (Children <12 years) with parasitemia >10,000 p/μL ■ Hb < 7 g/dL, or Hct < 20% (Adults) with parasitemia >10,000 p/μL							
Severe renal impairment*	■ Urine output <12 mL/kg/24 h, or plasma creatinine concentration above the age-related normal values (Children)/Urine output <400 mL/kg/24 h, or/and a serum creatinine >3 mg/dL (Adult), and despite adequate volume repletion/rehydration							
	■ Serum creatinine concentration > 265 μmol/L (3 mg/dL)							
	■ Serum creatinine concentration > 265 μmol/L (3 mg/dL) or blood urea >20 mmol							
Shock/Circulatory collapse*	■ Systolic blood pressure (SBP) < 70 mmHg (Adults) and < 50 mmHg (Children 1–5 years)							
	■ SBP < 80 mmHg (Adults) and < 50 mmHg (Children)							
	■ *Compensated shock*: capillary refill ≥3 s or temperature gradient on leg (mid to proximal limb), but no hypotension. *Decompensated shock*: SBP < 70 mmHg (Children) or < 80 mmHg (Adults) with confirmation of impaired perfusion (prolonged capillary refill or cool peripheries)							
Abnormal bleeding*	■ Spontaneous bleeding from gums, nose, gastrointestinal tract, and venipuncture sites. Clinical evidence of bleeding using tests (e.g., prothrombin time, platelet)							
	■ Recurrent or prolonged bleeding from nose gums, gastro-intestinal tract, or venipuncture sites; hematemesis or melena							
Disseminated intravascular coagulation (DIC)	■ Laboratory evidence (e.g., prothrombin time prolonged >3 s of the control)							
Multiple convulsions	■ More than 2 convulsions observed within 24 h							
Metabolic acidosis*	■ Plasma bicarbonate <15 mmol/L							
	■ Plasma bicarbonate <15 mmol/L or base excess (≥ 10 mmol/L)							
	■ A base deficit of >8 mEq/L or, if unavailable, a plasma bicarbonate of <15 mM or venous plasma lactate >5 mM							

(Continued)

TABLE 1 (Continued)

Signs/ symptoms	Definitions	1990	2000	2006	2010	2012	2014	2015
Hemoglobinuria*	■ Hemolysis not secondary to glucose-6-phosphate dehydrogenase deficiency							
	■ Urine is dark or black, and urinalysis dipstick test is positive for Hb, associated with absence of microscopic hematuria (i.e., presence of blood in urine)							
Impaired consciousness/ Cerebral malaria*,§	■ Rousable coma (impaired consciousness)/Unarousable come (cerebral malaria)							
	■ A Glasgow coma Score < 9 (Adults), or a Blantyre coma score < 2 (Children)							
	■ A Glasgow coma Score < 11 (Adults), or a Blantyre coma score < 3 (Children)							
Prostration*	■ Weakness so that the patient cannot sit or walk, with no obvious neurological explanation							
	■ The inability to sit upright or to drink (Children), Extreme weakness (Adults)							
	■ Generalized weakness so that the patient is unable walk or sit up without assistance							
Clinical jaundice*	■ Plasma/serum bilirubin concentration > 50 µmol/L (3 mg/dL)[a]							
	■ Plasma/serum bilirubin concentration > 50 µmol/L (3 mg/dL)[b]							
	■ Plasma/serum bilirubin concentration > 50 µmol/L (3 mg/dL) with parasite density > 20,000 parasites/µL[c]							
	■ Plasma/serum bilirubin concentration > 50 µmol/L (3 mg/dL) with parasite density > 100,000 parasites/µL[a,c]							
Multi-organ dysfunction	■ Clinical jaundice, and evidence of other vital organ dysfunction							
Pulmonary oedema*	■ Diagnosed upon radiological examination							
	■ Diagnosed upon radiological examination, or oxygen saturation < 92% on room air with a respiratory rate > 30/min, often with chest indrawing and crepitations on auscultation							
Acidotic breathing	■ Deep breathing and respiratory distress							
Hypoglycaemia	■ Whole blood glucose concentration < 2.2 mmol/L (40 mg/dL)							
Hyperpyrexia	■ Core body temperature > 40°C							
Hyperlactatemia*	■ Plasma lactate 5 mmol/L (Children) and > 6 mmol/L (Adults)							
	■ Plasma lactate >5 mmol/L							

(Continued)

TABLE 1 (Continued)

Signs/ symptoms	Definitions	1990	2000	2006	2010	2012	2014	2015
Failure to feed	■ n.a (observable)							
Hyperparasitemia*	■ No parasitemia threshold defined[a]							
	■ Parasitemia >4% (unstable malaria endemicity), > 20% (stable malaria endemicity)[a]							
	■ Parasitemia >5% (low malaria endemicity), > 10% (high malaria endemicity)[a]							
	■ Parasitemia >2% (low malaria endemicity), > 5% (high malaria endemicity)[a]							
	■ Parasitemia >20% in any epidemiological context[a]							
	■ Parasitemia >10% in any epidemiological context[a]							
	■ Parasitemia >2%[c]							

DIC, Disseminated intravascular coagulation; Hb, Hemoglobin; Hct, Hematocrit; n.a, Not applicable; *Pf*, *Plasmodium falciparum*; *Pk*, *Plasmodium knowlesi*; *Pv*, *Plasmodium vivax*; SBP, Systolic blood pressure; WHO, World Health Organization. *These signs/symptoms have been revised by the WHO. [&]Defined as severe normocytic anemia in the previous guidelines (1990–2012). [§]Impaired consciousness (rousable coma) and cerebral malaria (unarousable coma) were clinically differentiated before advent of coma scales (16). *Pv* and *Pk* have been first mentioned as able to induce severe malaria in the 2006 and 2012 WHO guidelines, respectively. Manifestations of severe malaria due to *Pv* parasites may present to with some symptoms similar to severe *Pf* malaria. Manifestations of severe malaria due to *Pk* parasites are similar to those of severe *Pf* malaria with the exception of coma. [a]Used for *P. falciparum* severe malaria only. [b]Used for *P. vivax* severe malaria only. [c]Used for P. knowlesi severe malaria only. The color is related to linking the definition of a given sign/symptom to the period where this definition was retained in global WHO guidelines to diagnose severe malaria.

Temporal analysis of burden of MiP was limited in regions such as Madhya Pradesh, Jharkhand, and Chhattisgarh (Figures 3, 4). In Madhya Pradesh, the prevalence of peripheral *Pf* infection at ANC decreased from ~13% in 1991 to ~7% in 1995–1996, while prevalence of peripheral *Pv* infection at ANC levels off at ~5% between 1991 and 1997–1998. In Jharkhand, the prevalence of *Pv* infection at ANC increased from ~1% in 2006–2007 to ~5% in 2015. The same trend was observed for peripheral *Pf* infection among women attending ANCs in Chhattisgarh where the prevalence of this species increased from ~0.5% in 1984–1985 to ~1.5% in 2007–2008 (Figure 3). In contrast, an increase in prevalence of peripheral *Pv* infection at DU was noted in Jharkhand (~0.5% in 2006–2007 to ~4% in 2015). Regarding placental infection, the burden of *Pf* infection slightly decreased from ~1.5% in 2006–2007 to ~1% in 2012–2015 in women living in Jharkhand (Figure 4). There are few studies that analyzed temporal trends of MiP burden in other settings such as sSA and Latin Americas where malaria endemicity patterns are different. One study reported an apparent reduction in *Pf* MiP burden from ~60 to 5% during years 1994–2019 in different malaria eco-epidemiological regions (coastal savannah zone, middle forest zone, and northern savannah zone) in Ghana (59).

3.4. Clinical and parasitological features of MiP

Clinical presentation of MiP in India is diverse with *Plasmodium* infections ranging from asymptomatic carriage of the parasites to severe clinical forms.

3.4.1. Asymptomatic malaria

The detection of *Plasmodium* parasitemia of any density, in the absence of fever or other acute symptoms, in individuals who have not received recent antimalarial treatment is known as asymptomatic malaria (60). All plasmodial species can elicit asymptomatic infections during pregnancy with higher parasitemia in *Pf* infections compared to non-*Pf* infections (50). Data on asymptomatic MiP are greatly lacking from India. The burden of asymptomatic malaria varies across Indian areas, and this heterogeneity is related to several factors such as using diagnostic techniques with varying sensitivity and specificity. Using RDT, Corrêa and colleagues reported that ~10% of *Plasmodium spp*-infected women were asymptomatic in tribal areas of Chhattisgarh, Telangana, and Andhra Pradesh (49). In Jharkhand state, it was reported that >70% of MiP cases were asymptomatic in a randomized trial (53). Another study in the same state reported same results both, at ANC (70.6%) and DU (75.7%) (46). Similarly, a community based study found that asymptomatic cases accounted for 76.7% of all malaria cases in rural areas in Chhattisgarh (54). There is a link between asymptomatic infections and parasite density, with many asymptomatic infections being found at submicroscopic levels and thus can only be detected using molecular tools (61). A recent meta-analysis reported overall prevalence of submicroscopic *Plasmodium* infections of 0.4–38.4% in general Indian population (62), which is lower estimates as seen in MiP. In Chhattisgarh, Singh and colleagues found significant proportion of both peripheral and placental *Plasmodium* spp. submiscroscopic infections, for which the extent was species-dependent, among majorly primigravidae/secundigravidae women (47). More than 60% of peripheral and placental infections were submicroscopic with higher rates seen in *Pv* compared to *Pf* infections (placental: 66.7 vs. 64.7%, peripheral: 90.5

vs. 50%) (47). Tracking asymptomatic and submicroscopic *Plasmodium* infections is vital for malaria elimination strategies in endemic areas (63), especially in India where malaria burden significantly decreased in last years.

3.4.2. Uncomplicated malaria

The fraction of asymptomatic infections that will become clinically symptomatic is shaped by a cocktail of factors such as level of transmission, control measure coverage, comorbidities (e.g., human immunodeficiency virus—HIV, malnutrition), host and parasite factors (64, 65). Uncomplicated malaria (UM) encompasses non-pathognomonic and flu-like signs and symptoms which often comprise fever, nausea, rigors, chills, headache, muscle pains, etc. (66). Symptoms usually occur 7–30 days after mosquito bite and last for 6–10 h sequentially three stages: cold, hot, and sweating (67). The same clinical malaria symptomatology is seen in pregnant women living in endemic areas such as India. Most of the *Pf* and *Pv* UM-related symptoms reported in Indian pregnant women attending ANC and/or DU included fever, weakness/fatigue, body/joint pain, headache, loss of appetite, diarrhea, dizziness, hepato-splenomegaly, and nausea/vomiting (33, 43, 44, 46, 49, 50, 52, 54).

Malaria is also associated with cell and biochemical changes in multiple organs and tissues. One of the most dominant MiP-associated signs is anemia for which risk is increased in pregnant women and malaria-infected pregnant women (46, 55). The pathophysiological mechanism includes hemolysis of both *Plasmodium*-infected and uninfected red blood cells (RBCs) and impaired/suppressed hematopoiesis (67, 68). The sequestration pathophysiological phenomenon is known and described well in *Pf* parasites (69), but not for *Pv* and *Pk* though few reports showed ability of *Pv*- and *Pk*-infected RBCs to cytoadhere to endothelial cells in placenta, bloodstream vessels and brain even though low cytoadherence of *Pk*-infected RBCs to cerebral microvascular endothelial cells was found (70–72). In India, malarial anemia during pregnancy is due to *Pf* and *Pv* with overall prevalence of 36.6–100% varying across the country (39–43, 46, 47, 50, 58, 73), with mild and moderate forms accounting for >85% of all anemia cases (28, 41, 46, 49). The extent of MiP-associated anemia seems to be higher in primigravidae/secundigravidae and ANC patients. On ANC visits anemia prevalence of ~67–88.3% against ~59.6–83.9% at DU through studies conducted in Jharkhand, Chhattisgarh, and Rajasthan (39, 46, 47, 50).

3.4.3. Severe malaria

Pregnant women are particularly susceptible to *Plasmodium* infections and its severe forms in malaria endemic regions of India (31, 57, 74). Available data outline that *Pf* is the principal contributor to severe MiP cases and maternal/fetal outcomes, and few reports about *Pv* causing SM attacks (75, 76). No severe MiP case due to non-*Pf*/*Pv* species has been documented in the world so far. Using systematic review and meta-analysis approach, we recently showed that the overall prevalence of SM in individuals with *Pv* mono-infection was 29.3% in India, with lowest and highest rates in Karnataka (15.3%) and Uttarakhand (57.8%), respectively (5). In pregnant women, data on SM prevalence are greatly missing in India, and the studies on clinical patterns of severe MiP are focused and/or have evaluated few particular presentations only (e.g., ARDS, CM) (77, 78). One study from Karnataka reported that 32.4% of malaria-infected patients were diagnosed with SM, and *Pv* was the main contributor of SM cases (56.6%) (52). Most of severe *Pf* MiP cases occurred in primigravidae as reported by Singh et al. in Madhya Pradesh (29), and Kochar et al. in Rajasthan (79). No severe MiP case with *Po*, *Pm*, and *Pk* have been documented in India till now.

3.4.3.1. Severe malarial anemia

Malaria infection is a risk factor for severe anemia in Indian pregnant women (47, 50). Based on the available data, severe anemia is found at prevalence of 3–15.6% in *Plasmodium*-infected pregnant women (28, 39, 41, 46, 47, 49, 50, 52, 74). *Pf* and *Pv* as mono- and mixed infections are the species responsible for this severe hematological condition, with higher rates seen in *Pf*-MiP as reported in Karnataka state (52). However, these estimates do not reflect the real burden of MiP related SMA in Indian context for at least four reasons: (i) different thresholds for hemoglobin level were used for diagnosing severe anemia (e.g., Hb < 5 g/dL or < 7 g/dL), (ii) in some studies, moderate and severe anemia were collectively diagnosed with the same Hb threshold (e.g., Hb < 9 g/dL) (41), (iii) very few studies appraised other severe anemia-inducing conditions such as malnutrition (49), and (iv) none of the studies included parasitemia threshold for defining SMA as per WHO guidelines (Tables 2, 3). In this context, it is needed to document the real contribution of SMA in pregnancy in India.

3.4.3.2. Cerebral malaria

Cerebral malaria is a common severe manifestation in Indian pregnant women (38, 77, 84, 87, 88), as high CM prevalence of 60 and 76% reported among malaria-infected individuals from Orissa and Rajasthan states, respectively (79, 82). Routinely, CM is more frequently seen in primigravidae/secundigravidae compared to multigravidae, and is mostly associated with poor maternal and fetal outcomes (28, 31, 77, 88).

3.4.3.3. Hypoglycemia

Hypoglycemia is often reported at low rates in Indian children and non-pregnant adults diagnosed with SM. A systematic review and meta-analysis found a pooled proportion of hypoglycemia of 0.05% due to *Pv*-related mono-infections in the country (5). In SM pregnant women, prevalence of hypoglycemia is ~3–35% with higher rates found in *Pf* infected pregnant women compared to their *Pv* infected counterparts (Table 3). In Rajasthan, *Pv* induced hypoglycemia in 4% of malaria patients while a prevalence of 35% was reported among *Pf*-infected women in Uttar Pradesh (Table 3) (48, 74).

3.4.3.4. Acute renal failure

The etiology of acute renal failure (ARF) is multifactorial in pregnancy, and studies outline that malaria contributes toward a small fraction as reported from Gujarat state where ARF proportion is ~0–8% in pregnant women (83). A study from Rajasthan reported a *Pf*-induced ARF proportion of 20%, but this estimate was obtained on only 45 pregnant women (Table 3) (80).

