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Rabies, a long-standing one health example – progress, challenges, lessons and visions on the way to 0 by 30

Edited by
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Rabies, a long-standing one health example – progress, challenges, lessons and visions on the way to 0 by 30

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Editorial: Rabies, a long-standing One Health example – progress, challenges, lessons and visions on the way to 0 by 30

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Editorial on the Research Topic
Rabies, a long-standing One Health example – progress, challenges, lessons and visions on the way to 0 by 30

Rabies control requires a One Health approach

Rabies is a zoonosis that is endemic in most African and Asian countries and has one of the highest case fatality rates of any disease. More than 95% of the estimated 59,000 annual cases of rabies in humans are attributed to dog bites (1, 2). Death and suffering from rabies are preventable, and the ambition to eliminate dog-mediated human rabies by 2030 has been formulated as "Zero by 30", as manifested in a Global Strategic Plan by the United Against Rabies Coalition (3). However, even in regions that have made great strides in eliminating the disease, these successes remain fragile and hard to maintain if interventions are weakened or stopped because a country or region is no longer endemic (4).

One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems and to generate incremental value by joining interdisciplinary forces. This view recognizes that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent (5). One Health approaches were proven to contribute to resilient communities and health systems long before the world was hit by the COVID-19 pandemic that brought One Health to the forefront of public attention. In the case of dog-mediated rabies, the need for close collaboration between the animal and human sectors has been evidenced and acknowledged for many years. Rabies experts have been emphasizing the importance of collaboration between sectors in surveillance, prevention, control, and elimination efforts even before the One Health concept became an increasingly common position of the "Tripartite" international organizations,[1] in which rabies was given special relevance from the very beginning (6). The application of a One Health approach is also a central aspect of the global strategic plan towards elimination of dog-mediated human rabies not only as a means of successful rabies elimination but also because of the demonstrated economic savings.

This Research Topic called for papers presenting experiences, innovative perspectives, and visions for achieving the "Zero by 30" goal, with a special focus on the importance of One Health for rabies control, not only at the direct host-agent zoonotic interface but also considering environmental, sociological, and (geo)political aspects. A total of 147 authors responded to this call and contributed to 20 publications contained in this Research Topic, which include original research papers (15), brief research reports (1), perspectives (3), and opinion papers (1). The authors reported from the global (8) to the local level, with country and subnational examples from Benin (1), Brazil (2), Chad (2), Côte d'Ivoire (1), Indonesia (1), Mexico (1), Namibia (1), Thailand (2), and the Philippines (1).

Political empowerment of the veterinary sector

The recent COVID-19 pandemic has given the One Health paradigm an unprecedented boost in public awareness, but all too often only in the direction of pandemic preparedness and response, to the detriment of endemic and neglected diseases. Nadal, Abela-Ridder, et al. provide insights into the disruptive effects of the COVID-19 pandemic on rabies control, highlighting that mass dog vaccination in 2020 was carried out as planned in only 5% of countries surveyed, access to post-exposure prophylaxis (PEP) decreased, and under-reporting worsened in many places. The authors conclude that veterinary services now need to take the lead in ensuring that they become an irreplaceable and integral part of public health services at local, regional, and global levels. Djegu et al. give an example of capacity strengthening in Benin's Central Veterinary Laboratory through the joint efforts of national authorities and inter- and non-governmental actors, leading to such empowerment of the veterinary sector.

The importance of considering the cultural context

Social and cultural aspects play a paramount role when it comes to zoonotic disease prevention and control, notably for those that encompass close human-animal bonds, such as rabies. Nadal, Hampson, et al. highlight the importance of understanding the different cultural and religious contexts in which humans relate to animals. In particular, they refer to traditional approaches to treatment, such as faith healing, and religiously motivated attitudes toward rabies, i.e., accepting a disease as an expression of a divine will. N'Guessan et al. describe factors such as health-seeking behaviors and attitudes toward the concept of health and disease as determinants of rabies PEP dropout in the San-Pedro region of Côte d'Ivoire, and Mbaipago et al. emphasize, among others, religious motives such as the perceived impurity of the dog as barriers to treatment in the Republic of Chad. Premashthira et al. also take a look at the impact of socioeconomic factors on dog owners' knowledge, attitudes, and practices toward dog vaccination in Thailand.

Improving dog vaccination, PEP, and rabies treatment

As has been demonstrated for decades, rabies can be fully prevented by vaccinating dogs (to eliminate it from the main reservoir population) and humans (to prevent the development of rabies after exposure). In a study from Thailand, Thanapongtarm et al. shed light on the characteristics of dog populations by describing the ownership correlation of free-roaming dogs. This knowledge can help to model dog populations with little effort and thus facilitate the planning of dog vaccination campaigns. The work of Lugelo et al. and Wera et al. is also dedicated to the facilitation and impact of mass vaccination of dogs by investigating the effectiveness of a passive cooling device on vaccine stability during a field study in Tanzania and by looking into factors influencing immunity in Indonesian dogs, respectively. Molini et al. focus on the complementary aspect of parenteral vaccination with oral rabies vaccines; the authors tested the immunogenicity of an oral vaccine strain in a field trial in Namibia.

In the context of human vaccinations, da Silva et al. report on an ongoing reliance on PEP in Brazil, despite the fact that dog rabies has not been eliminated and dog bite incidence remains high. As the use of PEP can be optimized by assessing the individual rabies risk of the bite victim, the authors advocate for closing knowledge gaps on PEP administration in collaboration with health professionals while improving communication between health and veterinary authorities. This points to the cross-sectoral concept of "integrated bite-case management" (IBCM), which aims to improve case detection and treatment, as showcased by Rysava et al. and Swedberg et al. While the former describes the potential of IBCM using an implementation case in Albay province, Philippines, the latter tackles the issue through a qualitative (interview) approach, confirming that IBCM needs to be embedded in the local context and cannot be implemented independently. Madjadinan et al. also used qualitative methods such as focus group discussions to illustrate barriers to PEP application following an IBCM approach and call for improvements in rabies health services and public awareness. Knobel et al. spin the One Health concept and look at a potential canine or human rabies therapy from a One Medicine perspective. They argue that little progress has been made in treating rabies-infected patients and plead for investigational

1 The so-called Tripartite Collaboration, consisting of the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (WOAH, formerly OIE), and the World Health Organization (WHO), was joined in 2022 by the United Nations Environment Programme (UNEP) to form the "Quadripartite".

combination therapy of naturally infected dogs as a model for a human clinical scenario. Using the experience gained, rabies could ideally be transformed into a treatable disease. This proposal has made the paper the most read in this Research Topic to date.

The role of wildlife

Since 1980, thanks to the involvement of human health systems in mass canine vaccination campaigns, Latin America has made the greatest progress in the elimination of dog rabies, mainly through the governance mechanism of the National Rabies Program Directors (REDIPRA). In the majority of Latin American countries, there have been no human rabies deaths for years, mainly due to decades of mass dog vaccinations. Meanwhile, the focus of infection has shifted to another interface: the one between humans or domestic animals and wildlife reservoirs. Bats such as *Desmodus rotundus* remain vectors of rabies in countries like Brazil, as described by Megid et al., and Mexico, as highlighted by Ortega-Sánchez et al.. It is discussed that these species' epidemiological relevance for rabies transmission and elimination depends on various environmental factors, such as anthropogenic changes, and therefore requires continued surveillance. This underlines the importance of including the ecosystem as an integrated part of the One Health approach with regard to rabies control, particularly when rabies control in dogs is highly advanced.

One Health solutions

This Research Topic comes at a time when the One Health concept is better understood and more "popular" than ever before, as countries strive to implement One Health strategies and link them with their mandated plans to eliminate rabies. This can break down silos and encourage governments, authorities, and other stakeholders to integrate the "systems approach" into their policies, resulting in shared expertise, joint action, and pooled resources. The United Against Rabies Forum (UARF) provides since 2020 a collaborative international network of partners from different sectors and disciplines to improve cross-sectoral coordination, reduce fragmentation, and support countries in their rabies elimination efforts. Tidman, Thumbi, et al. present the background, concept, and strategic direction of the UARF, while Tidman, Fahrion, et al. describe the composition of the Forum and the work carried out in the first 2 years of its existence to generate and compile approaches, tools, and materials to support countries in their efforts to achieve Zero by 30.

At local, regional, and global levels, we need more than ever a strengthened and integrated (veterinary, public, and environmental) health workforce to address challenges such as epidemics and climate change. Rabies control programs can contribute to building One Health capacity, opening opportunities to address other zoonotic threats, such as those with pandemic potential. Improving One Health coordination, collaboration, communication, and capacity building for rabies will contribute to this public good. To quote Ghai and Hemachudha, "It is time to target the political sector, to ensure that temporary disease burden reduction is not misconstrued as progress, to ensure that a legal framework is in place and that the strategies account for the restrictions imposed by the COVID-19 pandemic. It is crucial that countries maintain pressure and preserve the priority status of the disease at the country level, so that rabies can be eliminated once and for all" [SIC].

Author contributions

AF, SD, CF, ML, TM, SR, and MV edited this Research Topic. FB, KH-T, SM, and MM supported the Research Topic as topic coordinators. AF and SD wrote this article, and all authors reviewed and complemented the manuscript and approved the submitted version.

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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The Impact of Socioeconomic Factors on Knowledge, Attitudes, and Practices of Dog Owners on Dog Rabies Control in Thailand

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Rabies is a deadly zoonotic disease responsible for almost 60,000 deaths each year, especially in Africa and Asia including Thailand. Dogs are the major reservoirs for rabies virus in these settings. This study thus used the concept of knowledge, attitudes, and practices (KAP) to identify socioeconomic factors that contribute to the differences in the canine rabies occurrences in high and low-risk areas which were classified by a Generalized Additive Model (GAM). Multistage sampling was then applied to designate the study locations and a KAP-based questionnaire was used to retrieve data and relevant perspectives from the respondents. Based on the responses from 476 participants living across four regions of Thailand, we found that the knowledge of the participants was positively correlated with their behaviors but negatively associated with the attitudes. Participants who are male, younger, educated at the level of middle to high school, or raising more dogs are likely to have negative attitudes but good knowledge on rabies prevention and control whereas farmers with lower income had better attitudes regardless of their knowledge. We found that people in a lower socioeconomic status with a lack of knowledge are not willing to pay at a higher vaccine price. Public education is a key to change dog owners' behaviors. Related authorities should constantly educate people on how to prevent and control rabies in their communities. Our findings should be applicable to other countries with similar socioeconomic statuses.

Keywords: epidemiology, KAP, public education, willingness to pay, zoonosis

INTRODUCTION

Rabies is a deadly zoonotic disease caused by Lyssaviruses belonging to the family Rhabdoviridae of the order Mononegavirales (1). With an almost 100% mortality rate, infected individuals are always fatal once symptoms develop (2). A wide variety of mammals were reported to harbor the virus, for example, bats, dogs, raccoons, and skunks (3). However, most of the human rabies cases are dog-mediated. It was estimated that canine rabies is responsible for around 59,000 human deaths annually and most of the endemic countries are located in Asia and Africa (4). Among those countries, Thailand had recently suffered from an unprecedented outbreak of rabies in animals. From the national active and passive surveillance program in which majority of the samples were passively collected, the Department of Livestock Development, Thailand (DLD) found positive results to rabies examination (fluorescent antibody technique and mouse inoculation test) at 15.3% (1,476/9,643) and 5.1% (377/7,321) in 2018 and 2019, respectively (5). Although the trend of rabies outbreaks is decreasing, there are still ongoing outbreaks in both humans and animals. In 2020, three human rabies deaths had been notified in three different provinces whereas the animal rabies cases had been recorded in 36 out of 77 provinces of Thailand. Dogs are the most active animals in the spread of the virus in these communities (5). Among the samples tested by DLD in 2020, most were retrieved from dogs (62.8%; 4,428/7,056). The submitted samples were primarily collected from animals suspected of symptoms of rabies. However, some died from other causes, such as car crashes and their samples were then sent to the laboratory as a part of the active surveillance.

In Thailand, dog owners must have a full responsibility for their dogs. Legally, the dogs must be vaccinated against rabies and kept from biting others. However, we do have a situation where stray dogs are fed without anyone claiming ownership. To get an overall herd immunity for rabies, DLD conducts a mass vaccination campaign annually for both owned and stray dogs.

The socioeconomic status of people living in a certain setting can directly affect three important aspects of health namely health care, environmental exposure, and health behavior (6). The problem of socioeconomic health disparities has been previously observed in many countries across the globe, for instance, United States (7), South Africa (8), Japan (9), and Indonesia (10). In Thailand, socioeconomic disparities among people classified in different social classes were previously pointed out. Several related health problems have been raised such as hypertension (11) and chronic respiratory diseases (12). Such disparity may also affect how people perceive and behave during rabies outbreaks in Thai communities. Regarding the rabies problem, it was found in a previous study that socioeconomic factors were associated with human rabies infection in China (13). It is worth exploring the influences of socioeconomics on rabies situations in Thailand.

The study of knowledge, attitudes, and practices (KAP) is on a curious basis that whether the increase of knowledge is correlated with attitudes and practices. The observed relationships will be then applied to tailor relevant policies to mitigate health problems (14). KAP has been previously used in the study of different infectious diseases, for instance, Leptospirosis (15), Brucellosis (16), and rabies (17–19). KAP would be an effective tool in exploring more insights into the behavioral aspects of dog owners. More understanding of these anthropogenic factors is helpful to target the right interventions to the right groups of people. In Indonesia, it was found that the attitude of dog owners was significantly associated with the intention to participate in a rabies control measure (20). Therefore, more understanding on the attitudes would be helpful in policy recommendation.

The present study, therefore, exploited KAP to identify the factors, especially on the socioeconomic aspects, relevant to the dog owners that contribute to the differences in the dog rabies occurrences in high and low-risk areas located in four different regions of Thailand.

MATERIALS AND METHODS

Selection of Study Sites

A spatial risk map of dog rabies infection was produced at the district level of Thailand. Briefly, we used a Generalized Additive Model (GAM) to quantify the relationship between rabies occurrences and a set of explainable factors at the sub-district level including human population, dog population, cattle population, length of the road, and distance from the case locations to the country border. Then, the model was used to predict the probability of rabies occurrences in all sub-districts in the country. Finally, the rabies risk map at the district level was produced by averaging the predicted values of the sub-district level and classified into three risk levels namely low, medium and high. All modeling processes were performed with

TABLE 1 | Overview of the KAP questions.

Category	Questions
Demographic data of the respondents	(1) district where the residence is located (2) age (3) gender (4) education (5) main occupation (6) religion (7) income (8) type and number of pets (9) house ownership status (10) fence and gate (11) number of house members and their age (12) community roles
Knowledge	The questions asked about knowledge on (1) rabid animal species (2) seasonal restriction of the rabies outbreak (3) clinical signs (4) transmission (5) treatment of infected animals or humans (6) prevention by vaccine (7) first shot of dog vaccination (8) repeated vaccination annually (9) vaccine retention (10) vaccination in sick animals (11) prevention by ears and tail cutting
Attitudes	The questions asked about opinion on (1) harmfulness of rabies to pets and humans (2) annual vaccination (3) destruction of bitten dogs (4) destruction of stray dogs (5) sterilization (6) responsibility for the cost of vaccination (7) notification of suspected rabid dogs (8) identification and registration of dogs and cats (8) offense to the law in case of dog releasing in public place (9) stray dog quarantine
Practices	(1) rabies vaccination history for their dogs (2) vaccination practices (3) vaccine price willing to pay (4) sterilization of dogs (5) area restriction of dogs (6) dog saliva exposure avoiding (7) experience of encountering rabid dogs and notification to government agency (8) history of rabid dog exposure of their dogs and their action taking (9) rabies case-finding action (10) channels to receive rabies outbreak information

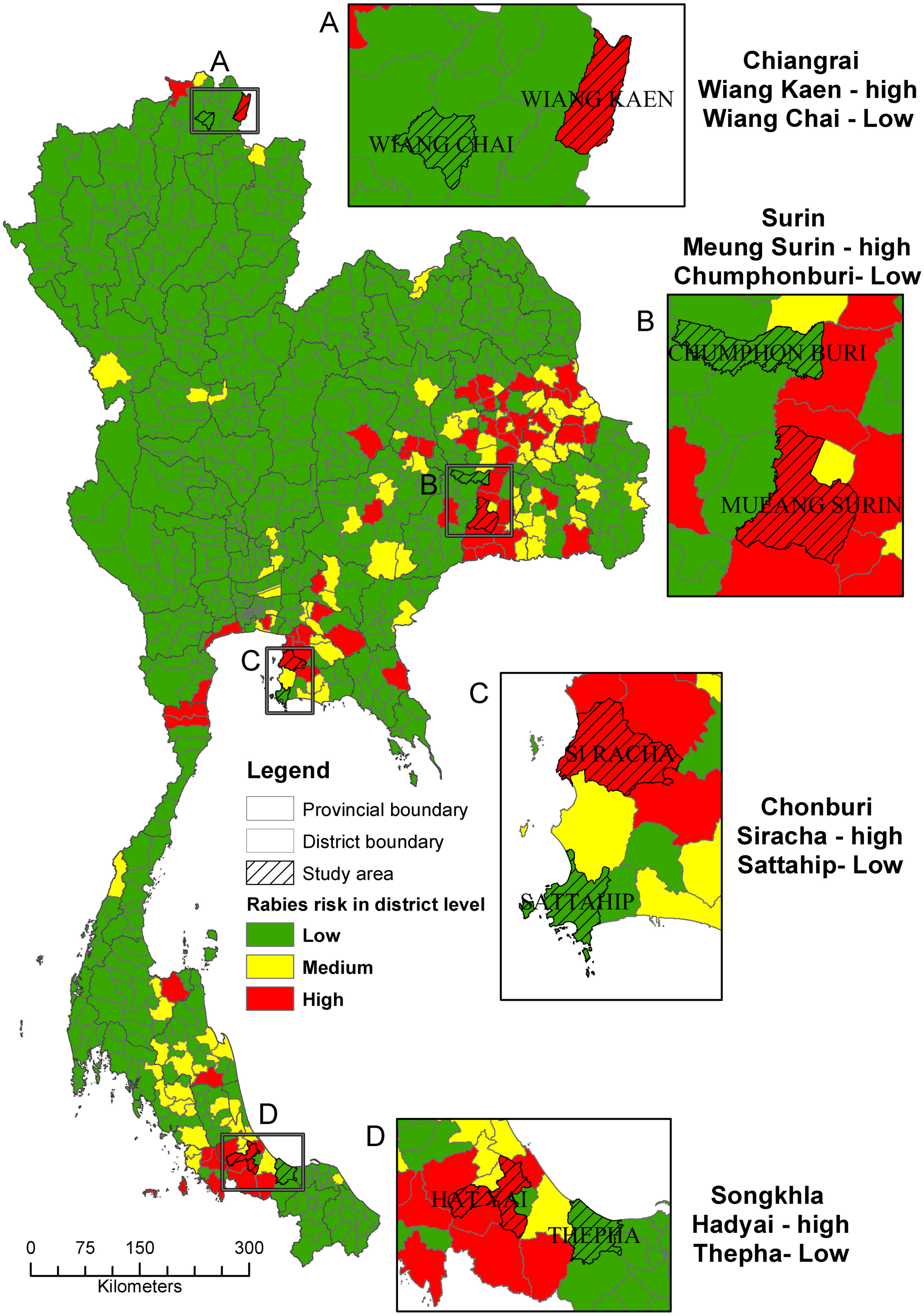

FIGURE 1 | A map depicting the risk level of canine rabies transmission at district level for the selection of study areas (shaded; **A–D**) in the four main regions of Thailand.

the package "mgcv" in program R version 4.0.3 (R Core Team, Vienna, Austria).

Multistage sampling was then used to designate the study locations. To deal with the culture and socioeconomic diversity, we performed our field investigation in four regions of Thailand. In each region, the province with the highest risk was chosen. Within each province, one high-risk and another low-risk districts were purposely selected.

KAP Survey

A KAP-based questionnaire was prepared and validated before use by the experts for the consistency of the content in the questions. The questions were divided into four parts including (1) demographic data of the respondents (14 questions), (2) knowledge (11 questions), (3) attitudes (11 questions), and practices (11 questions). The questionnaires were distributed equally to each district. The original questionnaire was prepared in Thai. We translated it into English and attached in the **Supplementary Material**. The overview of the questions we asked was summarized in **Table 1**.

The target population was the dog owners who had at least one dog at home. The sample size required for the survey was calculated following the formula proposed by Cochran, 1977 (21) with 95% confidence interval and 5% margin of error. We set the sample proportion at 16.8% as suggested by a previous study on KAP in Thailand (22). The participants were face-to-face interviewed and the responses were filled into ODK-open software (https://opendatakit.org/) and stored on the cloud database. The data was, later on, downloaded for further analysis.

Data Analysis

The demographic data were explored by descriptive analysis and K-means clustering. Cluster analysis helps identify structures within the data including their KAP scores. The homogenous groups of socioeconomic clusters of dog owners were identified. The principle of minimizing intra-cluster distance and maximizing the inter-cluster distance facilitated us to distinguish behavior in each study group (23). In our analysis, we use non-hierarchical cluster analysis or K-Means clustering. The logistic regression was applied to explore the association between factors and risk of areas (low and high-risk areas). Also, the multiple logistic regression was analyzed at the end to fit the best model. The scores of attitudes, knowledge, and practices were compared between both areas. Our scoring criteria were detailed in **Supplementary Table 1**. The correlations of knowledge, attitude and practice scores were analyzed using Spearman's correlation coefficient. The cut-off for the statistical difference was set at a p-value < 0.05. All data were analyzed with the packages "lmtest" and "zoo" in program R version 4.0.3 (R core team, Vienna, Austria) and the K-means commands equipped in the SPSS software version 19 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.) was used to classify clusters. The Euclidean distances were then calculated to assess the distance between the final clusters.