3.4.3.5. Acute respiratory distress syndrome/pulmonary edema

Acute respiratory distress syndrome is diagnosed in malaria infected pregnant women at lower rates compared to SMA, CM, and ARF, with overall proportion of ~0–4% in states including Rajasthan, Gujarat, and Andhra Pradesh (Table 3). ARDS can pose a veritable

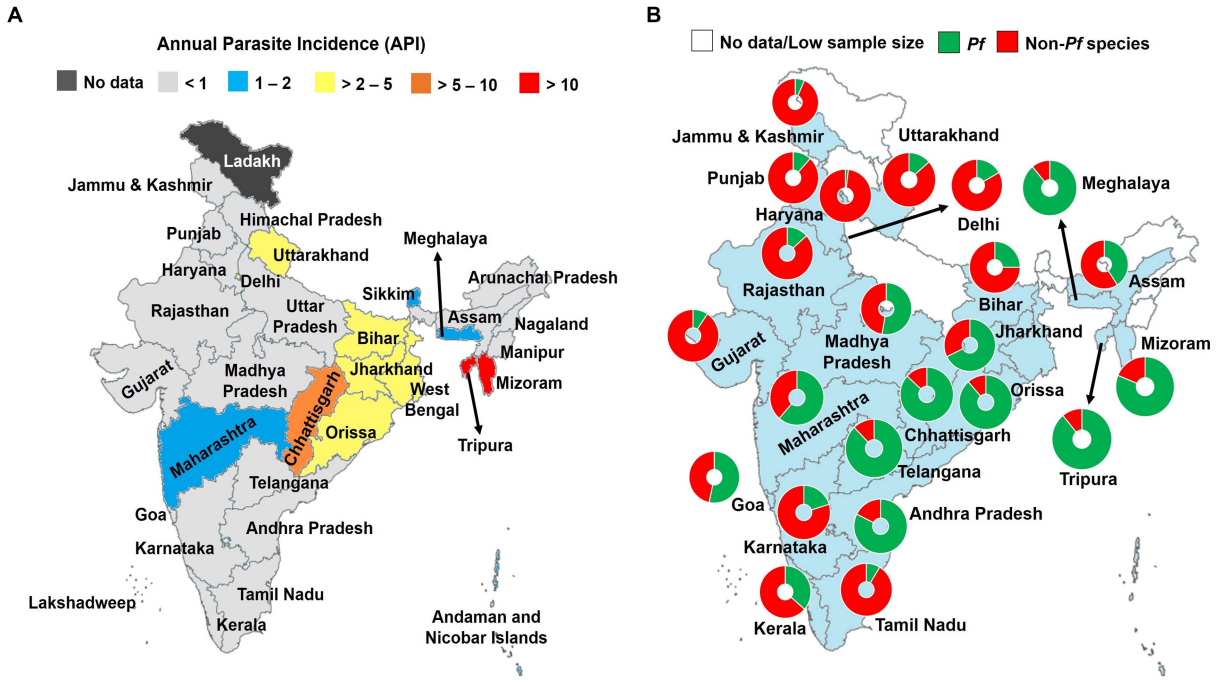

FIGURE 1
Burden of malaria in India, 2021. **(A)** Annual parasite incidence of *Plasmodium* spp. infections, and **(B)** contribution of *Pf* and non-*Pf* species. API, Annual parasite incidence; *Pf*, *Plasmodium falciparum*; *Pv*, *P. vivax*. Malaria species were identified using light microscopy Non-*Pf* species are mostly represented by *Pv* (>90%). The data were retrieved from official website of the National Vector Borne Disease Control Program (https://nvbdcp.gov.in). Pie charts depict the relative contribution of malaria species in total malaria cases. Only sample size of malaria positive slides >30 were retained and presented in **(B)** (17). The map was retrieved from the official website of the Ministry of External Affairs of Government of India (https://mea.gov.in/india-at-glance.htm).

diagnostic and therapeutic dilemma as previously reported in a *Pv*-infected preeclamptic pregnant woman (78). Kochar et al. (81) reported that pulmonary edema occurred in 13.3% of malaria infected pregnant women just after delivery in Rajasthan.

3.4.3.6. Clinical jaundice

Jaundice is the main clinical manifestation seen in *Pv* SM in Indian patients (5). This condition occurs in malaria infections due to intravascular hemolysis, disseminated intravascular coagulation, and rarely the occurrence of hepatocellular jaundice in *Pf* malaria infection also known as "malarial hepatitis" (89). Compared to severe *Pf* infections, the risk of jaundice is lower in severe *Pv* infections regardless of the living area and age group (5). In *Plasmodium* spp. infected pregnant women, the proportion of clinical jaundice ranges from ~4–14% with similar rates for *Pf* and *Pv* (Table 3).

3.4.3.7. Other severe malaria clinical manifestations

Severe clinical manifestations such as shock, prostration, metabolic acidosis, and abnormal bleeding can also occur in *Plasmodium* infected pregnant women, but at lower rates (≤ 5%) compared to the above mentioned clinical manifestations (Table 3).

3.4.4. Other severe clinical manifestations: severe thrombocytopenia

Severe thrombocytopenia defined as level of blood platelets below 50,000/μL, is not considered as clinical marker of SM in latest WHO guidelines (10). Previous works in India reported significant fraction of SM patients presenting this hematological disorder (5, 90, 91). *Pf* and *Pv* are able to induce severe thrombocytopenia, and available data in India on comparative analysis of these two species outline that the risk of this condition varies with area. In Karnataka, the risk of severe thrombocytopenia is nearly four times higher in *Pf*-infection compared to *Pv*-infections. In contrast, this risk is reduced by ~60% in *Pf*-infections in Rajasthan state (5). In pregnant women, few studies reported disparate findings for severe thrombocytopenia prevalence in Karnataka (6 and 26.8%) for *Plasmodium* spp. infections while 56 and 82% were found in Rajasthan and Gujarat for *Pv* mono-infections, respectively (45, 74, 84, 86).

3.5. Comorbidities and concurrent infections seen in MiP

Clinical course and outcomes of MiP are modeled by complex interaction between host, parasite, and environment (48). In addition, external factors such as comorbidities and concurrent infections can modulate interaction between host and *Plasmodium* parasites, and thus impact the natural history of malarial infection in pregnant women (Figure 5).

Concurrent infections included diseases caused by bacteria, viruses, parasites, and fungi. In late 80s, Mehta and Mehta reported toxoplasmosis in *Plasmodium* infected pregnant women from Kolkata, West Bengal (92). In the same period in Chandigarh, septicemia of bacterial origin was reported in one woman diagnosed with CM (57).

TABLE 2 Studies conducted on burden of MiP in India.

Design	Timing of screening	States (Areas)	Setting[†]	Year of collection	Screening method	*N*	*Plasmodium* spp. infection prevalence	Ref.
Case–Control	ANC	Chandigarh and neighboring villages	Rural area	1984–1985	LM	5,589	1.40%[#]	(25)
Cross-sectional	DU	Uttar Pradesh	Not specified	Not specified	Histology/LM	256	21.9%[‡&]	(26)
Cross-sectional	DU/ANC	Gujarat (Surat)	Not specified	1987–1988	LM	Not specified	57.7%[#]	(27)
Cross-sectional	ANC	Madhya Pradesh (Jabalpur)	Rural, tribal, and urban	1991	LM	Total (831), Dry (62), Monsoon (466), and Autumn (303)	Total (17.4%[#]), Dry (19.4%[#]), Monsoon (14.8%[#]), and Autumn (21.1%[#])	(28)
Cross-sectional	ANC	Madhya Pradesh (Jabalpur)	Rural, tribal, and urban	1991–1993	LM	1,000	20%[#]	(29)
Cross-sectional	ANC	Madhya Pradesh (Mandla District)	Rural and Forested	1995–1996	LM	456 + 325 (781)	12.9%[#] + 11.4%[#] (12.3%[#])	(30)
Cross-sectional	ANC	Madhya Pradesh (Jabalpur)	Rural, tribal, and urban	1992–1995	LM	1,598	17.9%[#]	(31)
Cross-sectional	ANC	Orissa (Koraput)	Rural, tribal and with perennial hyperendemic transmission	Not specified	LM	209	11.6%[#]	(32)
Cross-sectional	-	Madhya Pradesh (Mandla District)	Rural and Forested	1997–1998	LM	274	55.1%[#]	(33)[§]
Cross-sectional	-	Madhya Pradesh (Mandla District)	Rural and Forested	1996	LM	100	30[#]	(34)[§]
Cross-sectional	DU	Madhya Pradesh (Mandla District)	Rural and Forested	2002–2003	LM/RDT	182	29.3%[‡]	(35)
Cross-sectional	DU	Madhya Pradesh (Mandla and Satna districts)	Rural, forested and tribal	2002–2003	LM	209 (Mandla), 590 (Satna)	Mandla: 5.3%[#] and 14.4%[‡] Satna: 6.9%[#] and 10.8%[‡]	(36)
Cross-sectional	DU/ANC	Madhya Pradesh	Rural, tribal and urban	2006	LM	1,825 (ANC), 1,012 (DU)	Dry season: 1.9%[#] (ANC), 0.8%[#] (DU); Post-rainy season: 6.4%[#] (ANC), 2.9%[#] (DU) Dry season: 0.8%[‡] and Post-rainy season: 2.9%[‡]	(37)
Cross-sectional	ANC	Maharashtra (Mumbai)	Not specified	Not specified	LM	416	6.5%[#]	(38)
Cross-sectional	DU/ANC	Jharkhand (Ranchi, Konbir, and Gumla)	Rural, semi-urban, and urban	2006–2007	LM/RDT	Peripheral: 2,382 (ANC) and 717 (DU); Placental: 0 (ANC) and 712 (DU)	1.8%[#] (ANC) and 1.7%[#] (DU); (ANC) and 2.4%[‡] (DU)	(39)
Cross-sectional	DU/ANC	Madhya Pradesh (Jabalpur)	Rural, tribal and urban	2008–2009	LM	500	1.8%[#] and 2.2%[‡]	(40)

(Continued)

TABLE 2 (Continued)

Design	Timing of screening	States (Areas)	Setting†	Year of collection	Screening method	*N*	*Plasmodium* spp. infection prevalence	Ref.
Cross-sectional	DU/ANC	Chhattisgarh (Bastar, Rajnandgaon)	Rural and Forested (Bastar is high endemic, Rajnandgaon is low endemic)	2007–2008	LM/RDT	Rajnandgaon: 1,498 (ANC), 547 (DU); Bastar: 1,198 (ANC), 481 (DU)	Total: 1.3%[#] (ANC), 1.9%[#] (DU) Rajnandgaon: 0.1%[#] (ANC), 0.6%[#] (DU) and 3.2%[‡] (DU); Bastar: 2.8%[#] (ANC), 3.4%[#] (DU) and 3.6%[‡] (DU)	(41)
Cross-sectional	ANC	Chhattisgarh (Maita, Mallampeta, Dharmannapeta, Pusuguppa, Tippapuram, Yampuram, and Puttapalli)	Rural and Forested	2012	RDT	1,222	Total: 20.6%[#] (Maita: 47.6%[#], Mallampeta: 16.1%[#], Dharmannapeta: 15.2%[#], Pusuguppa: 16.9%[#], Tippapuram: 13.3%[#], Yampuram: 30.6%[#], and Puttapalli: 24.5%[#])	(42)
Cross-sectional	DU/ANC	Madhya Pradesh (Rewa)	Hyperendemic with 62–80% of cases due to *Pv*	2014	LM	203	35.5%[#]	(43)
Cross-sectional	DU	Madhya Pradesh (Katni, Maihar)	Katni (Semi-rural), Maihar (Rural)	2006–2007	Histology/LM/PCR/RDT	506 (histology), 504 (incision smear), 505 (impression smear), 504 (LM), 506 (RDT), and 110 (PCR)	10.3%[‡] (histology), 4.9%[‡] (incision smear), 3.6%[‡] (impression smear), 5.4%[#] (LM), 4.2%[#] (RDT), and 34.5%[#] (PCR)	(44)**
Retrospective	ANC	Karnataka (Mangaluru)	-	2014–2015	LM	12,600	0.3%[#]	(45)
Cross-sectional	DU/ANC	Jharkhand (Hazaribag)	Rural and semi-urban district with low but perennial transmission of malaria	Not specified	LM	1,271 (ANC), 870 (DU)	5.4%[#] (ANC), 4.3%[#] (DU)	(46)
Cross-sectional	DU/ANC	Chhattisgarh (Bastar, Rajnandgaon)	Rural and Forested (Bastar is high endemic, Rajnandgaon is low endemic)	2007–2008	LM/PCR	2,477 (ANC), 948 (DU)	LM: 1.2%[#] (ANC) and 1.7%[‡] (DU); PCR: 3.4%[#] (ANC) and 4.2%[‡] (DU)	(47)
Cross-sectional	-	Uttar Pradesh (Aligurgh)	Not specified	Not specified	LM/QBC/RDT	156	57.0%[#]	(48)[$]
Descriptive	ANC	Chhattisgarh, Andhra Pradesh, and Telangana	Forested	2015	RDT	563	29.3%[#]	(49)
Cross-sectional	DU/ANC	Rajasthan (Bikaner)	Seasonal transmission	Not specified	qPCR/LM	ANC: 2,021 (LM) and 298 (qPCR), DU: 1,206 (LM) and 297 (PCR)	LM: 1.3%[#] (ANC) and 0%[‡] (DU) qPCR: -[#](ANC) and -[#](DU)	(50)

(Continued)

TABLE 2 (Continued)

Design	Timing of screening	States (Areas)	Setting†	Year of collection	Screening method	*N*	*Plasmodium* spp. infection prevalence	Ref.
Case–Control	ANC	Jharkhand (Hazaribag)	Rural and semi-urban district with low and perennial transmission of malaria	2014–2015	LM/PCR/RDT	534	9.4%[#a]	(51)
Cross-sectional	ANC	Karnataka (Mangaluru)	-	2014–2017	LM/RDT	105	67.6%[#]	(52)
Cluster randomized controlled trial	ANC/DU	Jharkhand (Kamdara and Basia in Gumla district, Bano and Kolebira in Simgeda district)	Forested with malaria peak from June to October	2012–2015	RDT/PCR/ Histology	ANC	ANC	(53)
						ISTp: 3,163 (RDT) and 2,620 (PCR)	ISTp: 3.2%[#] (RDT) and 5.9%[#] (PCR)	
						PCD: 108 (RDT) and 2,706 (PCR)	PCD: 9.3%[#] (RDT) and 4.2%[#] (PCR)	
						DU	DU	
						ISTp: 1,405 (RDT) and 1,454 (Histology)	ISTp: 2.2%[‡] (RDT) and 6%[‡] (Histology)	
						PCD: 1,540 (RDT) and 1,560 (Histology)	PCD: 2.1%[‡] (RDT) and 4.2%[‡] (Histology)	
Cross-sectional	-	Chhattisgarh (Durg, Sarguja, Bilaspur, Raipur, and Bastar)	Rural and forested with high malaria burden	2019	RDT	21,572	0.8%[#] (from 0.03% in Durg to 4.4% in Bastar)	(54)[$]
Case–Control	ANC/DU	Madhya Pradesh (Maihar)	Rural and semi-urban, and ethnic tribal populations	2010–2012	LM	3,873	1.3%[#] & 1.3%[‡]	(55)
Cross-sectional	-	Karnataka (Mangaluru)	-	2015	LM/RDT	29	20.7%[#]	(56)[$]

ANC, Antenatal care visit; DU, Delivery unit; ISTp, Intermittent screening and treatment during pregnancy; MiP, Malaria in pregnancy; *Pf*, *Plasmodium falciparum*; *Pv*, *Plasmodium vivax*; LM, Light microscopy; PCR, Polymerase chain reaction; PCD, Passive case detection; QBC, Quantitative buffy coat; qPCR, Quantitative PCR; and RDT, Rapid diagnostic test. The list of studies used is presented as Supplementary Table 1. †Characteristics of the area at time of study. [#]Maternal peripheral blood was used for malaria parasite detection. [‡]Maternal placental blood was used for malaria parasite detection. [&]Placental infection included presence of malarial parasite and/or hemozoin (malarial pigment). [$]The study was conducted in community. [$]The study included women attending hospital and presenting signs suggestive of severe malaria. **Histology was the gold standard as method for malaria parasite detection. [a]Only *Pv* infections.

High diversity of viruses including dengue virus, HIV, and SARS-CoV-2 were concurrently found in malaria infected pregnant women in the country (Figure 5). In Uttar Pradesh, a case report of co-infection with *Pf*, *Pv*, and dengue virus was seen in one 6-month pregnancy (93). In Maharashtra, a co-infection case of *Pv* with SARS-CoV-2 responsible for the current COVID-19 pandemics was described (94).

Regarding comorbidities, mostly cardiovascular disorders such as eclampsia and pre-eclampsia were diagnosed in *Pv*–induced ARDS and CM cases in Delhi and Karnataka were seen (78, 95). The effect of poor nutritional status on malaria risk is still elusive (96). A study from three states (Chhattisgarh, Andhra Pradesh, and Telangana) found malaria infected pregnant women with poor nutritional status (i.e., mid-upper arm circumference < 230 mm) (49). Other comorbidities such as cancer, diabetes and HELLP syndrome have also been reported (Figure 5).

3.6. Factors associated with MiP in India

3.6.1. Parity and gestation trimester

Parity is one of the most important factors associated with both peripheral and placental MiP, with primigravidae much at risk of malaria infection and its deleterious consequences (97). Moreover, studies found that the level of antibodies inhibiting placental sequestration of *Pf* parasites is increasing over successive pregnancy, thereby supporting a parity-dependent acquired antimalarial immunity (98, 99). In India, many studies reported both higher peripheral and placental malaria prevalence in primigravidae/secundigravidae compared to multigravidae (27, 29, 31, 32, 39, 46, 73, 79). In Jharkhand, a study reported that peripheral infection risk was 4.23 times higher in primigravidae/secundigravidae compared to multigravidae (46). The same team reported that the risk of placental infection was increased by >3 times in primigravidae/secundigravidae, finding that was previously

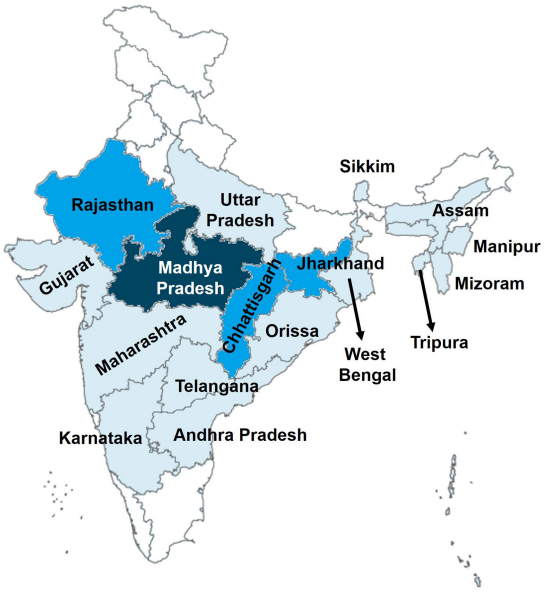

FIGURE 2
Geographical distribution of studies on MiP in India. The map was retrieved from official website of the Ministry of External Affairs of Government of India (https://mea.gov.in/india-at-glance.htm). Areas colored represent the places where studies on MiP were conducted. The darker the area higher is the number of conducted studies on MiP in those areas.

reported in a study conducted in the same state (Figures 6, 7) (39, 46). It has been reported that *Plasmodium* spp. infection rates are higher during second trimester of pregnancy (65). Data from several states including Madhya Pradesh, Rajasthan, Gujarat also support this observation (27, 31, 33, 80). However, one study conducted in Madhya Pradesh reported highest infection rates during third trimester (30).

3.6.2. Woman's demographical, clinical and genetic characteristics

The influence of pregnant woman's characteristics on malaria infection risk has been reported from studies conducted in India, with role of age, level of education, and clinical symptomatology. In Jharkhand, Hamer et al. (39) found that women aged <20 years attending ANC visits had 2.68 times more risk of peripheral *Plasmodium* infection compared to their older counterparts (Figures 6, 7). Similarly, an increased risk for peripheral *Plasmodium* infection was seen in women with lack of formal education in two studies conducted in Chhattisgarh and Jharkhand, respectively (39, 41). Studies reported higher *Plasmodium* infection in febrile pregnant women with signification association between diarrhea, fever or history of fever, moderate anemia and peripheral/placental infection (41, 46, 54). Fever was a stronger determinant of peripheral and placental *Plasmodium* infections both at ANC and DU (41, 46, 54).

3.6.3. Residence area

Several aspects of the residence area have been associated with MiP in India and include level of urbanization, forest cover and level of malaria endemicity (39, 41, 46, 54). In Jharkhand, MiP is more prevalent in rural areas compared to urban areas. Indeed, the risk of peripheral *Plasmodium* infection is ~4–6 times higher in rural women compared to their counterparts from urban areas. Again, the risk of placental *Plasmodium* infection is increased by ~3–4 times in rural areas compared to urban areas in Jharkhand (Figures 6, 7) (39, 46). Working in two areas of Chhattisgarh differing by malaria endemicity, Singh and colleagues reported peripheral *Plasmodium* infection risk increased by ~45 times in pregnant women living in Bastar (high endemicity area) compared to those living in Rajnandgaon (low endemicity area) (41).

3.6.4. Diagnostic methods

Given detection sensitivity of malarial diagnosis tools varies, it is expected to have higher chances of detecting low (very low) *Plasmodium* parasitemia with molecular tools which have higher sensitivity than LM and RDTs (63). Using PCR and LM for detecting peripheral/placental *Pf* and *Pv* infections, Singh et al. pointed out that chances of detecting peripheral/placental infections using PCR were increased by ~2.5–2.9 times for *Plasmodium* spp. infections, ~1.7–2.6 times for *Pf* infections and ~2.5–8.1 times for *Pv* infections among women attending ANC and DU in Madhya Pradesh (Figures 6, 7) (47).

3.7. Maternal outcomes of MiP

Malaria in pregnancy has a devastating impact on health of mothers and their babies, and is an important cause of maternal and infant mortality in malaria endemic regions. In malaria endemic areas *Plasmodium* infections are associated with adverse maternal outcomes such as miscarriage, stillbirth, abortion, and mortality. In India, *Pf* and *Pv* have been associated with these maternal outcomes which are frequently seen in primigravidae mothers (Figure 8; Supplementary Table 2) (30, 31, 33, 80). CM, pulmonary edema and hypoglycemia were cause of maternal death reported in two studies conducted in Chandigarh and Gujarat (57, 73). The overall prevalence of MiP-related maternal death in malaria-infected individuals is ~0–77.3% in India, with disproportion among different states. *Pf* is the main contributor to maternal death while few rare death cases associated with *Pv* infections have also been reported (50, 100). Abortions and stillbirths are reported at prevalence of ~7.2–16.7 and ~0–13.3% for *Pf*, and 0.3–8.4 and ~0–8% for *Pv*, respectively. The highest values of *Pf*-related abortions and stillbirths were reported in Rajasthan among pregnant women presenting with SM (80, 81).

Only two studies from Chhattisgarh and Rajasthan quantified the risk of maternal outcomes in MiP (47, 50). Using LM, Singh and colleagues found that pregnant women with peripheral *Plasmodium* spp. infection had 1.8 times higher risk of anemia and 13.7 times higher risk of severe anemia that their uninfected counterparts (Supplementary Table 3). Also, the risk of LBW was nearly six times higher in women with placental *Plasmodium* spp. infections compared to that with no infection (47). In Rajasthan, clinical *Pv* infection was associated with five-higher risk of maternal anemia (50). Likewise, the risk of maternal anemia was increased by four times in women with microscopic *Pf* infection compared to uninfected women. In the same vein, women with placental microscopic *Pf* infections were 4.28 times more at risk to give birth babies with LBW compared to those with no infection (50) (Supplementary Table 3).

3.8. Fetal/neonatal/infancy outcomes of MiP in India

Placental malaria infections are associated with adverse outcomes on fetus, newborns, and even during infancy. Malaria

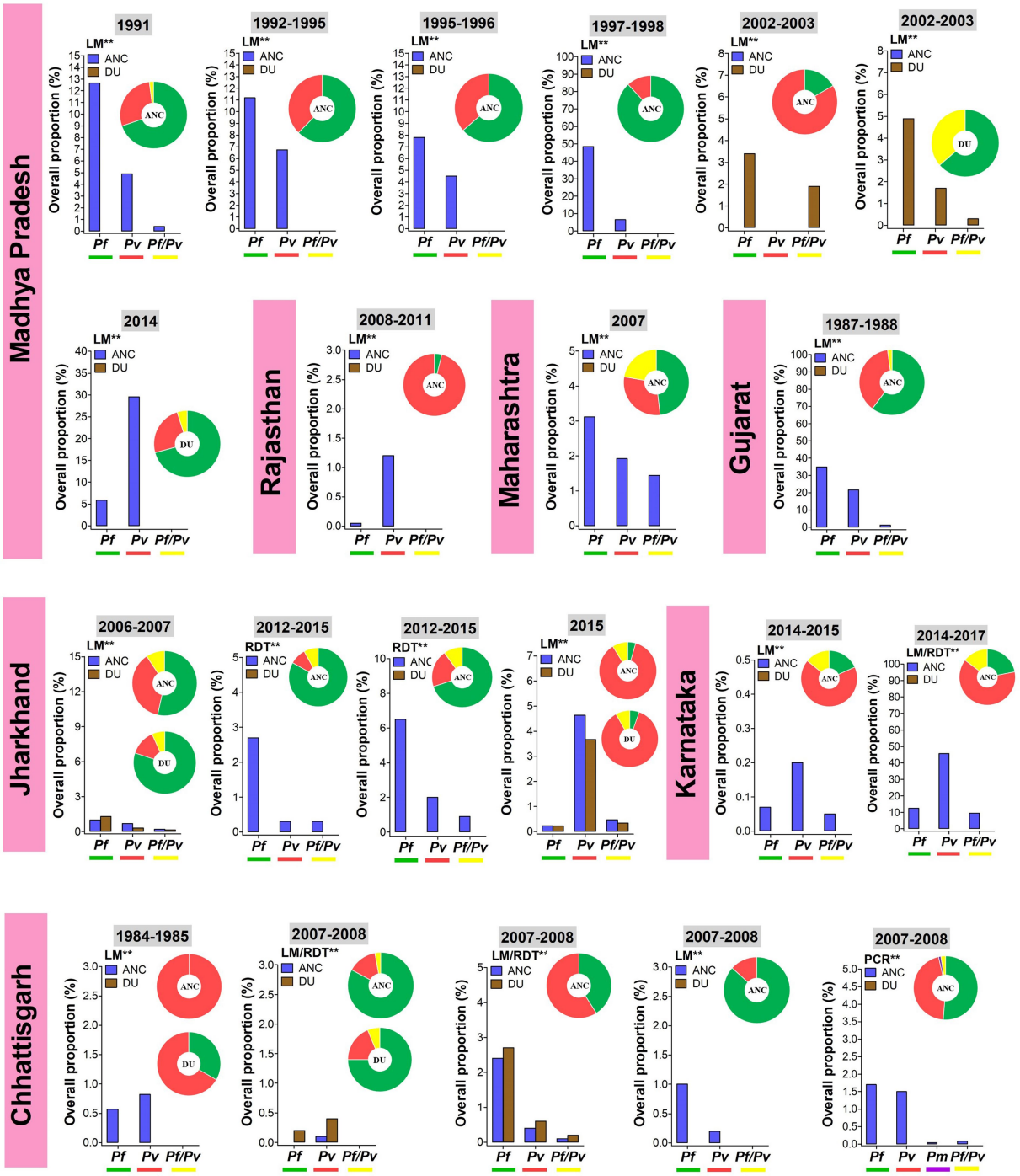

FIGURE 3
Overall proportion and contribution of *Plasmodium* species either as peripheral mono- or mixed infections in ANC and DU by states. ANC, Antenatal care visit; DU, Delivery unit; LM, Light microscopy; RDT, Rapid diagnostic test; PCR, Polymerase chain reaction; *Pf*, *Plasmodium falciparum*; *Pm*, *Plasmodium malariae*; *Pv*, *Plasmodium vivax*; *Pf/Pv*, Mixed infection with *P. falciparum* and *P. vivax*. Bars represent overall proportions of *Plasmodium* infections which are computed as ratio of number of patients with *Plasmodium* species either mono-infection or mixed infections to total number of patients. Pie charts represent the specific proportion of *P. falciparum*, *P. ovale*, *P. malariae*, and *P. vivax* species either mono- or mixed infections. These proportions were computed as ratio of total number of patients with either one *Plasmodium* species either mono- or mixed infections to total number of *Plasmodium*-infected patients. Findings were stratified by timing of screening (ANC and DU). *Pf* mono-infections, *Pv* mono-infections, and *Pf*/*Pv* mixed infections are depicted in green, red, and yellow, respectively. **Diagnostic method used for detection *Plasmodium* infections.

parasites, especially *Pf*, have a high tropism for placenta tissue in which the parasites develop and collaterally induce important histological changes (e.g., fibrinoid necrosis, calcification) (44). Such *Plasmodium*-induced placental changes impair fetal-maternal exchange and lead to disastrous consequences in babies such as low birth weight (LBW), prematurity, intrauterine growth retardation (IUGR), respiratory distress, and deaths in the worst case (Figure 9; Supplementary Table 3). LBW, IUGR and prematurity are the