RESULTS

Study Locations

We classified 926 districts in Thailand according to the risk of rabies outbreak occurrence as 65 high-risk, 101 medium-risk, and 760 low-risk districts, respectively. Within the four main regions, the provinces that contained the highest number of high-risk districts in each region were Chiang Rai in the North (two districts), Surin in the Northeast (nine districts), Chon Buri in the Central (five districts), and Songkhla in the South (six

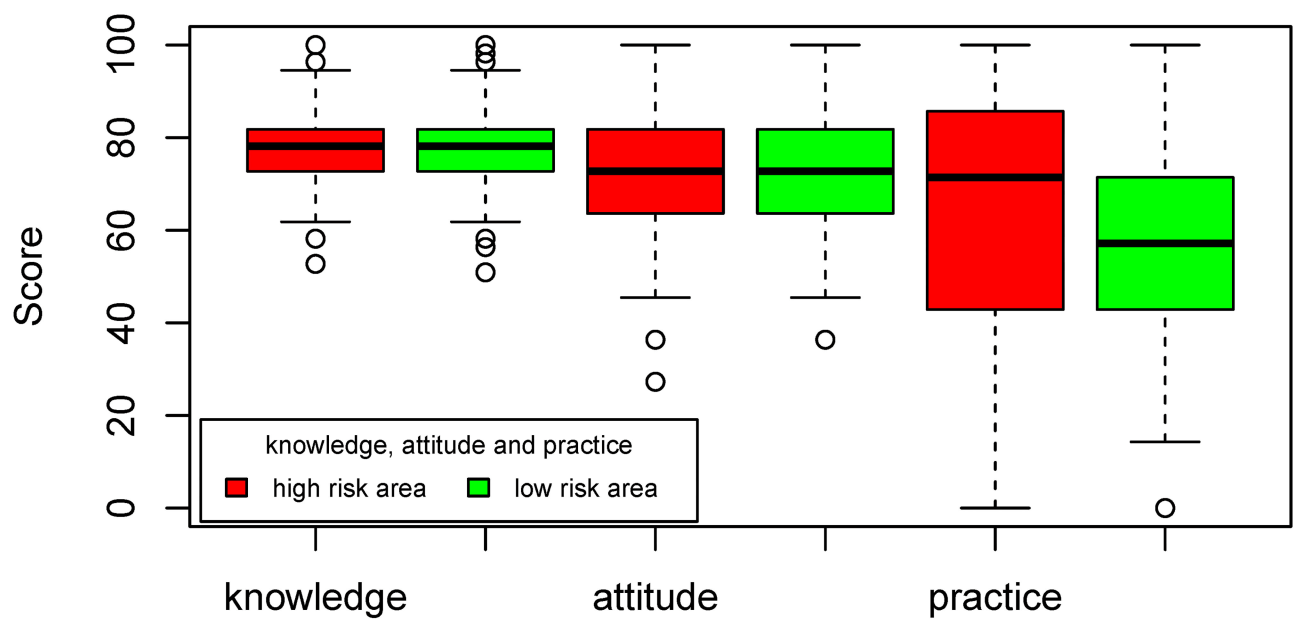

FIGURE 2 | Comparison of the adjusted scores of attitudes, knowledge, and practices between the high and low-risk areas for rabies transmission.

TABLE 2 | Demographic data of the KAP respondents.

Variables	Number (Percentage, 95% CI)		
	High-risk areas	Low-risk areas	Total
Number of respondents	243	233	476
Age (year)			
- Up to 20	8 (3.3%, 1.4–6.4%)	0 (0.0%, 0.0–0.0%)	8 (1.7%, 0.7–3.3%)
- 21–40	46 (18.9%, 14.2–24.4%)	47 (20.2%, 15.2–25.9%)	93 (19.5%, 16.1–23.4%)
- 41–60	116 (47.7%, 41.3–54.2%)	100 (42.9, 36.5–49.5%)	216 (45.4%, 40.8–50.0%)
- Over 60	73 (30.0%, 24.4–36.2%)	86 (36.9%, 30.7–43.5%)	159 (33.4%, 29.2–37.8%)
Gender			
- Female	166 (68.3%, 62.1–74.1%)	162 (69.5%, 63.2–75.4%)	328 (68.9%, 64.5–73.0%)
- Male	77 (31.7%, 25.9–37.9%)	71 (30.5%, 24.6–36.8%)	148 (31.1%, 26.0–34.4%)
Education			
- Below primary	32 (13.2%, 9.2–18.1%)	8 (3.4%, 1.5–6.7%)	40 (8.4%, 6.1–11.3%)
- Primary	111 (45.7%, 39.3–52.2%)	120 (51.5%, 44.9–58.1%)	231 (48.5%, 44.0–53.1%)
- Middle/high	55 (22.6%, 17.5–28.4%)	56 (24.0%, 18.7–30.1%)	111 (23.3%, 19.6–27.4%)
- Vocational	12 (4.9%, 2.6–8.5%)	24 (10.3%, 6.7–14.9%)	36 (7.6%, 5.4–10.3%)
- Bachelor or higher	33 (13.6%, 9.5–18.5%)	25 (10.7%, 7.1–15.4%)	58 (12.2%, 9.4–15.5%)
Main occupation			
- Farmer	90 (37.0%, 30.1–43.4%)	108 (46.4%, 39.8–53.0%)	198 (41.6%, 37.1–46.2%)
- Housewife	48 (19.8%, 14.9–25.3%)	40 (17.2%, 12.6–22.6%)	88 (18.5%, 15.1–22.3%)
- Merchant	54 (22.2%, 17.2–28.0%)	27 (11.6%, 7.8–16.4%)	81 (17.0%, 13.8–20.7%)
- Freelance	22 (9.1%, 5.8–13.4%)	17 (7.3%, 4.3–11.4%)	39 (8.2%, 5.9–11.0%)
- Public servant	12 (4.9%, 2.6–8.5%)	22 (9.4%, 6.0–14.0%)	34 (7.1%, 5.0–9.8%)
- Others	17 (7.0%, 4.1–11.0%)	19 (8.1%, 5.0–12.4%)	36 (7.6%, 5.4–10.3%)
Religion			
- Buddhist	214 (88.1%, 83.3–92.0%)	227 (97.4%, 94.5–99.1%)	441 (92.6%, 89.9–94.8%)
- Christ	12 (4.9%, 2.6–8.5%)	5 (2.1%, 0.7–4.9%)	17 (3.6%, 2.1–5.7%)
- Islam	3 (1.2%, 0.3–3.6%)	1 (0.4%, 0.0–2.4%)	4 (0.8%, 0.2–2.1%)
- Traditional ghost beliefs	13 (5.3%, 2.9–9.0%)	0 (0.0%, 0.0–0.0%)	13 (2.7%, 1.5–4.6%)
- No religion	1 (0.4%, 0.0–2.3%)	0 (0.0%, 0.0–0.0%)	1 (0.2%, 0.0–1.2%)
Income per month (THB)			
- Up to 10,000	115 (47.3%, 40.9–53.8%)	129 (55.4%, 48.7–61.9%)	244 (51.3%, 46.7–55.8%)
- 10,001–20,000	68 (28.0%, 22.4–34.1%)	51 (21.9%, 16.8–27.8%)	119 (25.0%, 21.2–29.1%)
- 20,001–30,000			
- Over 30,000	18 (7.4%, 4.5–11.5%)	27 (11.6%, 7.8–16.4%)	45 (9.5%, 7.0–12.5%)
(USD 1 ≈ THB 31.2)	42 (17.3%, 12.8–22.6%)	26 (11.2%, 7.4–15.9%)	68 (14.3%, 11.3–17.8%)
Animal keeping			
- Number of dogs	Mean = 2.8 (SD = 4.9)	Mean = 2.4 (SD = 2.1)	Mean = 2.6 (SD = 3.8)
- Breed Mixed	145 (59.7%, 53.2–65.9%)	156 (67.0%, 60.5–73.0%)	301 (63.2%, 58.7–67.6%)
Poodle	25 10.3%, 6.8–14.8%)	12 (5.2%, 2.7–8.8%)	37 (7.4%, 5.5–10.6%)
Bangkaew	13 (5.4%, 2.9–9.0%)	10 (4.3%, 2.1–7.8%)	23 (4.8%, 3.1–7.2%)
- Years of experience	Mean = 7.3 (SD = 7.8)	Mean = 7.0 (SD = 6.9)	Mean = 7.1 (SD = 7.4)
Type of house ownership			
- Owned	217 (89.3%, 84.7–92.9%)	224 (96.1%, 92.8–98.2%)	441 (92.6%, 89.9–94.8%)
- Rent	11 (4.5%, 2.3–8.0%)	3 (1.3%, 0.3–3.7%)	14 (2.9%, 1.6–4.9%)
- Dormitory	1 (0.4%, 0.0–2.3%)	1 (0.4%, 0.0–2.4%)	2 (0.4%, 0.1–1.5%)
- Others	14 (5.8%, 3.2–9.5%)	5 (2.2%, 95%CI 0.7–4.9%)	19 (4.1%, 95%CI 2.4–6.2%)
House fencing			
- Yes	87 (35.8%, 95%CI 29.8–42.2%)	81 (34.8%, 28.7–41.3%)	168 (35.3%, 31.0–39.8%)
- No	156 (64.2%, 57.8–70.2%)	152 (65.2%, 58.7–71.3%)	308 (64.7%, 60.2–69.0%)
- Number of family member	Mean= 4.7 (SD = 2.7)	Mean = 4.0 (SD = 1.7)	Mean = 4.3 (SD = 2.3)

districts). One high-risk and one low-risk districts located in these provinces were then chosen as our study sites (**Figure 1**).

Demographic Data of the Respondents

In total, we had 476 participants involved in our questionnaire survey, of which 243 (51.1%) resided in the high-risk areas. The average age of the participants is 53 years (range: 18–93). Overall, the majority of the participants are Buddhist females and educated to the level of primary school. The main occupation is farmer with an average income per month of <10,000 Thai Baht (≈ 320.5 USD). The average number of family members in their households is 4.3 persons. These participants keep around 2.6 dogs at home with an average raising experience of 7.1 years. Two-third of their dogs are mixed breed and raised without fencing. These demographic data are summarized in **Table 2**.

KAP Results

We found no statistically significant differences in the knowledge, attitudes, and practices scores between high and low-risk areas (**Figure 2**). The descriptive statistics of the overall scores were depicted in the **Supplementary Table 2**. The KAP overview scores; however, before proving statistical significance, reflected that respondents living in the low-risk areas had better knowledge and attitude scores compared to those in the high-risk areas. However, this was not the case for the practice scores. The knowledge scores in the high-risk areas (averaged 8.12 ± 1.74) were lower than in the low-risk areas (averaged 8.28 ± 1.54). The attitude scores in the high-risk areas (averaged 42.36 ± 3.99) were slightly lower than the low-risk areas (averaged 42.53 ± 4.06). In contrast, the practice scores in the high-risk areas (averaged 4.54 ± 1.51) were higher than the low-risk areas (averaged 4.36 ± 1.48). The differences in the practices of the dog owners comparing between high and low-risk areas were identified in the questions related to vaccination practices and house fencing (**Table 3**). After controlling confounding factors, we found the differences only in the vaccination practices. Participants living in the high-risk areas were not likely to buy vaccines for their dogs but they preferred to get a free vaccination service provided by the government staff (odds ratio: 0.410; 95% CI: 0.22–0.76) (**Table 4**). In **Table 5**, we found an overall positive correlation between knowledge and practices and a negative correlation between knowledge and attitudes.

Socioeconomic Clusters Regarding the KAP Scores

The sample population was clustered according to their KAP scores into three groups (**Table 6**) to compare socioeconomic factors affecting how dog owners control rabies in Thailand. The three classified clusters were: cluster 1—positive attitudes but poor knowledge, cluster 2—negative attitudes but good knowledge, and cluster 3—positive attitudes and good knowledge. The statistically significant difference between clusters was found in the knowledge and attitudes, but not in the practice scores. We found no difference between the clusters in the main indicator of this study, that is, living in the high and low-risk areas. Nevertheless, the statistical difference was denoted among clusters for the factors of age, gender, education, occupation, income, number of dogs raised, the average age of the youngest family member, and the average age of the oldest family member.