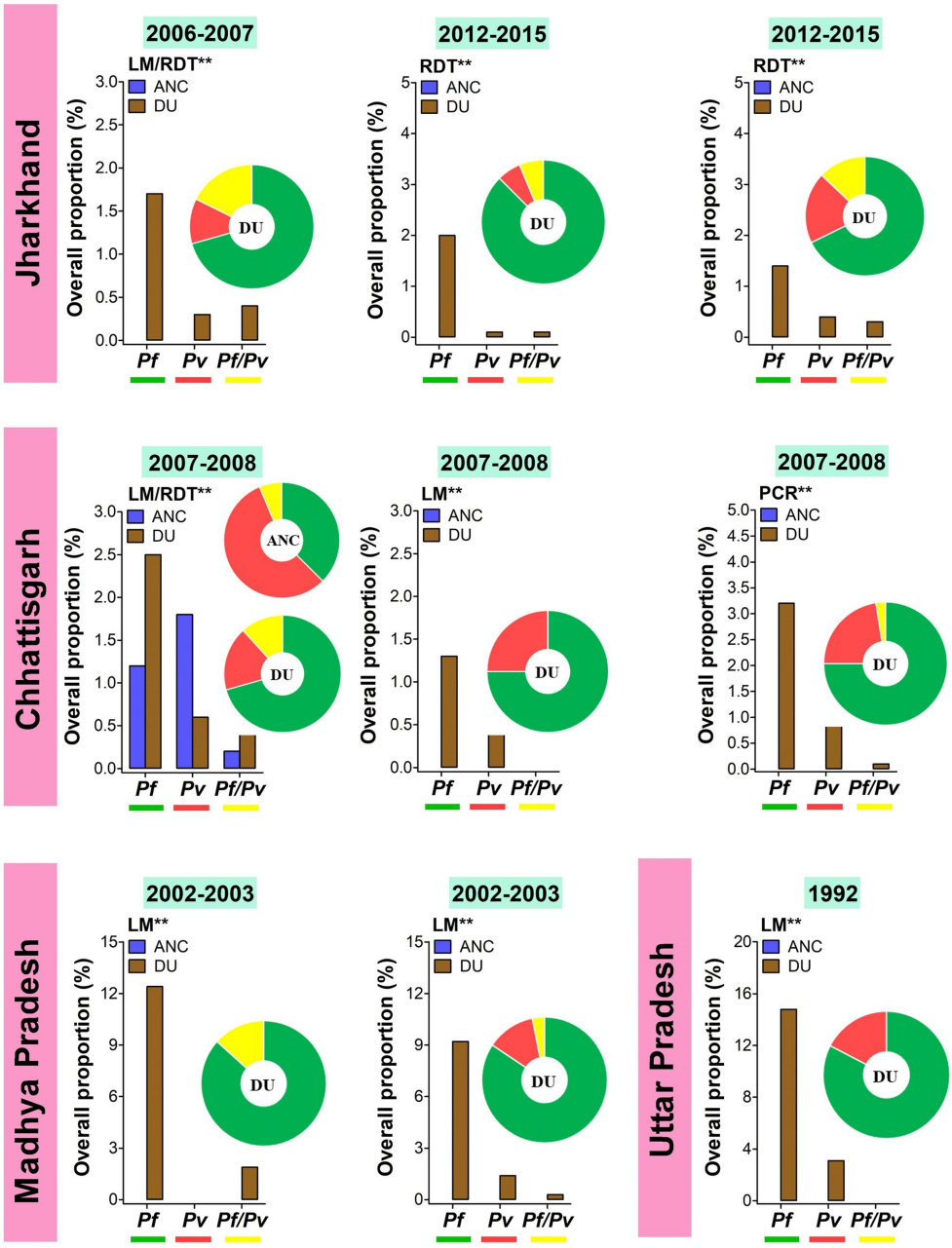

FIGURE 4
Overall proportion and contribution of *Plasmodium* species either as placental mono- or mixed infections in ANC and DU by states. ANC, Antenatal care visit; DU, Delivery unit; LM, Light microscopy; RDT, Rapid diagnostic test; PCR, Polymerase chain reaction; *Pf*, *Plasmodium falciparum*; *Pv*, *Plasmodium vivax*; *Pf/Pv*, Mixed infection with *P. falciparum* and *P. vivax*. Bars represent overall proportions of *Plasmodium* infections which are computed as ratio of number of patients with *Plasmodium* species either mono-infection or mixed infections to total number of patients. Pie charts represent the specific proportion of *P. falciparum*, *P. ovale*, *P. malariae*, and *P. vivax* species either mono- or mixed infections. These proportions were computed as ratio of total number of patients with either one *Plasmodium* species either mono- or mixed infections to total number of *Plasmodium*-infected patients. Findings were stratified by timing of screening (ANC and DU). *Pf* mono-infections, *Pv* mono-infections, and *Pf/Pv* mixed infections are depicted in green, red, and yellow, respectively. **Diagnostic method used for detecting *Plasmodium* infections in the studies.

predominant adverse outcomes in India, with *Pf* and *Pv* as main contributors.

Studies outlines that ~5.4–89 and ~ 14–80% of *Pf*- and *Pv*-infected women gave birth babies with LBW, respectively. In Madhya Pradesh, it was reported that almost all babies (95.2%), born to malaria infected women, had LBW (33, 36). Similarly, the prevalence of prematurity can often surpass 50% for both *Pf* and *Pv* in Indian women. It was reported that severe maternal anemia was risk factor for both LBW and prematurity in Jharkhand (101). Growth restricted babies are also frequently seen during MiP with prevalence of ~12.8–54, ~0–9.8, and ~ 0–25.3% for *Plasmodium* spp., *Pf*, and *Pv*, respectively (Figure 9; Supplementary Table 4). It is now recognized that *Pv* can also cause poor birth outcomes including perinatal and intrauterine mortality, but at lower extent than its *Pf* counterpart. In Indian context, the prevalence of *Pv*-induced intrauterine death during MiP ranges from 0 to 8% while that of *Pf* is estimated at ~7.2–31.1% (Figure 9; Supplementary Table 4).

TABLE 3 Severe clinical manifestations of MiP in malaria infected individuals in India.

States	Severe anemia	CM	ARDS/ PE	Hypoglycemia	Jaundice	Shock	ARF	Prostration	Multiple convulsions	Acidosis	Bleeding	Multiorgan dysfunction	Malaria species[f]	Ref.
Chandigarh	-	7%	-	-	-	-	-	-	-	-	-	-	*Plasmodium*	(57)
Madhya Pradesh	11.5%[c]	-	-	-	-	-	-	-	-	-	-	-	*Plasmodium*	(28)
Rajasthan	20% [a]	75.5%	4.4%	6.7%	13.3%	-	20%	-	11.1%	-	-	13.3%	*Pf*	(80)
Rajasthan	-	-	13.3%[&]	-	-	-	-	-	-	-	-	-	*Pf*	(81)
Orissa	-	60%	-	-	-	-	-	-	-	-	-	-	*Pf*	(82)
Rajasthan	-	76%	-	-	-	-	-	-	-	-	-	-	*Pf*	(79)
Madhya Pradesh	-	7%	-	-	-	-	-	-	-	-	-	-	*Pf*	(31)
Multiple states[d]	38%	-	3%	17%[#]	7.4%	-	8%[#]	-	13%	-	-	-	*Plasmodium*	(58)
Gujarat	-	-	-	-	-	-	4.2%[‡]	-	-	-	-	-	*Plasmodium*	(83)
Jharkhand	3.9–4.5%[b]	-	-	-	-	-	-	-	-	-	-	-	*Plasmodium*	(39)
Madhya Pradesh	-	-	9.5%[&]	-	-	4.7%	-	-	-	-	-	-	*Plasmodium*	(40)
Rajasthan	60%[a]	0%	4%	4%	12%	-	8%	0%	-	-	-	4%	*Pv*	(74)
Gujarat	-	2%	0%	20%	4%	2%	0%	2%	-	-	0%	0%	*Pv*	(84)
Chhattisgarh	10.7–15.6%[b]	-	-	-	-	-	-	-	-	-	-	-	*Plasmodium*	(47)
Jharkhand	7.8–13.6%[b]	-	-	-	-	-	-	-	-	-	-	-	*Plasmodium*	(46)
Uttar Pradesh	-	26.9%	-	35%	-	-	0%	-	16.8%	-	-	-	*Pf*	(48)
Rajasthan	7.5–8.6% [b]	-	-	-	-	-	-	-	-	-	-	-	*Pv*	(50)
Multiple states[e]	6.9%[b]	-	-	-	-	-	-	-	-	-	-	-	*Plasmodium*	(49)
West Bengal	-	-	-	29%	6%	-	3%	-	-	-	-	-	*Pf*	(85)
Karnataka	11.3%[a]	-	-	-	-	-	-	-	-	-	-	-	*Plasmodium*	(52)
Jharkhand	0.7–1.1% [b]	-	-	-	-	-	-	-	-	-	-	-	*Plasmodium*	(53)
Andhra Pradesh	12%[b]	22%	3%	3%	14%	4%	8%	-	12%	4%	5%	-	*Plasmodium*	(86)

ARDS, Acute respiratory distress syndrome; ARF, Acute renal failure; CM, Cerebral malaria; PE, Pulmonary edema; *Pf*, *Plasmodium falciparum*; *Pv*, *Plasmodium vivax*; WHO, World Health Organization; Ref., References. Estimates are percentages of each severe clinical manifestation among malaria-infected pregnant women, unless otherwise indicated. [#]Only reported in *Pf* parasites. [&]Pulmonary edema. [‡]Malaria was responsible for 4.2% of all ARF analyzed in the study. [a]Hemoglobin < 5 g/dL was used for defining for severe anemia. [b]Hemoglobin < 7 g/dL was used for defining for severe anemia. [c]Hemoglobin threshold for defining for severe anemia was not specified. [d]The study was conducted in nine Indian states (Orissa, Meghalaya, Tripura, Assam, Mizoram, Manipur, Sikkim, Andhra Pradesh, and Chhattisgarh). [e]The study was conducted in four Indian states (Chhattisgarh, Andhra Pradesh, and Telangana). [f]The estimates in the studies were found for *Plasmodium* spp., *Pf*, or *Pv*.

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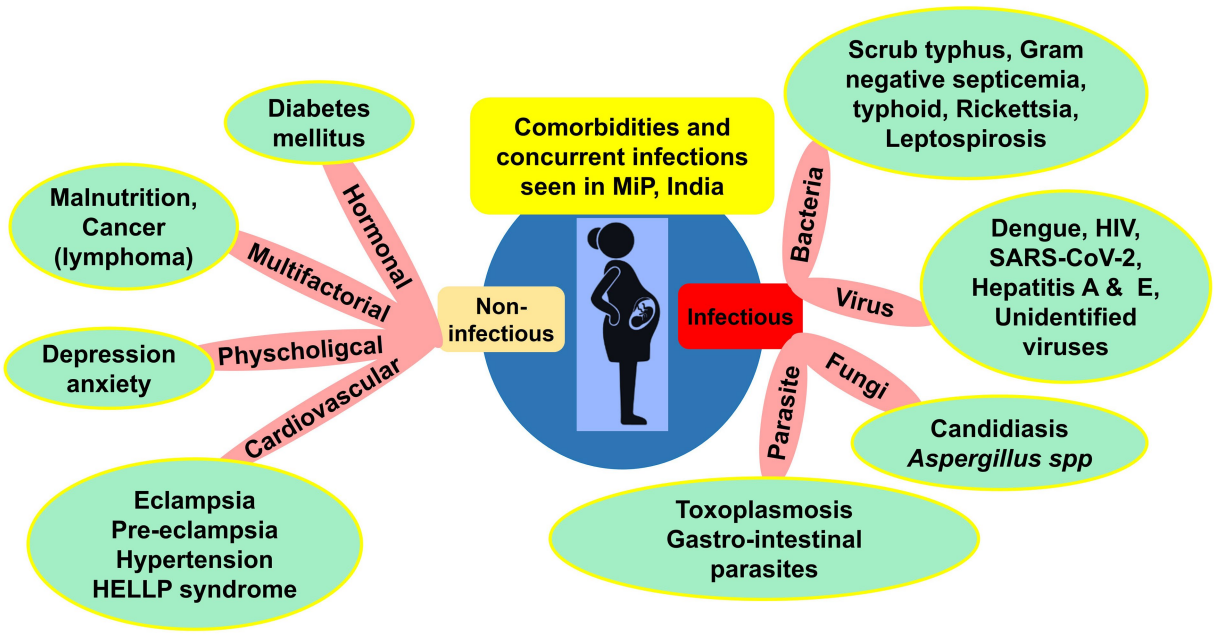

FIGURE 5
Comorbidities and concurrent infections reported in MiP in India. HELLP, Hemolysis, elevated liver enzymes, low platelet count; HIV, Human immunodeficiency virus infection; MiP, Malaria in pregnancy; SARS-CoV-2, Severe acute respiratory syndrome Coronavirus 2; UTI, Urinary tract infections. Some examples of comorbidities are presented in the green round shapes based on published literature and unpublished reports from health facilities. *Gestational hypertension is included in hypertension cases.

Malaria in pregnancy and its birth consequences are consistently correlated with increased malaria risk during infancy (102, 103). A study found an increased 2-fold risk for malaria infection and clinical malaria in small-for-gestational age babies born to Beninese women (104). Such data are scarce in India, but in one study conducted in Madhya Pradesh, mothers and their infants were followed up for 1 year. The authors reported increase in malaria prevalence, intensity and frequency during the follow up, and three of all *Pf*-infected infants died before their first birthday (33).

3.9. Neonatal and congenital malaria

In clinical practice, congenital malaria is defined as presence of *Plasmodium* asexual stages in cord blood and peripheral blood of the baby during first week of life (105). In neonatal malaria, *Plasmodium* asexual stages are found in neonates aged ≤28 days (105). A recent meta-analysis estimated global NCM prevalence at 40.4 and 12%, due to several variable factors including area and detection methods (106).

The prevalence of congenital malaria in India ranges from 0 to 12.9% (Figure 9). Prevalence data on neonatal malaria are absent in India, but some case reports outlined its occurrence in the country (Supplementary Table 5). NCM cases have been reported throughout India especially in states such as West Bengal, Madhya Pradesh, Rajasthan and Uttar Pradesh (Supplementary Tables 3–5). In India, NCM cases are mostly born to primigravidae women, have LBW and are aged 26 days on average with male predominance. On admission, babies present at hospital with mosaic of signs/symptoms mostly including pallor, hepatosplenomegaly, fever, jaundice/icterus, and irritability. Anemia, thrombocytopenia, leucopenia and clinical jaundice are frequently seen in newborns infected with malaria parasites having parasitemia in ranges from 0.1 to 25% and *Pv* mono-infections account for ~60% of all LM/RDT-detected NCM cases (Figure 10; Supplementary Table 5).

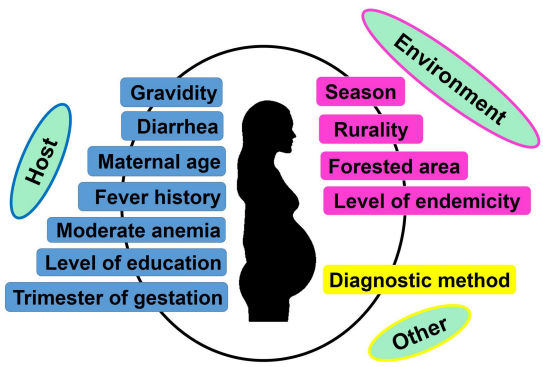

FIGURE 6
Factors associated with peripheral and/or placental *Plasmodium* spp. infection during pregnancy. Only factors identified based on multivariate logistic regression as reported in each included study were retained to create the image.

3.10. Prevention of MiP

In last 2 decades, India greatly scaled up large number of malaria prevention methods throughout the territory to achieve elimination objectives by 2030 (107, 108). Malaria prevention in India relies essentially on free provision of ITNs/LLINs and IRS (109). LLINs are

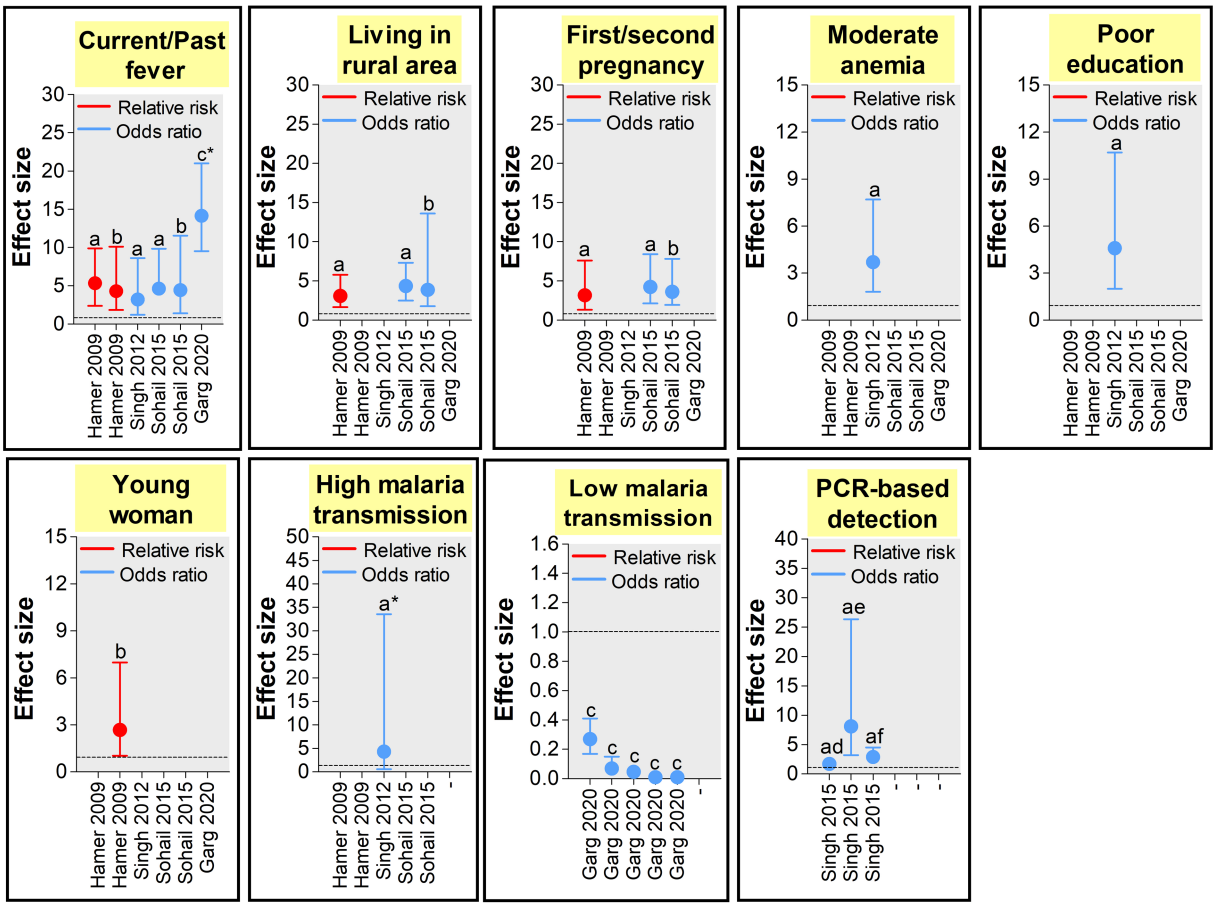

FIGURE 7
Size effects of peripheral *Plasmodium* spp. MiP determinants in India (39, 41, 46, 47, 54). Effect size was appraised as odds ratio (OR) and relative risk (RR) as reported in each study used to build the graphs. Only study that reported statistically significant effects sizes were retained. Dashed line represents a OR or RR = 1 (neutral factors). Factors with OR/RR < 1 and > 1 represent protective and risk factors of malaria infection, respectively. [a]Estimated from pregnant women attending antenatal care visits. [b]Estimated from pregnant women attending delivery units. [c]Estimated from pregnant women in community. [d]Estimated for *Plasmodium falciparum* infections. [e]Estimated for *Plasmodium vivax* infections. [f]Estimated for *Plasmodium* spp. infections. *The estimates presented in the graph are 1/10 of real estimates found in the study.