Socioeconomic Cluster Regarding Income, Education, and Willingness to Pay for Rabies Vaccines

After removing missing data, 470 dog owners were clustered regarding their income, education, and rabies vaccine prices that they are willing to pay. Three clusters were designated namely (1) willing to pay the highest cost of the vaccine, (2) moderate cost of the vaccine and (3) lowest cost of the vaccine (**Table 7**). The average prices that the participants in each cluster are willing to pay were 251.60, 91.09, and 38.58 Thai Baht, respectively. The differences between these three clusters were identified for the main indicator of this study (living in high and low-risk areas). Participants living

TABLE 3 | Determinants of the level of risk areas for rabies (high/low) in the univariate analysis regarding the practices of the dog owners (n = 476).

Practices of the dog owners	Odds ratio (95% CI)	*P*-value
Frequency of dog rabies vaccination		
- Annually	1.28 (0.65–2.54)	0.478
- Irregularly	1.04 (0.44–2.46)	0.927
- No vaccination	Reference	
Vaccination practice		
- Purchased vaccines by dog owner	0.40 (0.22–0.74)	0.004
- Paid service at veterinary hospitals/clinics	2.07 (1.04–4.35)	0.044
- Free service of government staff	Reference	
First rabies vaccine shot at few-month-old dogs		
- Yes	0.97 (0.68–1.40)	0.883
- No	Reference	
Dog population control		
- Yes	1.16 (0.80–1.68)	0.441
- No	Reference	
House fencing		
- Yes	1.58 (1.09–2.30)	0.017
- No	Reference	
Avoiding dog saliva exposure		
- Yes	1.06 (0.73–1.52)	0.768
- No	Reference	
Experience of encountering rabid dogs		
- Yes	1.16 (0.79–1.69)	0.457
- No	Reference	
Notification of rabid dogs to government agency		
- Yes	1.14 (0.57–2.28)	0.709
- No	Reference	
Rabid dog exposure to their dogs		
- Yes	1.39 (0.52–3.88)	0.515
- No	Reference	

TABLE 4 | Determinants of the level of risk areas for rabies (high/low) in the logistic multivariable regression model regarding the practices of the dog owners (n = 476).

Practices of the dog owners	OR (95% CI)	*P*-value
Vaccination practice		
Purchased vaccines by dog owner	0.41 (0.22–0.76)	0.005
Paid service at animal hospitals/clinics	1.79 (0.88–3.82)	0.12
Free service of government staff	Reference	
House fencing		
Yes	1.43 (0.95–2.14)	0.08
No	Reference	

TABLE 5 | Spearman's correlation coefficient between knowledge, attitudes and practices of dog owners regarding rabies control in Thailand.

	Knowledge	Attitudes	Practices
Knowledge	1.000	−0.100*(0.030)	0.206**(0.000)
Attitudes	−0.100*(0.030)	1.000	0.037 (0.422)
Practices	0.206** (0.000)	0.037 (0.422)	1.000

***Correlation is significant at the 0.01 level (2-tailed).*
**Correlation is significant at the 0.05 level (2-tailed).*

in the high-risk areas are willing to pay more compared to those who lived in the low-risk areas. Besides the risk levels, other factors that significantly influence the willingness to pay are geographical region, age, education, occupation, and income.

DISCUSSION

The present study used KAP techniques to cross-sectionally explore socioeconomic impacts on the spread of rabies virus among dog populations in the high and low-risk areas in Thailand. Subsequently, different statistical methods were employed to analyze socioeconomic factors that may contribute to rabies transmission.

We found that the high-risk areas for rabies propagation were identified in all regions of Thailand. However, the majority of the risky areas were disproportionately found in the Northeastern region (**Figure 1**). Our finding was in line with a previous study (24) that identified the hotspots of the rabies outbreaks in the same region. What we found here and the findings of the previous study are only a descriptive aspect of the outbreaks. An in-depth analysis is needed to find out the factors contributing to this observation.

Interestingly, our findings pointed out a different practice on how people get their dogs vaccinated (**Table 4**). This reflects the difference in social responsibility among people living in high and low-risk areas. It may also explain why the number of rabies cases was higher in the high-risk areas as the dog owners, in case they were unable to bring the dogs to the clinics, usually wait for a free vaccination service provided by the government whereas people in the low-risk areas actively purchase the vaccines for their dogs. Geographically, the high-risk areas visited in this study are located more remotely compared to the low-risk areas. Some participants also complained that the governmental services had not reached their premises. This may result in a low level of herd immunity. Indeed, the local administrative organization (LOAs) in Thailand have been working closely with the DLD to facilitate the distribution of the rabies vaccines to reach the rural and remote communities. However, there are still some unreachable areas as we found in our study. The uses of local leaders and administrators have been evidenced in the prevention and control of infectious diseases, for example, in the cases of Corona Virus Disease (COVID-19) (25) and Ebola (26). This approach should also be applicable in the case of rabies.

In **Table 5**, we found that the knowledge of the participants was positively correlated with their behaviors but negatively associated with their attitudes. A similar finding was also denoted in a previous study on rabies in Congo that poor knowledge of general people can lead to malpractices in the community (27). Our findings may direct the rabies control policy to focus more on providing knowledge and information on rabies prevention and control to the public rather than trying to change their perceptions. Nonetheless, a significant difference in the educational level of participants living in high and low-risk areas was observed in this study. Compared to the low-risk areas, we found 3.03 times (95% CI: 1.23-8.09, p-value = 0.02) higher in the number of people educated below primary level compared to the number of people with the bachelor or higher education living in the high-risk areas. The lower educational level of people living in the high-risk areas also affects how people comprehend messages announced by the government. In a previous study carried out in India, it was found that the low level of formal education is inversely linked to the knowledge of farmers regarding zoonotic diseases (28). To improve fundamental education is helpful to increase knowledge on rabies control and change relevant practices accordingly.

According to **Table 6**, we found multiple socioeconomic factors significantly influencing the knowledge and attitudes of the dog owners toward rabies control in their settings whether they were living in high or low-risk areas. It seems that participants who were categorized as male, younger age, educated at the level of middle to high school or raising more dogs tend to have negative attitudes but good knowledge whereas farmers with lower income had a better attitudes compared to other occupations regardless of their knowledge. Our findings reflect the complexity of how socioeconomic status impacts what people know and how they think about the control of rabies in Thailand. As the majority of people living in the study areas are farmers with primary education, we recommend, again, related authorities to constantly provide knowledge on how to prevent and control rabies to the general public, especially those who own dogs. In the policy implementation, the areas with poor people, aged higher and educated at the lower level should be firstly prioritized.

Different socioeconomic status of people included in this study also impacts on how much they are willing to pay for a dose of rabies vaccine (**Table 7**). Overall, we found that people who are younger, with higher education or higher income tended to pay more for the rabies vaccines. Moreover, people with higher knowledge scores are more willing to pay higher prices (p-value = 0.046). In a previous study on the willingness to pay for social health insurance in Vietnam, it was found that people with more knowledge on the issue are willing to pay more (29). Besides, our findings indicate that people living in high-risk areas are willing to pay higher. It implies that people who face directly with a crisis are more aware of the danger and

TABLE 6 | KAP score clusters to describe socioeconomic status of dog owners regarding rabies control in Thailand.

Factors	Cluster percent count percent			
	1	2	3	Significance[a]
Number of cases in the cluster	99	105	272	
Group describe	Positive attitudes but poor knowledge	Negative attitudes but good knowledge	Positive attitudes and good knowledge	
Total practice score	4.46	4.42	4.46	0.964[ns]
Total attitude score	47.90	37.11	42.51	0.000**
Total knowledge score	7.67	8.35	8.33	0.001**
Risk area	High 50.5%	High 54.3%	High 50.0%	0.751[ns]
	Low 49.5%	Low 45.7%	Low 50.0%	
Region	South 28.3%	South 32.4%	NE 29.0%	0.055[ns]
	NE 26.3%	East 30.5%	South 26.5%	
	North 26.3%	North 23.8%	North 23.2%	
	East 19.2%	NE 13.3%	East 21.3%	
Average age (year)	58.05	50.21	52.16	0.000**
Gender	Female 73.7%	Female 55.2%	Female 72.4%	0.003**
	Male 26.3%	Male 44.8%	Male 27.6%	
Education	Primary 62.6%	Primary 41.0%	Primary 46.3%	0.038*
	Middle to high 17.2%	Middle to high 33.3%	Middle to high 21.7%	
	Bachelor to higher 8.1%	Bachelor to higher 12.4%	Bachelor to higher 13.6%	
Occupation	Farmer 45.5%	Farmer 32.4%	Farmer 43.8%	0.008**
	Housewife 22.2%	Housewife 21.0%	Housewife 16.2%	
	Merchant 16.2%	Merchant 16.2%	Merchant 17.6%	
Income	Below 10,000 = 50.5%	Below 10,000 = 38.1%	Below 10,000 = 56.6%	0.006**
	10,000–20,000 = 30.3%	10,000–20,000 = 26.7%	10,000–20,000 = 22.4%	
	20,000–30,000 = 10.1%	20,000–30,000 = 16.2%	20,000–30,000 = 6.6%	
	Over 30,000 = 9.1%	Over 30,000 = 19.0%	Over 30,000 = 14.3%	
Number of dogs raised	2.32	3.73	2.2	0.002**
The average age of the youngest family member	26.42	21.65	19.10	0.009**
The average age of the oldest family member	62.78	57.94	61.66	0.039*
Rabies vaccine price that willing to pay	76.64	73.71	74.08	0.927[ns]

[a]*Statistically significant differences across groups were tested using Pearson's* χ^2 *test (*indicates statistically significant relationships for* $p < 0.05$, ***for* $p < 0.01$ *and ns stands for non-significant relationships).*

ready to pay higher for their safety. This circumstance was also observed in the case of COVID-19 that people having family members infected with the virus are more likely to pay for the vaccines (30). Nontheless, a contradict result was observed in our study. Participants living in the high-risk areas were usually wait for a free vaccination service whereas they are still willing to pay more. This might be related to the availability of the vaccines in the areas. This observation should be further investigated. The socioeconomic disparity has previously been pointed out regarding rabies problems (31, 32). For example, a study in Cameroon suggested that more wealthy people with better knowledge of rabies are more likely to seek medical treatment and post-exposure prophylaxis (33). The inequality of socioeconomic status of people living in different areas should be seriously considered in the tailoring of rabies prevention and control programs as well as designing public education campaigns and risk communication.

Like other studies, we faced some potential limitations. First, we tried to include people in all regions of Thailand. However, with limited resources, we carried out our survey in only eight districts across the country. There might still be some variations of the socioeconomic factors that were not identified. A future study extending to cover a larger geographical area is recommended. Moreover, the participants involved in this study were recruited purposively. An ideal random sample is not feasible as there is no official registration of the dog owners in Thailand. Nevertheless, the relevant authorities have been now working together to set the system up. With the animal registration system, a survey study like this would be performed more effectively. Besides, it would be also beneficial to the proper allocations of the resources related to rabies control such as vaccines. In this study, we identified an important factor that can directly contribute to the better control of rabies epidemics, that is, public education. The impact of

TABLE 7 | Clusters of income, education, and rabies vaccine price that willing to pay of dog owners regarding rabies control in Thailand.

Factors	Cluster percent count percent			
	1	2	3	Significance[a]
Number of cases in the cluster	31	196	243	
Group Describe	willing to pay the highest cost of the vaccine	willing to pay the moderate cost of the vaccine	willing to pay the cheapest cost of the vaccine	
Rabies vaccine price that willing to pay (Baht)	251.60	91.09	38.58	0.000**
Total practice score	4.32	4.46	4.34	0.100[ns]
Total attitude score	43.35	41.93	42.71	0.054[ns]
Total knowledge score	8.35	8.18	8.00	0.046*
Risk area	High 74.2%	High 53.1%	High 46.0%	0.013*
	Low 25.8%	Low 46.9%	Low 53.1%%	
Region	North 3.26%	North 17.9%	North 32.1%	0.000**
	NE 32.3%	NE 9.7%	NE 37.0%	
	East 22.6%	East 32.1%	East 16.0%	
	South 41.9%	South 40.3%	South 14.8%	
Average age	49.65	50.95	55.19	0.004**
Gender	Female 74.2%	Female 72.4%	Female 65.0%	0.197[ns]
	Male 25.8%	Male 27.6%	Male 35.0%	
Education	Primary 35.5%	Primary 42.3%	Below primary 10.7%	0.000**
	Middle to high 38.7%	Middle to high 24.0%	Primary 56.0%	
	Bachelor to higher 22.6%	Bachelor to higher 17.9%	Middle to high 21.0%	
Occupation	Farmer 32.3%	Farmer 33.2%	Farmer 50.2%	0.000**
	Housewife 22.6%	Merchant 22.4%	Housewife 18.1%	
	Merchant 22.6%	Housewife 18.9%	Merchant 11.1%	
Income	Below 10,000 = 25.8%	Below 10,000 = 43.46%	Below 10,000 = 61.3%	0.000**
	10,000–20,000 = 48.4%	10,000–20,000 = 25.0%	10,000–20,000 = 21.8%	
	20,000–30,000 = 9.7%	20,000–30,000 = 11.2%	20,000–30,000 = 8.2%	
	Over 30,000 = 16.1%	Over 30,000 = 20.4%	Over 30,000 = 8.6%	
Number of dogs raised	1.96	2.24	2.91	0.120[ns]

[a] *Statistically significant differences across groups were tested using Pearson's χ^2 test (*indicates statistically significant relationships for $p < 0.05$, **for $p < 0.01$ and ns stands for non-significant relationships).*

public education on rabies prevention has been addressed in a previous study conducted in Azerbaijan. It was found that people participating in the rabies awareness campaign are more likely to get their pets vaccinated (34). Therefore, we should identify channels that are the most effective ways in conveying the knowledge and governmental message to the genal public. This will increase their awareness and help controling the problem in long run.

In conclusion, the canine rabies outbreak is a complex problem involving multiple socioeconomic factors. Public education is a key to change the owners' behaviors regarding the control of rabies in Thailand. Related authorities should rigorously and constantly educate people on how to prevent and control rabies in their settings. Our findings should also be applicable to other countries with similar socioeconomic status.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Mahidol University—Center of Ethical Reinforcement for Human Research (MU-CIRB 2019/157.0606). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SP and AW conceived the study. SP, SS, OS, CS, and AW participated in the collection of field data. SS, WT, and SP conducted the statistical analyses. AW oversaw the study and coordinated the drafting of the article. AW, SS, and SP were the main reviewers. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Investigating the Efficacy of a Canine Rabies Vaccine Following Storage Outside of the Cold-Chain in a Passive Cooling Device

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Background: Thermostable vaccines greatly improved the reach and impact of large-scale programmes to eliminate infectious diseases such as smallpox, polio, and rinderpest. A study from 2015 demonstrated that the potency of the Nobivac® Rabies vaccine was not impacted following experimental storage at 30°C for 3 months. Whether the vaccine would remain efficacious following storage under more natural, fluctuating temperature conditions remains unknown. We carried out a randomised controlled non-inferiority trial to compare serological responses in dogs following vaccination with doses stored under cold chain conditions with those stored within a locally made Passive Cooling Device ("Zeepot") under fluctuating temperature conditions.

Materials and Methods: Nobivac® Rabies vaccine was stored under either cold-chain conditions or within the Zeepot for 2 months. Daily ambient temperatures and temperatures within the Zeepot were recorded every 3 h. Following storage, 412 domestic dogs were randomly assigned to receive either cold-chain or Zeepot stored Nobivac® Rabies vaccine. Baseline and day 28-post vaccination blood samples were collected. Serological analysis using the Fluorescent Antibody Virus Neutralisation assay was carried out with a threshold of 0.5 IU/ml to determine seroconversion. In addition, the impact of dog Body Condition Score, sex, and age on seroconversion was examined.

Results: The serological response of dogs vaccinated using Nobivac® Rabies vaccine stored within the Zeepot was not inferior to the response of dogs vaccinated using cold-chain stored vaccine ($z = 1.1$, $df = 313$, p-value $= 0.25$). Indeed, the 28-day post-vaccination group geometric mean titre was 1.8 and 2.0 IU/ml for cold-chain vs. non-cold-chain storage, respectively. Moreover, the percentage of dogs that seroconverted in each arm was almost identical (85%). There was a positive linear trend between Body Condition Score (O.R. 2.2, 95% CI: 1.1–5.1) and seroconversion, suggesting dogs of poor condition may not respond as expected to vaccination.

Conclusions: Our study demonstrated the potency of Nobivac® Rabies vaccine is not impacted following storage under elevated fluctuating temperatures within a Zeepot. These results have potentially exciting applications for scaling up mass dog vaccination programmes in low-and-middle income countries, particularly for hard-to-reach populations with limited access to power and cold-chain vaccine storage.

Keywords: dog-mediated rabies, mass vaccination, thermotolerance, passive cooling device, cold-chain, body condition score, vaccine storage

INTRODUCTION

Canine rabies causes ∼60,000 human deaths every year worldwide, most of the victims being children under 15 years of age (1). More than 95 per cent of these fatalities occur in developing countries. Since the first rabies vaccine was developed in the 1880s by Louis Pasteur, considerable efforts have been invested in the development of high-quality rabies vaccines for humans and animals (2, 3). Epidemiological and operational research has shown that domestic dogs are the reservoir for rabies in countries where human rabies remains a concern, and that mass dog vaccination is a cost-effective way of controlling and ultimately eliminating rabies from dog and human populations. However, implementing vaccination programmes at scale requires functioning cold chain systems which are often not available in the countries where rabies is still endemic. Indeed, the high cost of installation, training of personnel, and unreliable electricity have been identified as major challenges that hinder establishment of the cold chain system in developing countries (4, 5).

Challenges of transporting and storing vaccines hinder the last mile of immunisation, and as such considerable efforts have been invested to tackle these challenges on many fronts (5–7). For example, in recent decades stakeholders in the field of public health have encouraged vaccine manufacturers to produce thermotolerant vaccines as one way of addressing the problem. As a result, a number of thermotolerant vaccines have been developed and are already available (8–11), whilst several others are at different stages of development and approval (12). For example, thermotolerant vaccines have been developed and used to effectively control smallpox, meningitis A, Newcastle disease, and rinderpest (11–13). In addition to these well-known examples, a study conducted in 2015 demonstrated that the potency of Nobivac® Rabies canine vaccine was not impacted following storage under *fixed* experimental conditions at 25°C for 6 months and 30°C for 3 months (14). This later example provides hope that this rabies vaccine can be used following local storage outside of cold chain conditions in remote places where rabies remains endemic.

In addition to efforts to develop thermotolerant vaccines, innovations to develop equipment suitable for storage of vaccines in isolated areas is also ongoing (15, 16). These innovations have led to the production of a variety of off-grid equipment ranging from simple cold boxes, used to transport vaccines from the storage facility to the immunisation site, to solar-powered refrigerators (16). Further, the development of off-grid refrigerators, such as solar refrigerators, have helped to bring life-saving vaccinations to hard-to-reach populations who would otherwise be lost to vaccine preventable diseases. Despite their benefits, however, solar refrigerators are costly to establish and maintain. Additionally, solar refrigerators are sophisticated pieces of equipment and require specialist technicians, who are typically not available in remote regions of Sub-Saharan Africa and Asia, to repair problems that occur (17, 18). Thus, the use of this equipment has been constrained to a limited number of places. In response to these issues, manufacturers have directed their efforts to develop another generation of cold chain equipment known as passive cooling devices (PCDs). These devices do not need electricity to keep the internal compartments cool, instead they rely on effective insulation and cooling media to create a cool environment. For example, the Arktek® passive vaccine storage device, which was used to transport and store vaccines during the 2014 Ebola outbreak in west Africa, uses dry ice for cooling and has the ability to keep the vaccine compartment at 2–10°C for 35 days. However, the high purchase price of the Arktek® device which ranges from US$1,200 to US$2,400 and the lack of availability of ice in remote areas has limited the widespread utility of this tool in developing countries (19). These limitations have encouraged designers to focus their efforts into developing inexpensive and simple PCDs. One promising outcome of this challenge has been the development of the very low cost PCD called a Zeepot which is built from local materials such as clay and wood. A single Zeepot costs ∼$11 and is able to keep, on average, the internal temperature at 20°C below ambient temperature (19).

Availability of thermotolerant vaccines and affordable PCDs such as the Zeepot have the potential to transform ongoing rabies control efforts in resource-poor settings. The primary aim of this study, therefore, was to investigate whether the potency of the thermotolerant Nobivac® Rabies vaccine was impacted by long-term storage inside the locally made Zeepot under field conditions where temperatures naturally fluctuate. A secondary aim of the study was to investigate the impact that body condition score and age of domestic dogs have on immunogenicity. It is expected that the outcomes from this study will inform alternative cost-effective means of delivering rabies vaccination to dogs at scale in remote regions which frequently suffer from cold chain constraints.

MATERIALS AND METHODS

A controlled and randomised non-inferiority study was carried out to compare the serological response in dogs following vaccination with doses of Nobivac® Rabies vaccine (MSD, Boxmeer, Netherlands) stored under either cold-chain conditions (cold-chain storage) or within the Zeepot (non-cold-chain storage). The Zeepot was made of clay and wood as previously described by Lugelo et al. (19). The Nobivac rabies vaccine is inactivated and contains ≥2 I.U. rabies virus strain Pasteur RIV per dose and thiomersal 0.01% as preservative.

Preparation of Vaccines

Prior to the commencement of the field trial, Nobivac® Rabies vaccines were stored under cold-chain or non-cold chain (Zeepot) conditions for a 2-month period. The study began 6 months after the vaccines' date of manufacture. The Zeepot was placed in the living room, which is generally in the shade. The daily temperature inside the Zeepot was collected at 3 hourly intervals using a digital temperature data logger (Sensormetrix®). Similarly, the ambient temperature of the storage room in which the Zeepot was kept was recorded using the same device.

Study Location and Dog Population

The field trial was carried out in eight villages within the Serengeti district of northern Tanzania. These villages are Gesarya, Kebanchebanche, Kemgesi, Ngarawani, Nyamatare, Nyamoko, Nyamatoke, and Kwitete. Based on a complete census carried out between 2010 and 2015 as part of a research study, ∼26,756 people and 6,203 dogs live in these eight villages. Dogs are kept mostly for household security, hunting and protecting livestock from predators (20, 21). Despite being owned, the vast majority of these dogs roam freely and they rarely receive veterinary care. This is a common situation in many parts of the country and other areas of rural Africa (22).

Enrolment of Dogs

Two teams of researchers accompanied by a local community leader walked on foot to locate dog keeping households within each study village. Upon arriving at a household, the researcher with the help of the community leader explained the purpose of the study and asked the head of the household whether they were happy for their dog to participate in the study. Following the signing of Informed Consent by the owner, all unvaccinated dogs present at the household, irrespective of their body condition score (BCS) and ages, were enrolled in the trial. The BCS used a scale of 1 (emaciated) to 5 (obese). Previously vaccinated dogs were not enrolled in the study, instead they received a standard-cold chain stored vaccine.