recommended in high malaria risk areas even though no formal system exist for their distribution during ANC visits (110). Complementary control strategies such as chemoprophylaxis, sensitization campaigns for behavior changes, reduction of breeding sites for mosquito vectors and early case detection and prompt treatment are also encouraged by the Government of India. Ownership and use rates of LLINs vary greatly within and between states, and even when LLINs are present in household they are not adequately used by Indian population (111, 112). A large scale household based study reported LLINs use rates of 89 and 91% among pregnant women in the Odisha state (113). Similar rate (88.6%) was reported in pregnant women in another study conducted in the same state (114). In contrast, available data from health facility-based studies suggest that ITNs/LLINs use rates are lower than those seen in community studies. The proportion of pregnant women using ITNs/LLINs most of nights is ~18.3–82.8% and that of women sleeping under ITNs/LLINs the last night is ~0.05–82.5% (39, 41, 44, 47, 50, 52, 115). Also, the utilization of untreated ITNs/LLINs by pregnant women was common in few areas (39, 46). IRS is generally less used in households by pregnant women compared to ITNs/LLINs, with use rates of ~0–58.5% (39, 41, 44, 47, 50). Taking malaria chemoprophylaxis is very uncommon in pregnant women as per the studies conducted in forested and tribal areas of Jharkhand, Chhattisgarh, Rajasthan, and Madhya Pradesh (39, 41, 44, 46, 47, 50).

3.11. Therapeutic approaches for MiP control and drug resistance in India

Prevention of MiP with IPTp-SP is not implemented in India, and control of the disease in pregnancy relies on passive case detection during ANC visits. Until 2010, the Indian national guidelines recommended the utilization of quinine for *Pf* malaria and CQ for *Pv* malaria regardless the trimester of gestation (116). Since then, this MiP treatment policy changed with regard to Indian state, malaria species, severity of the infection and trimester of gestation. For uncomplicated *Pv* infections, CQ is administered in all trimesters. The utilization of primaquine (PQ) for radical cure of *Pv* infection is not recommended to pregnant women and her fetus. The treatment of uncomplicated *Pf* MiP is CQ for first trimester women while ACTs artemether + lumefantrine (AL) or artesunate + sulfadoxine/pyrimethamine (AS + SP) are used for

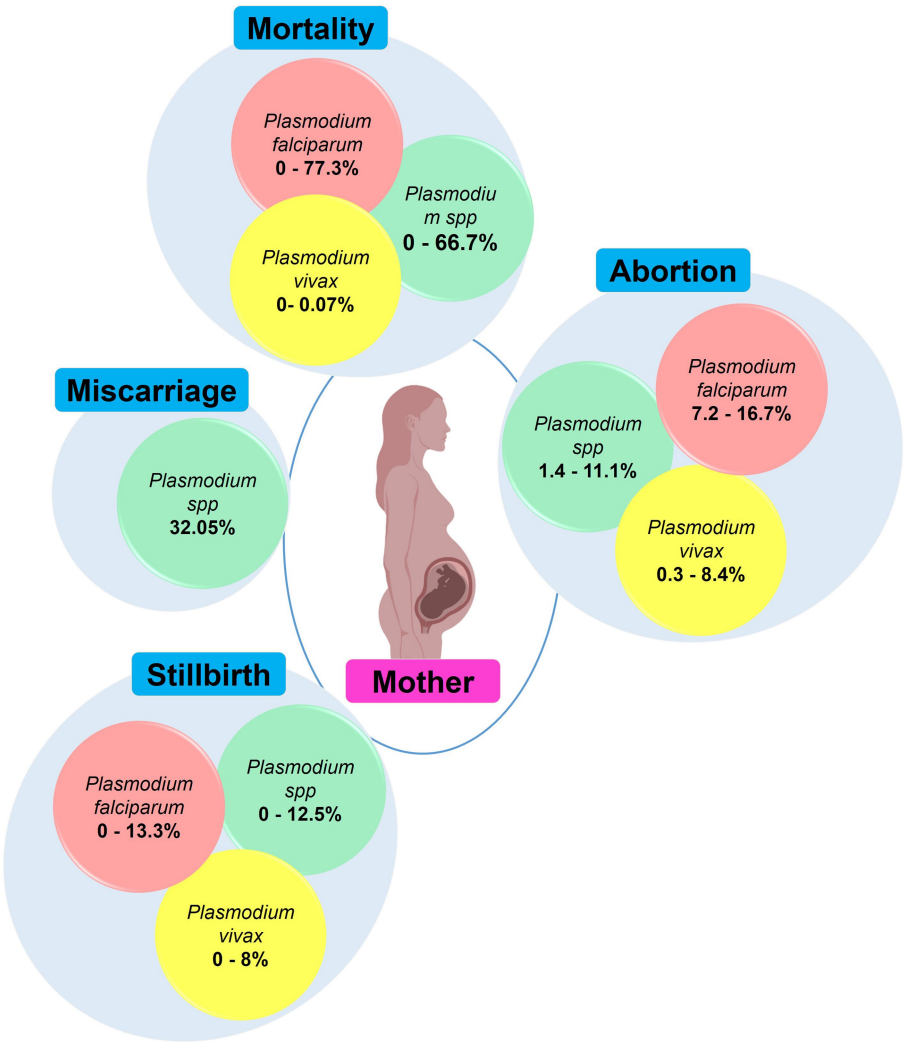

FIGURE 8
Main maternal outcomes during malaria in pregnancy. The values in round shapes are proportions of each maternal outcome among pregnant women infected with *Plasmodium* spp. (red), *P. falciparum* (red), and *P. vivax* (yellow). The values represent range (minimum and maximum values) for each maternal outcome as reported in each study included in this review. In *Plasmodium* spp., the malarial species was not specified during association analysis with maternal outcomes in the included studies. Created with Biorender.com.

second and third trimesters. Due to high level of SP resistance in Northeastern states, AL is recommended for treatment while AS+SP is used in other states. Parenteral quinine, artesunate, or artemether are given for SM (6, 116, 117).

Drug efficacy studies are limited in MiP in the country, but existing data outline both high efficacy and safety of AS+SP and AS+MEF (118). CQ and SP resistance are both well established, and studies reported low frequency *Pfk13* mutations associated with artemisinin resistance (i.e., 446I, 539T, and 561H) in Arunachal Pradesh and West Bengal (119–121). Several mutations in the *Pfk13* gene—i.e., F446I, N458Y, C469Y, M476I, Y493H, R539T, I543T, P553L, R561H, P574L, C580Y, R622I, and A675V—are strongly associated with resistance of *Pf* parasites to ACTs, the current antimalarial drugs recommended for treatment of uncomplicated *Pf* malaria (1, 119). There is a dearth of data on antimalarial drug resistance status in MiP. Using *in vitro* and *in vivo* studies, some authors found that 100 and 31.4% of *Pf* isolates collected from Madhya Pradesh and Uttar Pradesh states were resistant to CQ (34, 48). Although not purposely designed for appraising drug resistance, other studies reported adequate clinical and parasitological response of ~19.7–79% in *Pf*-infected pregnant women treated with CQ at health facilities (28, 30, 58).

3.12. Challenges and future directions on MiP in India

In this quest for achieving malaria elimination objectives, MiP should also be taken into account and a certain number of challenges ranging from prevention to treatment should be investigated in future by Indian researchers. Missing links on MiP research and proposed solutions are presented below and summarized in Figure 11.

Indian researches on epidemiology of MiP have carried out in few states such as Madhya Pradesh and Chhattisgarh, and there is a dearth of cross-sectional/longitudinal studies from other areas including

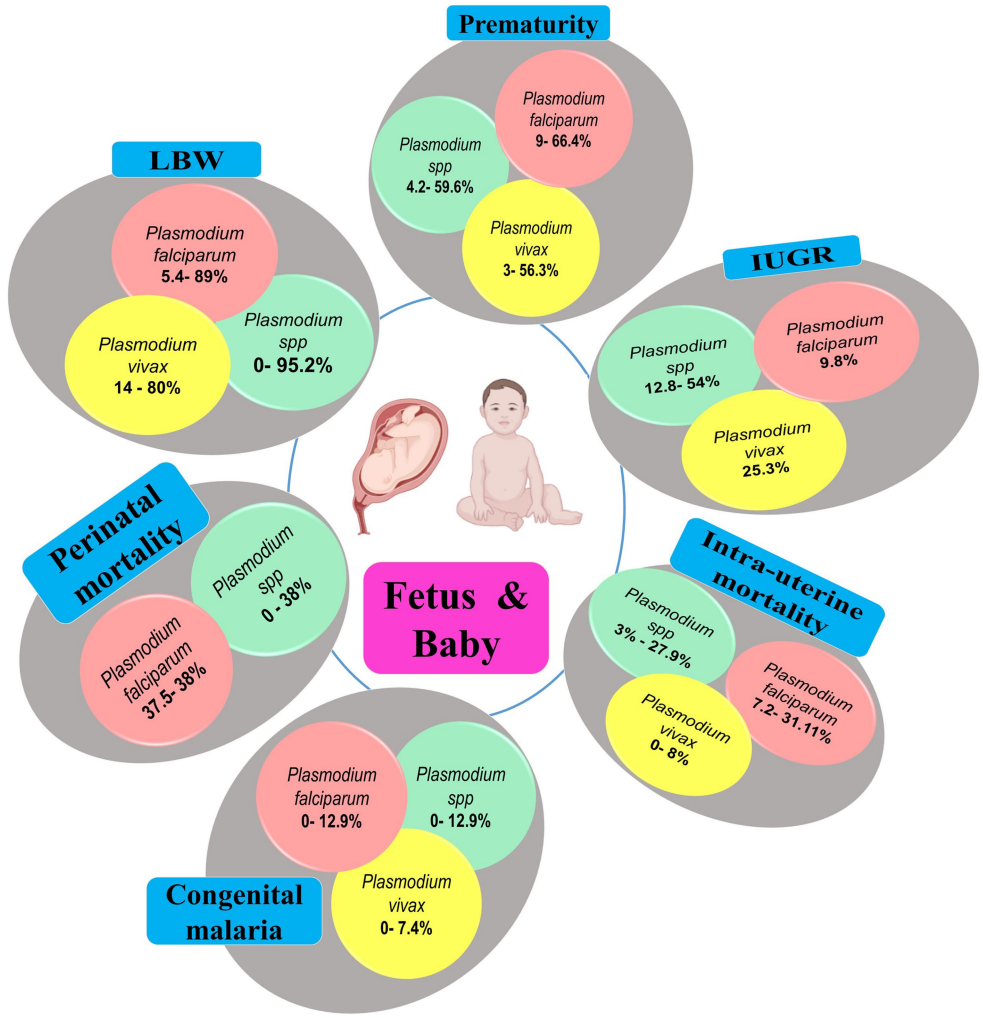

FIGURE 9
Main fetal/neonatal outcomes during MiP, India. IUGR, Intra-uterine growth retardation; LBW, Low birthweight; MiP, Malaria in pregnancy. The values in round shapes are proportions of each fetal/neonatal outcome among babies infected with *Plasmodium* spp. (red), *P. falciparum* (red), and *P. vivax* (yellow). The values represent range (minimum and maximum values) for each fetal/neonatal outcome as reported in each study included in this review. In *Plasmodium* spp., the malarial species was not specified during association analysis with fetal/neonatal outcomes in the included studies. Created with Biorender.com.

Northeastern states (e.g., Mizoram, Meghalaya, and Tripura), Bihar and Uttarakhand where malaria endemicity is still high. Also, more studies on MiP and NCM in tribal and forested settings are also greatly needed (122, 123).

Minor species including *Po* and *Pm* are also associated with MiP in India (22). LM is the standard and reports pinpointed that *Po* and *Pm* infections are misdiagnosed as *Pv* and *Pf* infections using LM in the country (124, 125). It is worth determining the extent and characterizing these species in MiP as previous studies showed their ability to elicit SM and noted pathophysiological differences between *P. ovale curtisi* and *P. ovale wallikeri* (4, 126–129).

In general, MiP studies are conducted in health facility settings either during ANC or DU periods. It would be helpful to more document MiP in community setting especially the extent of asymptomatic and/or submicroscopic parasitemia and their impact on clinical course of MiP. In addition, non-*Pf* low density infections (LDI) especially *Pv* parasitemia are problematic given efficient transmission of *Pv* LDI to *Anopheles* vectors (130, 131).

Clinical presentation of MiP in India encompasses asymptomatic, uncomplicated and severe malaria. In the latter clinical form, SMA is predominantly seen during pregnancy in India. However, other factors such as malnutrition and helminthiasis may also cause SMA, but these are rarely concomitantly addressed in MiP studies. Macronutrient undernutrition is a big contributor to severe adverse outcomes during pregnancy (132), but such studies are lacking in India.

As above discussed national guidelines for treatment of MiP are different for *Pf* and *Pv* species. However, studies report high rates of mixed infections on field that can hinder control of malaria in India (133, 134). Also, treatment of malaria in presence of comorbidities such as HIV may also be tricky and impact pregnancy outcome (e.g., drug–drug interactions). It was reported that severity and mortality are increased in patients co-infected with *Plasmodium* parasites and HIV (135, 136).