Sample Size Calculations

Sample size calculations were the same as those performed in the prior controlled and randomised non-inferiority themotolerance study carried out by Lankester et al. (14). In brief, the sample size for this study was estimated to be 50 dogs per group. This would give at least 80% power at the 5% significance level to detect non-inferiority of serological responses (the primary measurable output) to non-cold chain relative to cold-chain-stored vaccine, assuming a non-inferiority margin of $-1.2 \log^2$ titre units and a standard deviation of $1.8 \log^2$ titre units. We also planned to carry out an exploratory analysis to assess the impact of BCS and age as determinants of immunogenicity. Given this exploratory analysis, and given the aim of increasing confidence in the results and the availability of large numbers of local dogs, the sample size was increased to ∼200 dogs per group.

Sample Collection and Vaccination

Prior to sample collection, each enrolled dog was registered, had key biodata collected (e.g., age, BCS, sex), and a microchip inserted into the scruff of the neck. Whole blood samples were collected from the cephalic vein in S-Monovette® tubes (Sarsted AG, Nümbrecht, Germany). The blood samples were placed in a cool box for transportation to the laboratory.

Vaccination

The vaccines were prepared in the following manner: equal quantities of Group 1 (cold-chain) and Group 2 (Zeepot) stored vaccine were loaded into syringes each day prior to commencing field activities. Dogs were randomly assigned to receive vaccine from either group. The randomisation was achieved through the random assortment of syringes loaded with vaccine and subsequent inoculation to each dog being enrolled. Vaccination was carried out through subcutaneous injection of 1 ml of Nobivac® Rabies vaccine. The vaccines prepared each day were all used to ensure the number of dogs recruited in each group were equal. This process was repeated on the following day until a total of 200 dogs per group was reached. The vaccines were coded so that neither dog owners nor research staff knew the treatment given to each individual dog. Dog owners were told to contact the research team veterinarian via the telephone number written on the consent form if they observed any adverse effects that were likely attributable to the inoculation.

Follow Up Visit

Post-vaccination blood sampling was carried out at day 28 (4 weeks) after the inoculation. The field team visited the same households, study dogs were identified via the microchip reader and blood samples were collected and stored in the same manner as at day 0. All dogs were then vaccinated with a cold-chain stored vaccine to ensure effective immunogenicity.

Sample Processing

Following storage in the cold box and transfer to the laboratory, all blood samples were kept at room temperature for 12 h before being centrifuged to separate the serum. Aliquots of serum (1 ml) were taken from each sample and were kept at −20°C prior to shipment to the Animal and Plant Health Agency (APHA, UK) for serological analyses.

Serological Analyses

The Fluorescent Antibody Virus Neutralisation assay (FAVN) was used to determine levels of antibody titres against rabies (23). In brief, serial 3-fold dilutions of the positive and negative serum controls as well as of the test serum were prepared in the microplates. Each serum was added to four adjacent wells and serially diluted four times. A 50-µL of a dilution of challenge virus standard (CSV-11) containing 50–200 TCID50/ml was then added to each serum dilution well. The microplates were incubated for 1-h at 37°C in a humidified incubator with 5% CO_2. Following incubation, 50-µL of the cell suspension with concentration of 4×10^5 BHK-21 cells/ml was added to each well and further incubated for 48 h at 37°C in a humidified incubator with 5% CO_2. After incubation, the plates were fixed in 80% acetone, dried, and stained with fluorescein isothiocyanate (FITC) anti-rabies monoclonal antibody (Fujirebio Diagnostics, Malvern, PA, USA). For each serum dilution, four wells were examined for the presence or absence of virus-infected cells. The 50% endpoint of the antibody (D50) content of test sera and virus titrations ($TCID_{50}$) were calculated according to the Spearman-Kärber method. To determine a titre, the control "cell only" wells needed a full monolayer of confluent cells >80%. If serum cytotoxicity was detected, the wells for that specific sample were not read and the sample was filtered and repeated. By international convention, this titre was converted to a value in IU/mL by comparison of results obtained with those of the positive standard. The level of rabies neutralising antibodies was expressed in International Unit per millilitre (IU/ml). A cut off value of 0.5 IU/ml was used to determine seroconversion (24).

Statistical Analysis

Titre measurements were statistically analysed using two-sample *T*-test. *Post-hoc* estimates of the differences between elevated storage conditions and the cold-chain stored vaccine were calculated with one-sided 95% confidence intervals. The null hypothesis, of elevated storage condition being inferior to the cold-chain storage, was rejected if the difference between the groups did not exceed $<1.2 \log_2$ titre units. Multivariate logistic regression analysis was used to determine whether storage conditions, BCS or age of dog impacted the likelihood of seroconversion (titres $\geq$ 0.5 IU/ml).

RESULTS

Study Population

The enrolment and vaccine group assigned to dogs is shown in the study flow chart (**Figure 1**). In total,

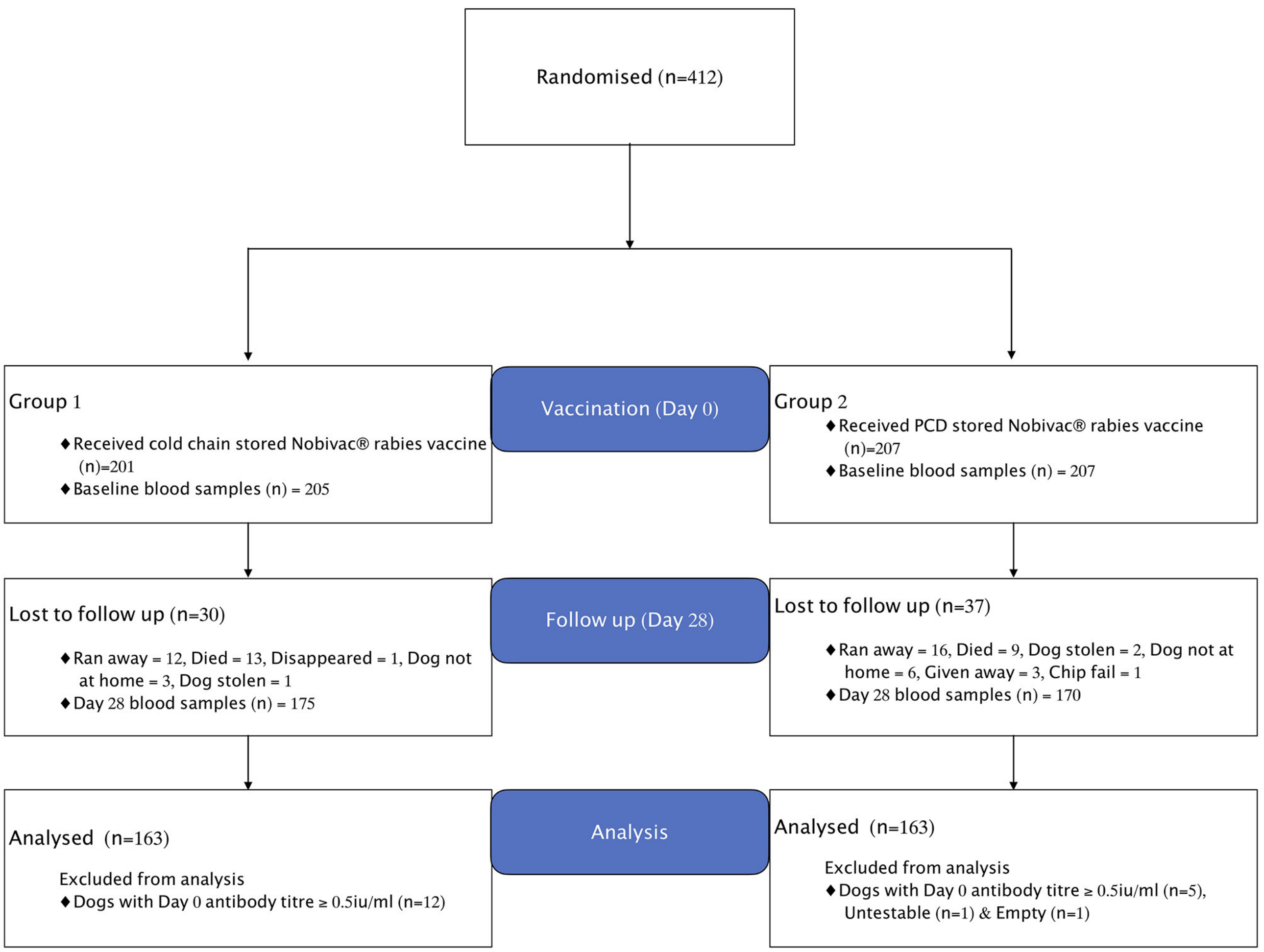

FIGURE 1 | Flowchart showing enrolment, treatments, and losses of dogs in the vaccine trial. Empty means vial without sample and Untestable means sample conditions were not appropriate for testing.

412 domestic dogs with no previous history of rabies vaccination were included in the study. Of these 412 dogs, 205 received cold-chain- and 207 received Zeepot-stored vaccine.

TABLE 1 | Summary showing the distribution of age, sex, and BCS of dogs in the two groups included in the final analysis.

Variable	Category	Group 1 (cold-chain stored vaccine)	Group 2 (Zeepot-stored vaccine)
Age (months)			
	0–3	12	15
	3–6	44	41
	6–12	52	53
	12–24	30	32
	24–36	11	10
	36–48	10	5
	48–60	2	2
	>60	2	5
Body condition score			
	1	28	47
	2	104	88
	3	31	28
	4	0	0
	5	0	0
Sex			
	Males	75	70
	Females	88	93

Vaccine-Related Adverse Events

During the follow up visit at day 28, a total of 22 dogs, 13 in Group 1 and nine in Group 2 were reported to have died. Of the 22 dogs, one died suddenly without showing any clinical signs and four dogs died due to fatal injuries caused by: severe bites from other dogs ($n = 1$), bites from a hyena ($n = 1$), being beaten by the owner ($n = 1$) and being trampled by cows ($n = 1$). The remaining 17 dogs died showing signs of illness, mostly within 2 weeks of the vaccination; 12 of these dogs (71%) were <6 months in age. Clinical signs included vomiting, diarrhoea, emaciation, coughing, anorexia, nasal discharges, dullness, and incoordination. Dogs in both groups presented similar clinical signs. Further investigation revealed that dogs from households not participating in the study within and outside the study villages exhibited similar clinical signs and died, suggesting that the cause of death among the study animals was not related to immunisation. Although the symptoms were suggestive of an infectious aetiology, a definitive diagnosis was not determined. Rabies could not be ruled out in four of the dogs that died, but none showed typical signs of rabies, although post-mortem samples were not collected for testing. No rabies cases were reported in any of the study villages of these dogs in the 2 years following the study, although four rabies cases were detected in the two of the villages in the 6 months prior to the study.

Lost to Follow Up and Post-inclusion Removal

Out of 412 dogs enrolled into the study at day 0, only 341 dogs were available at day 28 for re-sampling, with 67 lost to follow-up for the reasons shown in **Figure 1**. Seventeen out of

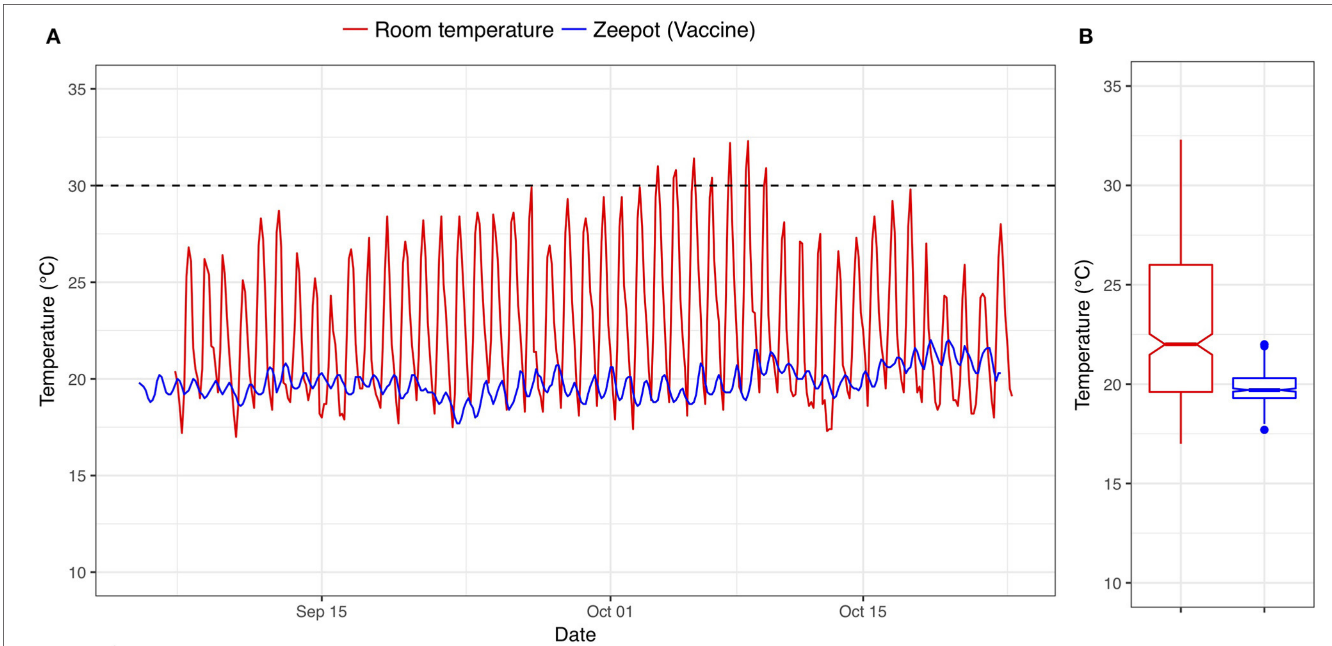

FIGURE 2 | (A,B) Time series and boxplot showing the temperatures of vaccines stored within the Passive Cooling Device (blue line) and the ambient room temperature (red line); dashed line indicates the maximum storage temperature below which Nobivac® Rabies vaccines have been shown to remain potent.

412 dogs sampled at day 0 were found to have pre-vaccination antibody titres ≥ 0.5 IU/ml and were thus excluded from the analysis. As a result, a total of 326 dogs were included in the final analyses, with 163 dogs in each treatment group as summarised in **Table 1** below.

Assessment of Vaccine Storage in the Zeepot

The room temperatures and the vaccine storage temperatures inside the Zeepot recorded over the 2-month storage period are shown in **Figure 2**. The mean room temperature was 22.84°C ranging from 17.00 to 32.2°C whereas the average temperature inside the Zeepot was 19.81°C fluctuating between 17.7 and 22°C. Despite ambient temperatures exceeding 30°C, the internal Zeepot temperature did not exceed 22°C, which was below the storage temperature at which the vaccines have been shown to remain potent (30°C) (14).

Day-28 Antibody Titre Comparison

The primary outcome variable was serological titre at day 28, and the predictor variable was the treatment group to which each dog belonged. The range of 28-day titres recorded for each group are shown in **Figure 3** and the 28-day $\log_2$ and geometric mean titres are shown in **Table 2**. The output from the T-test examining the difference in titre levels between the cold-chain stored vaccine group (Group 1) and the elevated-temperature stored vaccine group (Group 2) was 0.18 with the lower 95% confidence limit (one-sided) of −0.17. Since the lower limit of the confidence interval did not exceed the non-inferiority −1.2 $\log_2$ titre units, the null hypothesis that non-cold-chain stored vaccine are less potent than cold-chain stored vaccine was rejected.

The 28-day post vaccination group geometric mean titre was 1.8 and 2.0 IU/ml for cold-chain storage (Group 1) and non-cold-chain storage (Group 2), respectively.

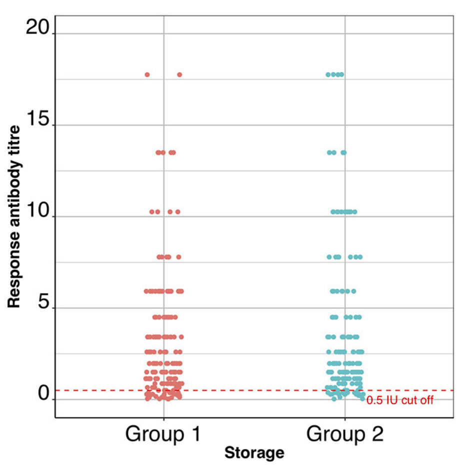

FIGURE 3 | A dot-plot showing the titres (IU/ml) of all the dogs in the two groups of the trial. The dashed red line indicates the 0.5 IU/ml cut off point above which dogs are considered to have seroconverted.

A secondary outcome variable was the percentage of dogs that seroconverted in each group (**Table 3**), which were almost identical (∼85%).

Exploratory Analysis: Impact of BCS and Age on Seroconversion at Day-28

Logistic regression analyses were performed to examine the impact that BCS, sex, and age might have on the likelihood of seroconversion. The results (**Table 4**) indicated that, in corroboration of the T-test results, storage conditions did not affect the proportion of dogs that seroconverted ($z = 1.1$, $df = 313$, $p\text{-value} = 0.25$). There was a positive linear trend between BCS (O.R. 2.2, 95% CI: 1.1–5.1) and seroconversion (**Figure 4**), implying that as BCS increased so did the likelihood of seroconversion. Neither sex nor age impacted seroconversion.

DISCUSSION

This randomised controlled field trial demonstrates that the Nobivac® Rabies vaccine stored within the Zeepot under

TABLE 2 | Group summary data–the number of dogs in each group and the day-28 log mean, standard deviation, and geometric mean values are shown; day-28 $\log_2$ mean and day-28 SD represent the average and standard deviation of the log (base 2) transformed data for each group.

Group	No. of dogs	Day-28 mean ($\log_2$)	Day-28 SD $\log_2$	Day-28 Geometric mean (IU/ml) $\log_2$
1	163	0.9	2.0	1.8
2	163	1.0	1.9	2.0

TABLE 3 | Group seroconversion data–the number of dogs in each group, the number that seroconverted (≥0.5 IU/ml) and the seroconversion percentage are shown.

Group	Total no. of dogs tested	No. of dogs seroconverted	% seroconversion
1	163	139	85.3
2	163	140	85.7

TABLE 4 | Multivariable logistic regression of a range of explanatory variables on the probability of seroconversion; Storage_Zeepot_ compares non-cold-chain storage with the baseline cold-chain storage; Age, age of dog; Sex_Male compares serological responses of male dogs with the baseline (female dogs); BCS, body condition score.

Variable	Odds ratio	95% CI	*P*-value
Storage_Zeepot	1.5	0.7–2.9	0.25
Age	1.1	0.9–1.6	0.35
Sex_Male	0.9	0.5–1.8	0.81
BCS	2.2	1.1–5.1	0.04*

**The p-value is significant at 0.05.*

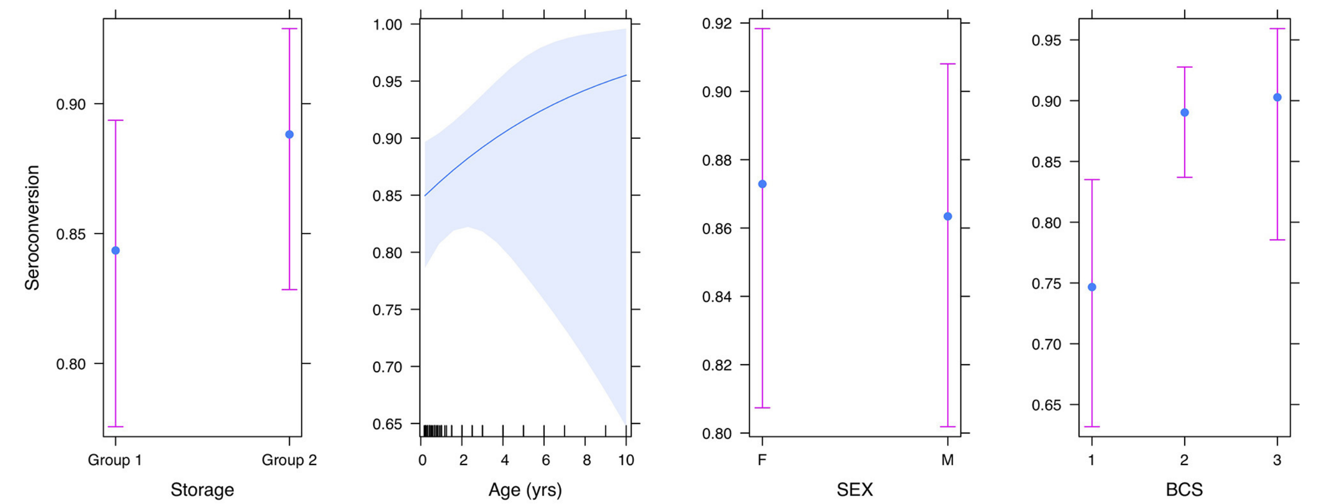

FIGURE 4 | Plot showing the effect of storage condition, age, sex, and body condition score on seroconversion.

fluctuating temperature conditions for up to 3 months elicited a neutralising antibody response equivalent to vaccine kept under stable cold-chain conditions. This conclusion is supported by the results from the logistic regression which showed storage condition had no effect on the seroconversion.

Following vaccination against rabies, dogs are considered protected when the immune system has elicited sufficient rabies virus-specific neutralising antibodies (RVSNA). RVSNA are a crucial component of adaptive immunity and contribute to the clearance of the viral infection (25). Globally, the antibody titre level of 0.5 IU/ml is recognised as the cut off above which humans, dogs and cats are classified as having developed a protective antibody response against rabies infection (24, 26). In this study, ∼85% of the dogs both in the cold chain and non-cold chain group passed this threshold (**Table 2**). This proportion is slightly less than reported in the preceding study by Lankester et al. in which 92% of vaccinated dogs successfully seroconverted (14). A possible explanation for this reduction could be the health status of enrolled dogs. In the Lankester et al. study the experimental conditions of the trial were carefully controlled with the vaccine storage temperature kept stable, the age of enrolled dogs kept between 6 months and 5 years and the BCS between 2 and 4. In contrast, the conditions in this field study were purposively less stringent so that the trial reflected natural conditions more closely. As a result, storage temperatures were allowed to fluctuate and dogs were enrolled regardless of their BCS and age. This allowed investigation of the impact that age and BCS might have on sero-conversion and resulted in the finding that there was a statistically significant effect of BCS on the likelihood of seroconversion, with dogs of good body condition being more likely to seroconvert than dogs with poor BCS. This supports a previous study that suggested that loss of body condition was associated with immunosuppression (27). As such, it is possible that the reduction in the percentage of dogs that seroconverted was due to the inclusion of dogs of low BCS.