Pregnant women are also reservoir for gametocytes, the transmission development stage of *Plasmodium* parasites. Singh and

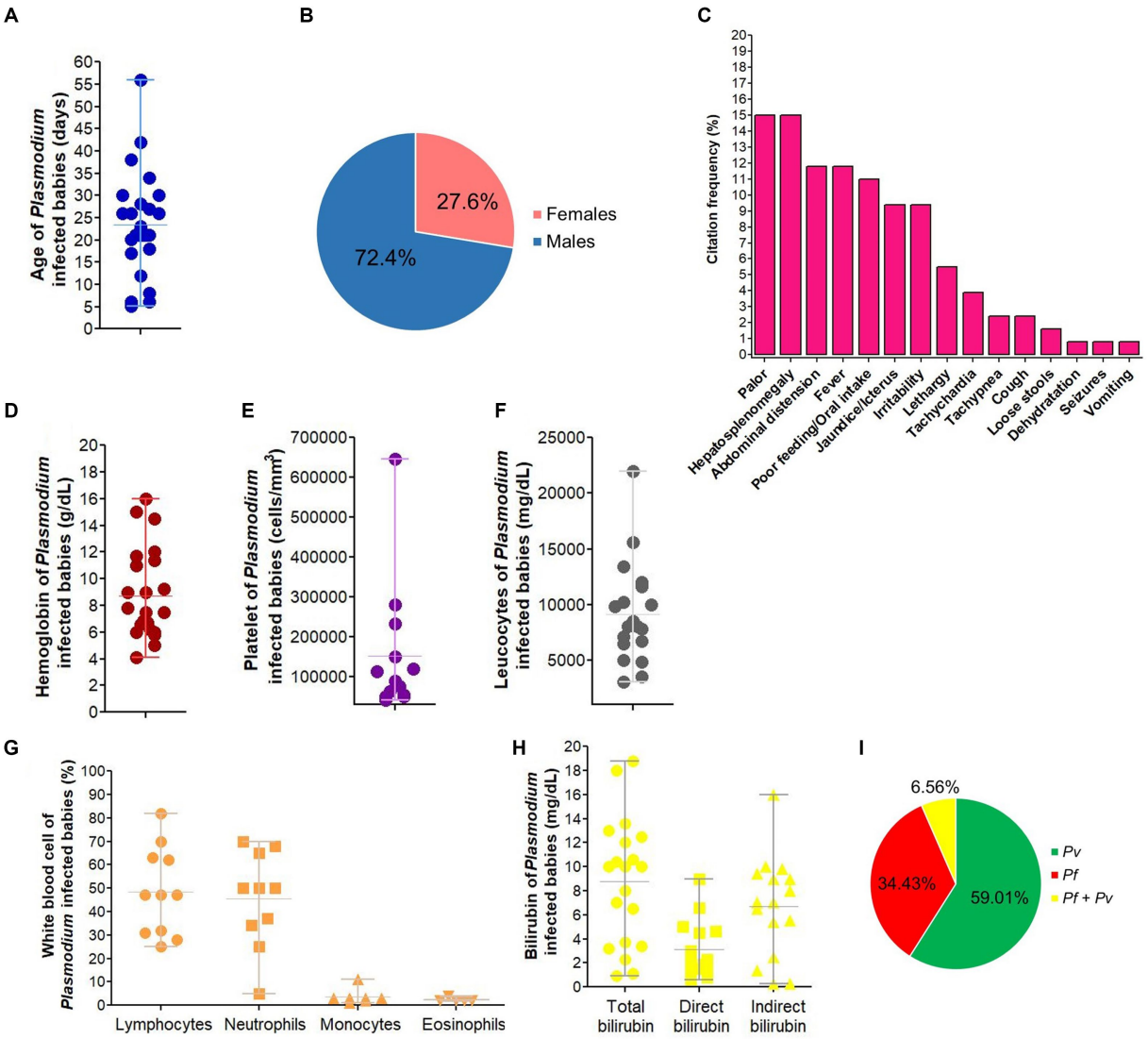

FIGURE 10
Characteristics of neonatal malaria cases in India. *Pf*, *Plasmodium falciparum*, *Pv*, *Plasmodium vivax*. The image depicts characteristics of *Plasmodium* infected babies for age **(A)**, gender-distribution **(B)**, signs/symptoms presented on admission/birth **(C)**, hemoglobin level **(D)**, platelet count **(E)**, total leucocytes **(F)**, white blood cell populations **(G)**, total, direct and indirect bilirubin level **(H)**, and malaria infection **(I)**. We excluded data from congenital malaria due to very low number of cases ($n = 3$). In panel **(C)**, percentage of each symptom on admission were computed by dividing the number of times that symptom was cited to the total number of citations for all symptoms. In panel **(I)**, malaria species were identified using LM and/or RDT.

coworkers reported gametocyte carriage rates of 54 and 73.8% in *Pf*-infected pregnant women from Madhya Pradesh (28, 30). This research area is understudied in MiP and should be investigated in future.

Plasmodium vivax parasites produce dormant stages called hypnozoites and responsible for malaria relapses, which are associated with transmittable gametocytaemia and delayed mortality (137, 138). PQ is currently recommended for preventing *Pv* relapses but its utilization is associated with risk of severe hemolysis in persons diagnosed with glucose-6-phosphate dehydrogenase (G6PD) deficiency (139). In India, G6PD testing is rarely performed at health facilities coupled with poor adherence to PQ-based 14-day regimen treatment and high prevalence of G6PD deficiency (e.g., Odisha) (5, 140, 141). This limits researches on evaluation of real burden of *Pv* relapses, effectiveness, and development of new hypnozoiticidal drugs.

Immunity against *Pv* is more rapidly acquired than that against *Pf*, that results in high proportion of *Pv* asymptomatic infections (142–144), which are often associated with high carriage of hypnozoites and undetected by LM and RDT (142, 144–146). Development of point-of-care tests based on biomarkers could be promising approach. We recently proposed a simple theoretical framework for identifying, evaluating and validating diagnostic, therapeutic prognostic and predictive biomarkers for malaria, and these could be translated to MiP (147). Longley et al. (148) proposed an interesting approach based on serological markers to detect recent *Pv* infection.

Another cause of lower sensitivity of RDTs in pregnant women could be likely deletions in the histidine rich protein 2 gene (*pfhrp2*)

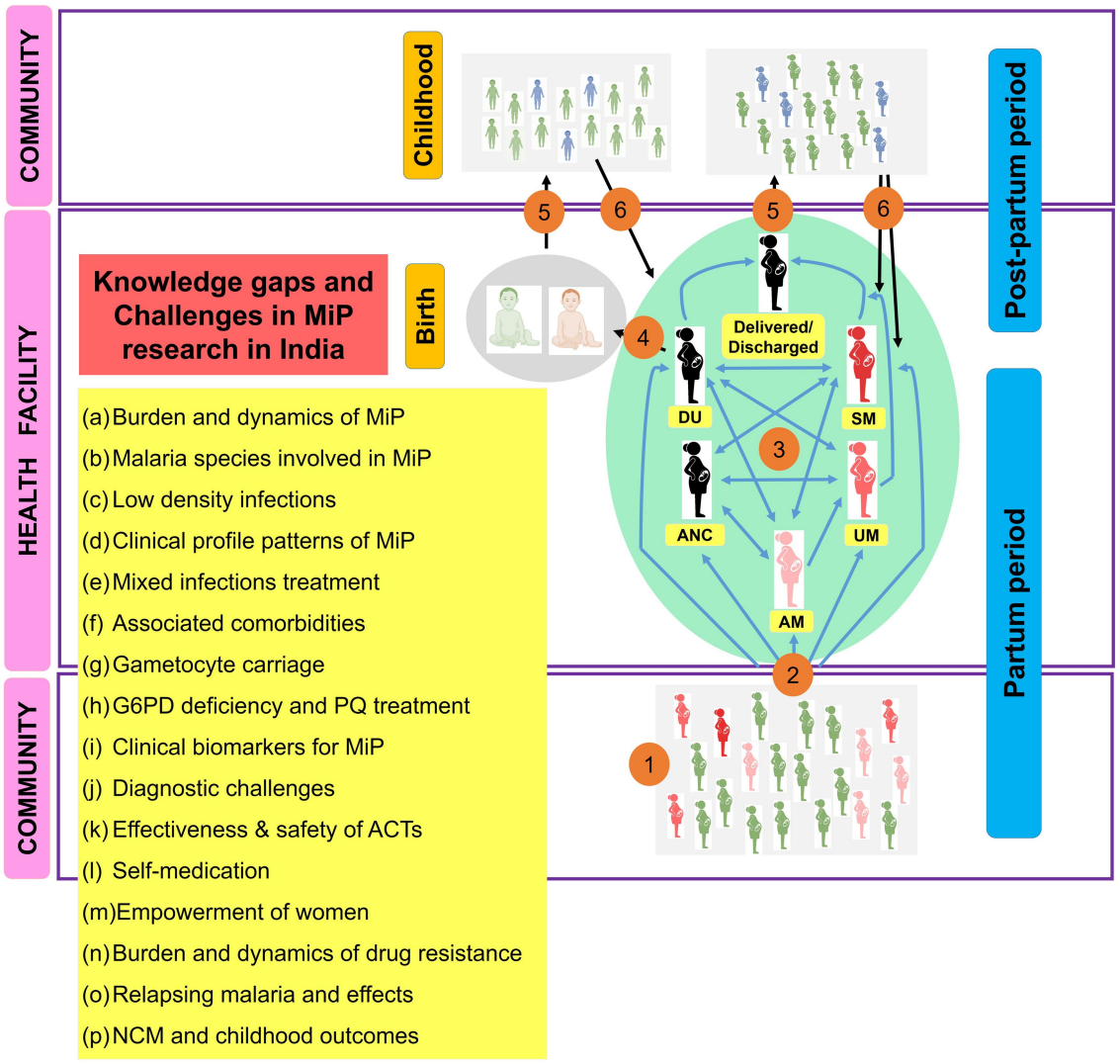

FIGURE 11
Main research gaps and challenges to be addressed on MiP in India. ACTs, Artemisinin based combination therapies; AM, Asymptomatic malaria; ANC, Antenatal care; DU, Delivery unit; G6PD, Glucos-6-phosphate dehydrogenase; MiP, Malaria in pregnancy; NCM, Neonatal and congenital malaria; PQ, Primaquine; UM, Uncomplicated malaria; SM, Severe malaria. The image presents the main knowledge gaps and challenges on MiP research in India. (1) At community, pregnant women are exposed to infecting *Anopheles* bites and some of them will be infected (red). The plasmodial infection may exist as asymptomatic and/or symptomatic. (2) Both asymptomatic and symptomatic (UM and SM), women can attend health facilities for diverse reasons (e.g., ANC, DU, and care seeking). (3) Women attending ANC/DU can present symptoms or not, and will be managed as per national health guidelines if malaria infection is diagnosed. (4) Women came for delivery can give birth to uninfected babies (gray) or babies diagnosed with congenital malaria (red), and these will be treated as per national guidelines. (5) Women and babies discharged after successful antimalarial treatment will go back home. (6) Women and babies are treated with CQ if *Pv* infection was diagnosed, but using PQ is prohibited for them. Thus, some of them (blue) can develop recurrent malaria episodes during post-partum period and childhood due to reactivation of hypnozoites. During this itinerary for women and babies, several challenges and knowledge gaps (yellow box) have been identified and should be taken into account in future researches in India.

which encodes a *Pf*-specific protein antigen (149). No studies have evaluated *pfhrp2* deletions in pregnant women in India (150, 151), and it would be interesting to appraise the prevalence profile of such deletions at ANC, DU, and community settings.

No studies on SP and artemisinin resistance in MiP in India have been carried out. Also, data on incidence of CQ resistance in MiP, which are probably may parallel CQ resistance in normal *Pf* infections occurring in the same region, are still needed. It is required to conduct more studies especially longitudinal studies to detect temporal variation of the drug resistance gene profile in pregnancy and its association with maternal outcomes.

Even though ACTs are recommended by National guidelines for treating MiP, there is a lack of studies on their effectiveness and safety in India (118). Systematic reviews and meta-analyses of prospective studies and clinical trials conducted in African and Asian settings have showed the efficacy and safety of ACTs during MiP (152, 153). In this context, further investigations are required to determine pharmacological aspects, effectiveness and inocuity profile of ACTs in MiP all around the country.

Self-medication both with traditional medicines and antimalarial drugs should also be addressed in pregnancy. Few studies reported pregnant woman were self-medicating, and this expose them to

adverse effects of drugs, often of poor quality as reported in several endemic sSA and SEA areas including India (154, 155).

Some studies reported poor awareness of pregnant women toward malaria, its treatment and prevention due to several causes including social barriers. Thus, there is need to empower pregnant and childbearing women toward malaria and preventive methods and this could be achieved through sensitization during community campaigns and ANC visits.

4. Conclusion

Malaria in pregnancy is still a serious public health concern in India. Its epidemiological burden is high in Indian pregnant women, with *Pv* and *Pf* as main causative agents while minor species (*Pm*, *Po*) are also involved. The epidemiology of these species in MiP is greatly varied with important role of mother's demographic and obstetrical characteristics, geography, and ecoclimatic features of the area. *Plasmodium* infections in pregnant women, often associated with comorbidities and concurrent infections, may progress from asymptomatic carriage of parasites to SM, which is mostly represented by SMA, CM, and hypoglycemia, and more frequently seen in *Pf* infections. MiP has deleterious effects on mother and her child that can often end with death. This review provided a comprehensive overview on epidemiological situation and identified important missing links in MiP and NCM to inform population, clinicians, and researchers. There is urgent need for further studies on the different above mentioned points addressed in the present review. If adequately addressed, the future findings could be greatly helpful for efficiently controlling MiP in India through development, implementation, and scale up of control strategies and policy makers, and thus achieve malaria control and elimination objectives in the country.

Author contributions

LPKF and VS conceptualized the paper. LPKF conducted literature review and extracted and analyzed data from papers, conceived the figures and maps, performed extraction and analysis data, and drafted the first version of the final manuscript. VS revised the manuscript for important intellectual content and supervised the work at all stages. All authors contributed to the article and approved the submitted version.

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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/fpubh.2023.1150466/full#supplementary-material

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Glossary

ACT	Artemisinin-based combination therapy
AL	Artemether + Lumefantrine
AM	Asymptomatic malaria
API	Annual parasite incidence
ARDS	Acute respiratory distress syndrome
ARF	Acute renal failure
AS	Artesunate
CM	Cerebral malaria
COVID-19	Coronavirus disease 2019
CQ	Chloroquine
DIC	Disseminated intravascular coagulation
G6PD	Glucose-6-phosphate dehydrogenase
HELLP	Hemolysis, elevated liver enzymes, low platelet count
HIV	Human immunodeficiency virus
IFA	Indirect fluorescence antibody
IL	Interleukins
IPTp	Intermittent preventive treatment during pregnancy
IRS	Indoor residual spraying
ISTp	Intermittent screening and treatment during pregnancy
ITN	Insecticide-treated net
IUGR	Intra-uterine growth retardation
LBW	Low birthweight
LDI	Low density infection
LLIN	Long lasting insecticide-treated net
LM	Light microscopy
MEF	Mefloquine
MiP	Malaria in pregnancy
n.a	Not applicable
NCM	Neonatal and congenital malaria
NVBDCP	National Vector Borne Disease Control Program
OR	Odds ratio
PCR	Polymerase chain reaction
Pf	*Plasmodium falciparum*
Pk	*Plasmodium knowlesi*
Pm	*Plasmodium* malariae
Po	*Plasmodium* ovale
Pv	*Plasmodium vivax*
PCD	Passive case detection
pfhrp2	*Plasmodium falciparum* histidine rich protein 2 gene
QBC	Quantitative buffy coat
RBC	Red blood cell
RDT	Rapid diagnostic test
RR	Relative risk
SARS-CoV-2	Severe acute respiratory syndrome Coronavirus 2
SEA	South East Asia
SM	Severe malaria
SMA	Severe malarial anemia
SP	Sulfadoxine + Pyrimethamine
sSA	sub-Saharan Africa
TNF	Tumor necrosis factor
UM	Uncomplicated malaria
WHO	World Health Organization

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Risky business: human-related data is lacking from Lyme disease risk models

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Used as a communicative tool for risk management, risk maps provide a service to the public, conveying information that can raise risk awareness and encourage mitigation. Several studies have utilized risk maps to determine risks associated with the distribution of *Borrelia burgdorferi*, the causal agent of Lyme disease in North America and Europe, as this zoonotic disease can lead to severe symptoms. This literature review focused on the use of risk maps to model distributions of *B. burgdorferi* and its vector, the blacklegged tick (*Ixodes scapularis*), in North America to compare variables used to predict these spatial models. Data were compiled from the existing literature to determine which ecological, environmental, and anthropic (i.e., human focused) variables past research has considered influential to the risk level for Lyme disease. The frequency of these variables was examined and analyzed via a non-metric multidimensional scaling analysis to compare different map elements that may categorize the risk models performed. Environmental variables were found to be the most frequently used in risk spatial models, particularly temperature. It was found that there was a significantly dissimilar distribution of variables used within map elements across studies: Map Type, Map Distributions, and Map Scale. Within these map elements, few anthropic variables were considered, particularly in studies that modeled future risk, despite the objective of these models directly or indirectly focusing on public health intervention. Without including human-related factors considering these variables within risk map models, it is difficult to determine how reliable these risk maps truly are. Future researchers may be persuaded to improve disease risk models by taking this into consideration.

KEYWORDS

blacklegged ticks, data synthesis, human-related, Lyme disease, risk assessment, risk map

Introduction

Tick borne diseases are caused by pathogens transmitted by infected ticks to an uninfected host. As the climate warms, it becomes possible for ticks to have increased abundance, survival, and feeding activity, and to expand their geographic distribution northwards (1, 2). Human activities and their impacts on natural habitats are further altering the distribution of these disease vectors, and human-wildlife contacts are increasing through means of socio-demographics (globalization, urbanization, etc...) and public health systems [vector control and other health interventions (3)]. Due to the constant changes in the environment, it is challenging to assess the risks associated with these infections without predictive modeling. Many current and future scenario predictive risk maps have been developed to monitor zoonotic infections for public health interventions (4–7). Epidemiologic risk maps are effective visualization tools used to identify

geographical areas of high risk for disease transmission and potential future high-risk regions. Used as a communicative tool for risk management, these maps provide a service to the public, conveying information that can raise risk awareness and encourage mitigation strategies (8–10). Since climate and land use changes are constantly altering the dynamics between vector and host, continuous monitoring of the emergence and expansion of the disease vector is required. This is true of the spread of Lyme disease and other tick-borne diseases (2, 3).

In eastern North America, Lyme disease is typically caused by an infection of the spirochaete *Borrelia burgdorferi* via blacklegged ticks [*Ixodes scapularis* (11)]. In humans, the infection can result in a multisystem illness that substantially affects the individual's quality of life, if left untreated (12, 13). As a common and widespread disease (14–16) that can lead to severe conditions (17), effective communication tools for risk management of this infection are necessary.

Risk maps for Lyme disease in North America tend to focus on the impact climate change has on the distribution of blacklegged ticks (18–22), since these ectoparasites are vectors for *B. burgdorferi* (23) and their geographic range is increasing (1, 24). The geographic range of blacklegged ticks is heavily dependent on environmental variables such as temperature and precipitation (25–28). The geographical ranges of their hosts also play a major role (25). Blacklegged ticks require a single host for each life stage (29, 30), and will migrate with these hosts, such as small mammals, birds, and ruminants (31, 32).

Different variables have been considered to affect Lyme disease distribution. These include ecological variables related to blacklegged ticks [tick density, dispersion (33–35)] and their small mammal reservoir and migratory avian hosts (36, 37), as well as environmental variables such as temperature, humidity, and forest fragmentation (20, 38, 39). Recent risk map publications have adopted "One Health" approaches, which incorporate sociological, ecological, and biological knowledge into their research (40, 41). This approach aims to examine and integrate human-related or anthropic variables that may influence human health or risk (22, 42) including those variables beyond human demographics.

For instance, one may expect that human exposure and the risk of becoming infected by Lyme disease is also dependent on individual human behavior (e.g., knowledge, activity). As such, outdoor workers have been found to be more at risk for zoonotic diseases than those who are outdoors recreationally, due to their degree of exposure to the environment (43), and those with immune deficiencies may be more at risk for severe symptoms (44). Studies have included surveys of a population to gauge their knowledge on their risks to Lyme disease or tick infections (22, 42, 45, 46). Socio-economic status and ethnicity have also been found to play a role in Lyme disease risk (42, 47, 48). Knowing that not all individuals are equally at risk for being infected with diseases, including Lyme disease, it should be expected that studies in which Lyme disease risk maps are developed would include variables associated with human characteristics and behaviors. For these studies to be relevant to public health, variables associated with humans (i.e., social, economic, risk perception) should be taken into consideration, as these factors directly affect the risk posed to the public.

Here, we reviewed the literature to identify the variables past research has considered influential to the distribution of Lyme disease via blacklegged ticks in North America. Variables that researchers routinely included in risk models were examined, and those human variables that were often disregarded but may be informative were highlighted. By calling attention to the lack of human variables found in previous risk maps, future researchers may be persuaded to enhance models by including anthropic factors to improve disease risk prediction.

Methods

Collection of data

We focused on past studies that are comparable due to similarities in geography [same continent, overlapping tick populations (49, 50)], disease vector, and spirochaete strain (*B. burgdorferi*). For this reason, we focused on one tick vector (black-legged tick) which is endemic to eastern North America (17). On March 31, 2023, a descriptive literature review was conducted following methods by Paré and Kitsiou (51) using Google Scholar, PubMed, and CrossRef with the inclusion criteria terms: "*Ixodes scapularis*," "blacklegged ticks," "risk map" (exact phrase), "Lyme disease," "risk assessment," and "*B. burgdorferi*." This literature review concentrated on eastern North American (across Canada, the United States, and Mexico) risk assessments of Lyme disease transmitted by blacklegged ticks only. Geographical scale varied across studies, with some focusing on areas at the municipality, provincial/state, regional, or national scale. However, it should be noted that there are several risk assessments for Lyme disease concentrated in Europe and western North America where other tick species and spirochaete vectors can transmit Lyme disease (17, 20, 52). Only studies in which analyses included at least one risk map in their results was considered for this review, as we were specifically interested in comparing studies that produced risk map models to evaluate risk. We performed a systematic review following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (53). The initial search consisted of 145 studies published between 2000 and 2022 with the above criteria. Twenty studies were excluded, as they did not include a risk map in their results. Twelve more studies were excluded since their results were based outside of North America, another two studies were excluded as they focused on different vectors or bacteria and diseases and one article was removed from the literature review as it was retired and no longer considered relevant. Finally, 49 government reports, reviews, and theses were removed, leaving a total of 61 peer-reviewed articles meeting our criteria to be included in analyses (Figure 1; Supplementary Table 1).

Once the literature review was completed, data on the models within the articles were collected. This included collecting information on the number of ecological, environmental, and anthropic variables used in the studies to develop risk map models. Frequency counts for the number of each ecological, environmental, and anthropic variable in a risk map model were recorded. Ecological variables included variables that influence or dictate the relationship between an organism and its environment

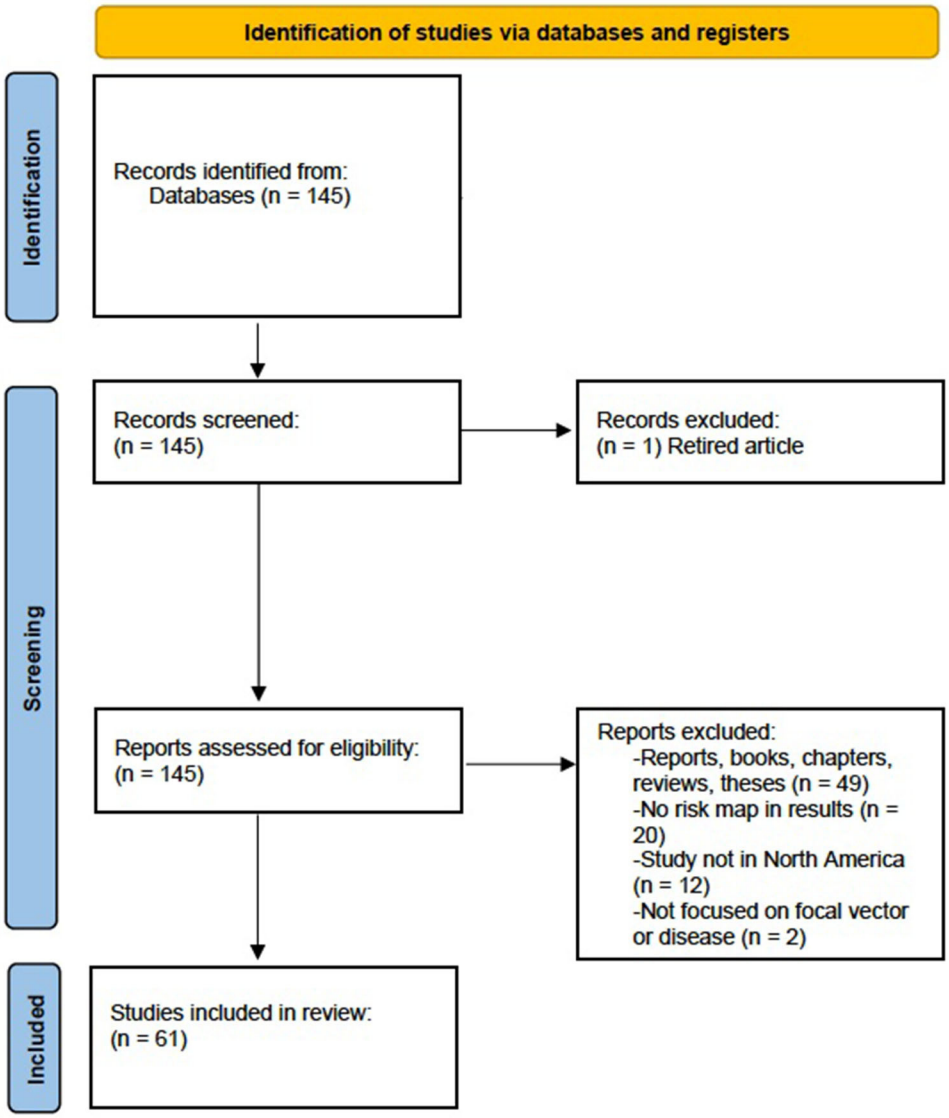

FIGURE 1
Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flow diagram for systematic review which included searches of databases for risk maps related to Lyme disease and blacklegged tick (*Ixodes scapularis*) distributions.

(i.e., tick occurrence, *B. burgdorferi* prevalence). Environmental variables included natural resource factors that define an ecosystem or habitat (i.e., temperature, humidity, land cover). Anthropic variables were defined as variables related to human beings (i.e., population density, sex, age). As Lyme disease is primarily transmitted via tick vectors (11), these observations do not provide any information on how the disease circulates within a human population. Further, certain predictor variables were simplified to allow comparisons more easily. For example, forest cover and vegetation index were categorized together, as were vapor pressure and humidity, elevation and altitude, human population size and density, and tick abundance and density (Supplementary Table 2).

Additional elements that characterized these risk models were collected and recorded, including the year of publication (Supplementary Table 3), the study's focal location (Country), Map Type (predictive vs. surveillance), the period of the study (year), the focal Scale of study (local, regional, national), the distribution of the study considered– vector (tick) vs. host (human or otherwise) vs. vector and host (both considered)—the Tick Surveillance methodology used for the model (passive vs. active vs. no tick surveillance), and the Tick Life Stage the tick data was based on (immature ticks vs. adult vs. all stages vs. no tick data; Table 1; Supplementary Table 4). Map Type (predictive vs. surveillance) was also included, whereby "predictive" maps referred to future predictive map models, as they predict future scenarios, while current predictive maps will be referred to as "surveillance" models, as they pertain to current risks.

Most risk map models are predictive as they use modeling to create these maps. However, some models are used to predict risks associated with potential future geographical ranges and

TABLE 1 The cumulative number of anthropic, environmental, and ecological variables used across map elements in the reviewed studies.

Map element		Anthropic	Environmental	Ecological
Map Type	Surveillance (38)	17	40	52
	Predictive (25)	3	62	30
Map distribution	Host (16)	14	24	19
	Vector (33)	5	62	38
	Host and vector (15)	2	16	26
Map scale	Local (38)	14	58	54
	Regional (17)	6	30	18
	National (9)	0	14	10
Tick life stage	Adult (3)	1	3	4
	Immature (12)	1	30	16
	All stages (32)	10	51	49
	No tick data (16)	8	18	13
Tick surveillance	Passive (18)	4	23	23
	Active (19)	5	27	26
	Active and passive (10)	0	15	16
	No surveillance data (18)	14	22	10
Country	Canada (27)	12	41	34
	USA (31)	5	55	45
	USA and Canada (3)	2	4	1
	USA and Mexico (1)	1	0	1
	USA, Canada, and Mexico (1)	0	2	1

The total number of variables for each variable group is the sum of those variables used across studies (n = 61). Numbers in brackets indicate the number of studies for that specific map element. USA, United States of America.

distributions of Lyme disease (18, 38, 39, 54, 55), while others are used to predict the current risk or prevalence (56–59). In addition, Map Scale was considered and categorized by whether the study focused their spatial scale by country (national), province or state (regional), or a smaller unit (e.g., census division; local).

Synthesis of data

A non-metric multidimensional scaling (NMDS) analysis was performed in R [(60); version 1.4.1717] using the "*vegan*" package (version 2.6-2) to determine any significant differences in the frequency of ecological, environmental, and anthropic variables used across studies ($n = 61$), depending on their map elements: Map Type, Map Scale, Map Distribution, Year, Tick Life Stage, Map Surveillance method, and Country. Here, the NMDS, which is commonly used as an ordination for community ecology (61), was performed where "sites" were the individual studies, and "environmental data" were the map elements that influenced the abundance of "species" (ecological, environmental, and anthropic variables).

Linear models were then used to determine if groups of variables (ecological, environmental, and anthropic) differed in frequency across the different map elements identified by the NMDS. A binomial linear regression was conducted with Map Type as the response variable (surveillance vs. predictive) and the number of ecological, environmental, and anthropic variables used in the studies as predictors. An ordinal linear regression with the "logit" function and "equidistant" threshold was conducted (62) with Map Distribution (as a factor) as the response variable (host vs. vector. vs. host and vector), using the package "*ordinal*" [version 2019.10 (63)]. *Post-hoc* tests were conducted, and box plots were used to visualize variation within those map elements that were identified as significant by the NMDS.

Results

Comparatively, certain map elements were more frequently used across the studies assessed in this literature review. For Map Type, there were more predictive maps than surveillance. For Map Distribution, there were more vector (blacklegged tick) distributions considered than human or both human and tick distributions. Maps at the local scale were most common across studies. All stages for Tick Life Stage were most frequently considered vs. specific life stages. Both active and passive data for Tick Surveillance was most frequently included in risk map models and most studies in this assessment were based in the United States (Table 1). The most common variable included in

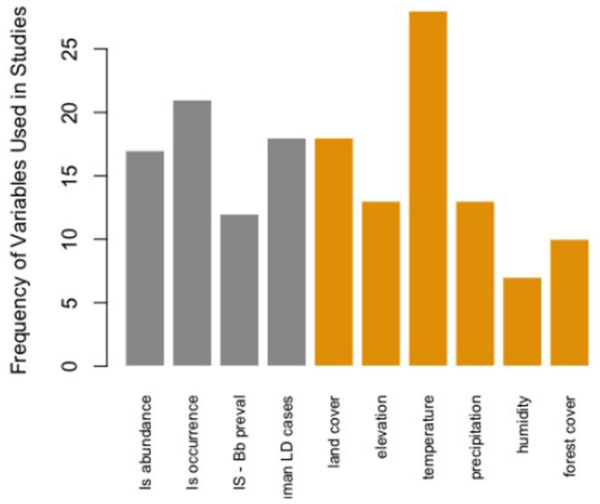

FIGURE 2
Frequency of the top 10 variables used within the 61 Lyme disease risk map models in North American studies published between 2000 and 2022. Is abundance, *Ixodes scapularis* abundance; Is occurrence, *Ixodes scapularis* presence/absence; Is—Bb preval = *Borrelia burgdorferi* prevalence in *Ixodes scapularis*; LD, Lyme disease.

TABLE 2 **Non-metric multidimensional scaling (NMDS) goodness of fit results of map elements classifying studies based on the frequency of ecological, environmental, and anthropic variables incorporated in each risk map model ($n = 61$).**

Variables	r^2	Pr (>r)
Year of publication	0.0421	0.289
Map Type	0.2268	**0.001**
Distribution	0.1221	**0.012**
Scale	0.0350	0.372
Tick life stage	0.0415	0.811
Tick surveillance	0.0352	0.653
Country	0.0468	0.730

Variables include Year of publication, Map Type, Map Distribution, Tick Life Stage, Country. Number of permutations = 999. Statistically significant results are in bold.

Lyme disease or blacklegged tick risk maps was temperature ($n =$ 28 studies). Of the 10 most frequently used variables in these map models, six were environmental variables, four were ecological, and none were anthropic (Figure 2). The results of the NMDS analysis suggested that there were differences in the frequency of ecological, environmental, and anthropic variables used across Map Type and Map Distribution (Table 2; Figure 3; Supplementary Table 5).

When comparing Map Types (surveillance vs. predictive), the difference in use of environmental variables was statistically significant ($p < 0.0173$), with predictive maps using more of these types of variables. For both types of risk maps, anthropic variables were rarely used (Table 3; Figure 4A). Comparing across Map Distributions, studies that included host distributions in risk models considered more anthropic variables than studies that included vector distributions ($p < 0.0152$). Meanwhile studies that included both host and vector distributions tended to include more ecological variables ($p < 0.0291$; Table 4; Figure 4B). There was no significant difference across Year of publication, Surveillance Type used, Map Scale, Country of study origin, or Tick Life Stage focused on in studies (Table 2).

Discussion

Although there is a plethora of literature dedicated to identifying factors that can influence an individual's risk for Lyme disease via tick vectors directly and indirectly (48, 64, 65), risk maps that demonstrate the spatial breadth of these risks are less common. The results of this review have shown that within this subset of risk maps, there is no standardized risk score, or variable being used across studies. Temperature was the most common variable used in risk map models, however, it was included in less than half of the risk maps considered. There is extensive research on the relationship between blacklegged ticks and temperature, as it affects a tick's development, survival, and host-seeking behavior (19, 66, 67). These are significant factors that influence tick abundance and distribution, and therefore influence the distribution and incidence of Lyme disease (19, 68). In general, the most common variables included in Lyme disease (or blacklegged tick) risk map models were environmental and ecological, while anthropic were lacking. It should be noted that certain studies, such as Slatculescu et al. (69) considered many anthropic variables, including population density, walkability scores in an urban setting, median income, and drew conclusions about the contribution of an individual's variability on their Lyme disease risk, however, they did not express these results spatially in a risk map model. Similarly, several other ecological variables often studied and considered influential to Lyme disease and/or blacklegged tick distributions were rarely included in these risk map models. For instance, blacklegged tick distributions are affected by reservoir and migratory host distributions (36, 70) while Lyme disease distributions can be influenced by the genetic diversity of *B. burgdorferi* strains (49, 71). Without considering these variables within risk map models, it is difficult to determine how reliable these risk maps truly are.

Depending on the Map Type and Map Distribution used, studies significantly differed in the number of ecological, environmental, and anthropic variables used to produce risk maps. Studies that utilized different Map Types also significantly varied in their usage of environmental variables, where studies that produced predictive maps used this group of variables more often. Interestingly, predictive map studies also used anthropic variables less often. This suggests that when research focuses on future scenarios, they are reliant on how the environment may change, but do not consider human behavior. Although it is difficult to assess future trends in human behavior, it is still possible to include anthropic variables such as socio-economic status and demographic information (22) to gain a better understanding of the patterns in human attributes that could influence their risk for Lyme disease.

Across Map Distribution types, studies that included host distributions in their risk maps had more anthropic variables than other distributions (vector only or host and vector distributions). Risk maps that included vector only or both host and vector

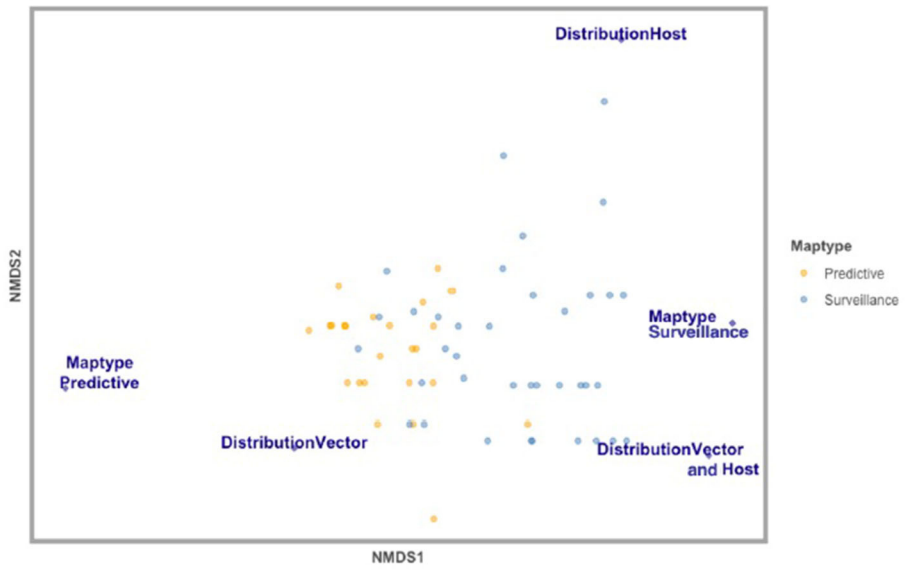

FIGURE 3
Non-metric multidimensional scaling (NMDS) analysis of map elements classifying studies based on the number of ecological, environmental, and anthropic variables incorporated in each study included in this review ($n = 61$). Variables displayed are significantly distinct variables across studies—Map Type (surveillance vs. predictive), Map Distribution (vector vs. host vs. vector and host), and Map Scale (local, regional, national). Studies that developed Predictive risk maps are in yellow, and studies that developed Surveillance risk maps are in blue.

TABLE 3 General linear model results for Map Type ~ anthropic + environmental + ecological, where family = "binomial" and the independent variables are counts (the number of variables in each category).

Variable type	Estimate	Standard error	*z* value	Pr (>|z|)
(Intercept)	0.5878	0.8051	0.730	0.4653
Anthropic	0.7311	0.6579	1.111	0.2665
Environmental	−0.6633	0.2129	−3.115	**0.0018**
Ecological	0.6571	0.5070	1.296	0.1950

Null deviance = 81.772 on 60 degrees of freedom, residual deviance = 64.676 on 57 degrees of freedom. AIC: 72.676, n = 61. Statistically significant results are in bold.

distributions tended to include more ecological variables. As most host distributions in these risk map models were human distributions, it is logical for human attributed variables to be included in these models. Meanwhile, research that incorporated both host and vector distributions, or only vector distributions may be more likely to include variables from the environment, as they are less directly focused on Lyme disease risk for humans specifically. Rather, they use the result of their risk map model that included disease vector distributions to indirectly make conclusions for public health risks (18, 19, 42).

This review was limited to research that included risk maps as results within a study, excluding studies that included maps within introductions and methods for context. In some cases, a single variable (e.g., tick distribution) was used as a proxy for Lyme disease distribution and correlated with environmental or human-related data [e.g., forest cover, urban development; (36, 72)]. Other studies considered several variables, but these distribution models were not applied to develop risk maps (38, 73). It should also be noted that despite only 20 incidences of human-related variables being included in these studies' models overall (Table 5), more recent research has begun to consider general public influence within their studies by including citizen science or "Google trends" to determine blacklegged tick and Lyme disease distributions (37, 89, 90). Other human demographic data can be acquired through public government agencies such as population densities and household incomes (69, 74–76). More personal and individualistic information can be acquired via questionnaire surveys (22, 42, 45, 46).

There is a clear understanding that environmental factors heavily influence the distribution of diseases and their vectors (21, 52, 77, 78). However, very few studies consider that human related factors may also influence these distributions (22, 69). Further, human population growth, urbanization, and travel can affect vector-borne distributions (3), particularly when human movement or land development influences animal movement, effectively altering the host dynamics of tick vectors (79, 80). This is concerning, as the risks related to Lyme disease directly affect human health, and human behavior can affect infection rates (46, 69, 81, 82). Predictive Map Type risk maps especially ignore how human-related factors may influence Lyme disease risks for humans, and this may be due to studies focusing on *B. burgdorferi* distribution rather than infection rates.

Overall, it was found that risk maps that focused on Lyme disease and blacklegged tick distributions differed across the types of variables used according to the study goal and intended use of the map. Because of these inconsistencies, it is difficult

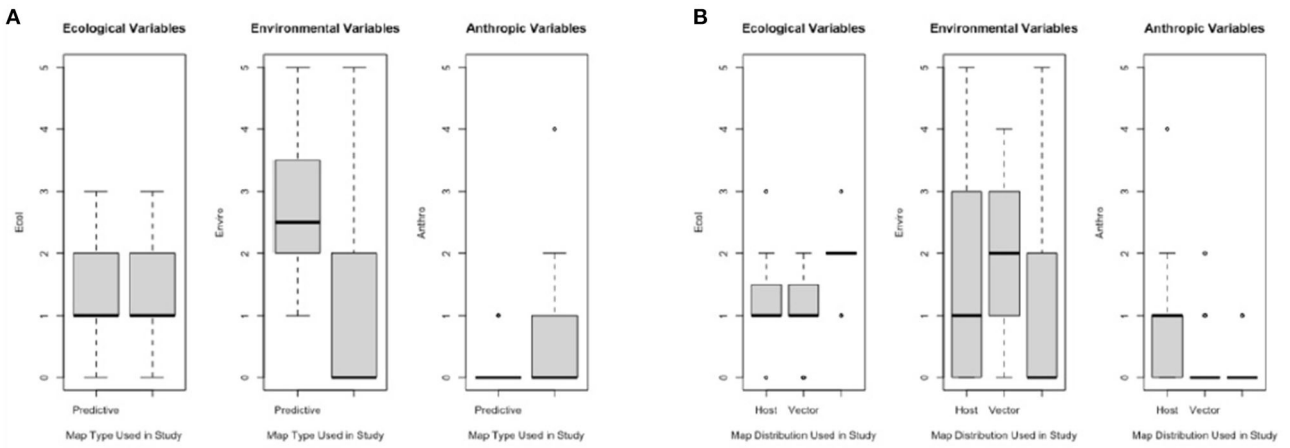

FIGURE 4
Frequency of ecological, environmental, and anthropic variables used across studies ($n = 61$) for varying map elements: **(A)** Map Types—predictive (future scenario; $n = 24$) and surveillance (current distribution; $n = 37$), and **(B)** Map Distributions—host ($n = 15$), vector ($n = 15$), vector ($n = 32$), or both vector and host (V & H; $n = 14$).

TABLE 4 Cumulative link model results for map distribution ~ anthropic + environmental + ecological, where link = "logit," threshold = "equidistant" and the independent variables are counts (the number of variables in each category).

Variable type	Estimate	Standard error	*z* value	Pr (>|z|)
Anthropic	−1.7432	0.6016	−2.897	**0.00376**
Environmental	−0.3410	0.1790	−1.905	0.05681
Ecological	1.0577	0.4504	2.348	**0.01887**
Threshold-coefficients				
Threshold	−1.1201	0.7432	−1.507	–
Spacing	2.9951	0.4817	5.657	–

logLik = −51.82, AIC = 113.64, n = 61. Statistically significant results are in bold.

to compare and validate these models to accurately forecast Lyme disease risks geographically or temporally. At the same time, geospatial data related to anthropic factors can be difficult to acquire. Our results bring attention to the fact that there is no consistent "risk" variable or assessment across studies, likely because these studies tend to vary in specific objectives, despite the general intent of public health intervention. For this reason, risk maps should be scrutinized more thoroughly. As our knowledge on blacklegged ticks and *B. burgdorferi* increases, we must continually re-assess how risk models have predicted their geographic distributions over time. Differences in tick exposure patterns and Lyme disease risk is likely across regions and can depend on the scale and socioeconomic factors included in the assessment (69). Future studies should consider improvements for forecasting these risks, as well as exploring risk assessments beyond comparison of blacklegged ticks and Lyme disease. Expanding the scope to other tick-borne diseases or co-infections of other bacteria including *Babesia* and *Anaplasma*

TABLE 5 List of peer reviewed articles included in the literature review that incorporated anthropic variables in their risk assessments where a map was produced as a result.

Paper ID	Anthropic variables used	References
MP9	- Human population density	Lieske and Lloyd (74)
MP15	- Human population size per county	Bisanzio et al. (76)
MP18	- Household income	Little et al. (75)
MS3	- Age and sex of Lyme disease patients	Tutt-Guerette et al. (84)
MS8	- Human population density - Hiking behavior and Lyme awareness survey	Tadiri et al. (45)
MS12	- Human population size per census division	Gasmi et al. (85)
MS17	- "Lifestyle" categories based on surveys	Ozdenerol et al. (42)
MS18	- Sociobehaviours; preventive behavior score, knowledge score, and risk perception score - Human population density	Bouchard et al. (22)
MS21	- Behavioral risk factors - Reported tick exposure (by survey respondents)	Aenishaenslin et al. (46)
MS25	- Human population density	Self et al. (86)
MS26	- Human population density	Glavanakov et al. (87)
MS28	- Human population density - Google trends for Lyme disease focused key words	Kutera et al. (88)
MS35	- Human population density	Diuk-Wasser et al. (36)
MS36	- Human population density	Larsen et al. (72)

Specific anthropic variables are described below along with their source.

sp. (16, 83) may demonstrate further patterns with spatial risk models.

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.

Author contributions

EF: conceptualization, methodology, formal analysis, investigation, data curation, writing—original draft, visualization, and project administration. MV: resources, writing—review and editing, project administration, and funding acquisition. VM: conceptualization, resources, writing—review and editing, supervision, project administration, and funding acquisition. All authors contributed to the article and approved the submitted version.

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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/fpubh.2023.1113024/full#supplementary-material

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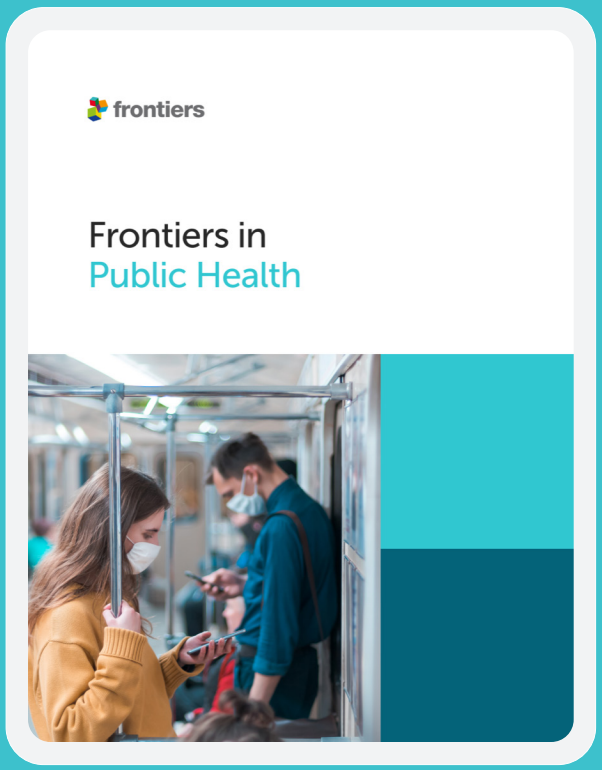

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