Though the seroconversion was lower in dogs with low BCS it was still high; ∼76% of dogs with BCS of 1 and 2 seroconverted. To improve seroconversion, a booster vaccination on day 28 could be given, but further investigation is needed to understand whether this is necessary.

The other factor that could have resulted in the lower seroconversion as compared to the Lankester et al. study was the fluctuating storage temperatures. But, as the percentage that seroconverted was the same for both treatment groups (cold-chain and non-cold-chain stored vaccine) this difference does not appear to reflect either an impact of non-cold-chain storage or temperature fluctuations. This finding is important as it indicates that the thermotolerance of the Nobivac Rabies vaccine is not dependent upon constant storage temperature conditions, as used in the Lankester et al. study, but rather is sustained despite the fluctuating storage temperatures as shown in **Figure 2**.

While the current study was not able to generate data on the longevity of the immune response in the non-cold chain group, previous studies have shown that when RVSNA are produced the immune memory B-cell immunity persists even after RVSNA drop below 0.1 IU/ml (28). Since both treatment groups elicited similar levels of antibody response, it seems unlikely that dogs vaccinated with the non-cold chain stored vaccine will have significantly shorter duration of immunity compared to those vaccinated with cold chain stored vaccine.

The multivariate analysis also demonstrated that sex had no effect on the seroconversion or titre. This result is consistent with findings from previous studies Berndtsson et al. (29) and Mansfield et al. (30).

The finding that the potency of the Nobivac vaccine was not impacted following storage in the Zeepot for the duration of 3 months has important implications for developing new immunisation strategies in remote or hard-to-reach areas facing logistical challenges in the transportation and storage of vaccine. As a result, decentralised storage strategies, involving vaccines being stored outside of cold-chain conditions for up to 3 months can be designed, implemented and tested for cost-effectiveness with the knowledge that such storage will not impact potency. Thermotolerance was a key factor that contributed to the successful campaigns to eradicate smallpox and rinderpest (31, 32). For example, with a shelf life of 8 months when kept at 37°C, it was possible to keep the rinderpest vaccine outside the cold chain for 30 days at a time, which gave sufficient time for vaccinators to travel on foot to implement vaccinations in extremely remote areas. This would not have been possible if conventional vaccine storage of 2–8°C was required. Likewise, the vaccination campaign against meningitis using the thermotolerant MenAfriVac's vaccine delivered in a controlled temperature device resulted in very high population coverage levels being achieved (11). The use of thermotolerant rabies vaccines stored without the need for electrical power in locally made Zeepots, outside of cold chain conditions, could bring similar benefits, in coverage and cost-effectiveness, to the control of dog-mediated human rabies.

CONCLUSION

The results obtained in this study clearly demonstrated that the Nobivac® Rabies vaccine kept in locally made Zeepots elicited protective neutralising antibody responses equivalent to those from vaccine stored under cold chain conditions. These findings will help in the design of cost-effective vaccine delivery strategies for use in resource-poor settings and will be of benefit to nations designing national elimination plans in an attempt to hit the international commitment of zero human deaths by 2030, "Zero by 30" (33).

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and Use Committee, Washington State University and Sokoine University of Agriculture. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

AL, KH, and FL made substantial contributions to the study design. AL, AC, and MB collected the data. AL, FL, KH, LM, and DM analysed and interpreted the data. AL led the writing of the manuscript, which all authors contributed to. AL, FL, RK, and KH contributed to reviewing and proof-reading the manuscript as well as interpretation and synthesis. All authors read and approved the version of the manuscript to be published.

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Serological Surveillance of Rabies in Free-Range and Captive Common Vampire Bats *Desmodus rotundus*

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The control of vampire bat rabies (VBR) in Brazil is based on the culling of *Desmodus rotundus* and the surveillance of outbreaks caused by *D. rotundus* in cattle and humans in addition to vaccination of susceptible livestock. The detection of anti-rabies antibodies in vampire bats indicates exposure to the rabies virus, and several studies have reported an increase of these antibodies following experimental infection. However, the dynamics of anti-rabies antibodies in natural populations of *D. rotundus* remains poorly understood. In this study, we took advantage of recent outbreaks of VBR among livestock in the Sao Paulo region of Brazil to test whether seroprevalence in *D. rotundus* reflects the incidence of rabies in nearby livestock populations. Sixty-four *D. rotundus* were captured during and after outbreaks from roost located in municipalities belonging to three regions with different incidences of rabies in herbivores. Sixteen seropositive bats were then kept in captivity for up to 120 days, and their antibodies and virus levels were quantified at different time points using the rapid fluorescent focus inhibition test (RFFIT). Antibody titers were associated with the occurrence of ongoing outbreak, with a higher proportion of bats showing titer >0.5 IU/ml in the region with a recent outbreak. However, low titers were still detected in bats from regions reporting the last outbreak of rabies at least 3 years prior to sampling. This study suggests that serological surveillance of rabies in vampire bats can be used as a tool to evaluate risk of outbreaks in at risk populations of cattle and human.

Keywords: rabies, serology, RFFIT, vampire bats, antibodies, virus neutralizing antibodies

INTRODUCTION

Rabies is an infectious disease of viral etiology that causes acute encephalitis, with rapid and usually fatal evolution in all mammals (1). The etiological agent is the RNA virus belonging to the family *Rhabdoviridae* and genus *Lyssavirus*, and it is a zoonosis distributed worldwide that now affects mainly low-income countries (2). Rabies virus is transmitted by contact with infected saliva through bites or scratches. Dogs are the main reservoir of rabies and are responsible for most fatal cases in humans worldwide (2). However, in Latin America, with success obtained by the countries to control rabies mediated by dogs through the mass vaccination campaign (3, 4), bats have become the main reservoir of rabies over the last decade and are responsible for thousands of cases in livestock and for most of the remaining cases in humans (1, 4). Within bats, the common vampire bat *Desmodus rotundus* is the most important reservoir because of the high occurrence of spillover of rabies from this species of bat to other animals and humans, and vampire bat rabies (VBR) remains unpredictable and uncontrolled in several areas of the continent.

By feeding every night on livestock, VBR causes significant economic losses in Latin America, particularly to small-scale farmers (5). *D. rotundus* can also feed on the blood of human beings, resulting in occasional and unpredictable outbreaks of VBR in remote settings such as the Amazon region (6). The epidemiology of VBR circulation among *D. rotundus* that results in rabies cases among livestock is driven by several natural factors that are still poorly understood. Anthropogenic and ecological features favor the presence of bats (e.g., distribution of cattle herd and land occupation) (7), and factors favoring VBR among bats are also involved. However, understanding VBR circulation requires the capacity of measuring rabies exposure or infections within the bat population.

Most studies understanding rabies circulation among bats rely on official reports of livestock mortality, but this data is often biased by variable levels of under-reporting across the landscape (8, 9). Herbivores are accidental hosts of the rabies virus. Because they are a dead-end host, they only contribute as sentinels in the existence of the virus in the bat population (7). Despite its limitations, studies based on livestock mortality have shown that rabies can circulate in the landscape in different ways including wave-like spread into new areas, metapopulation dynamics, or endemically (9–11). However, the dynamics of the virus in endemic areas where the virus has been established for longer periods of time is less understood. Therefore, surveillance of rabies in bats, although logistically challenging, can bring unique insides into the circulation of the virus and our ability to predict and prevent future outbreaks in humans and livestock (9, 11).

The recognition of bats as reservoirs of the disease made epidemiological surveillance extend to these species. Rabies virus is rarely isolated from an infected bat because infected bats are often lower than 1% of the population (11). Because the presence of anti-rabies antibodies correlates with exposure to the virus, serological studies can contribute to understanding VBR circulation among bats. Several studies have shown the presence of antibodies to rabies in bats that do not die from the disease, as seen in other animals and also in humans (12, 13), implying that exposure to rabies in this species does not necessarily leads to mortality (14). For example, in the Botucatu region of Brazil, an endemic region of VBR, 45% of *D. rotundus* vampire bats had virus neutralizing antibodies (VNA) titers ranging from 0.10 to 0.20 IU/ml, 9.31% had between 0.20 and 0.3 IU/ml, and 11% had VNA levels >0.30 IU/ml (14). Similarly, *D. rotundus* bats from 16 colonies were tested in the endemic region of Paraiba

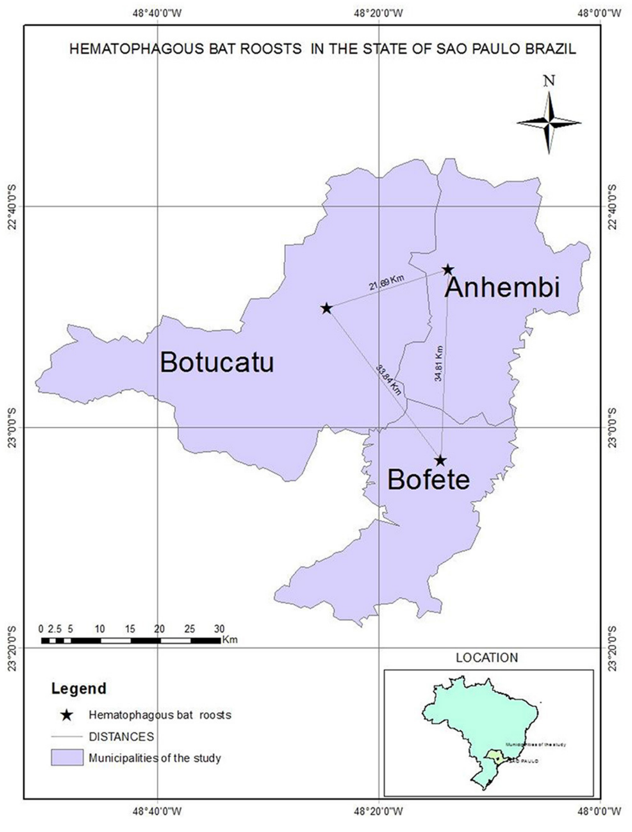

FIGURE 1 | Map showing the three municipalities with different epidemiological scenarios.

in Brazil and all animals presented anti-rabies antibodies titers in some level, with titers higher than 0.5 IU/ml in 30.1% of bats (15). Delpietro et al. (16) reported the detection of 30% antibodies in bats after an outbreak of bovine rabies in Argentina. Before the outbreak, 2% of the bats had the antibodies, and during the outbreak, the percentage increased to 4%, suggesting that the presence of high proportion of seropositivity results from a high circulation of the VBR among bats at the time of an outbreak.

Here, we took advantage of recent outbreaks of VBR among livestock in the Sao Paulo region of Brazil to further test whether seroprevalence in *D. rotundus* reflects the incidence of rabies in bat populations. Field work was combined with captive experiments to follow seropositive animals and increase our understanding of within-host anti-rabies antibodies dynamics among *D. rotundus*.

MATERIALS AND METHODS

Virus Neutralizing Antibodies (VNA) in *D. rotundus* Bats During and After Outbreaks

We captured and collected samples of 64 *D. rotundus* from December 2015 to June 2016 from colonies in areas with three different epidemiological scenarios (**Figure 1**) of outbreaks in livestock of nearby farms attacked by these bats (according to the Ministry of Agriculture, Livestock and Supply from Brazil). Bats were aleatorily captured using nets installed outside the roost. In the first location, 23 bats were caught from roosts located in the University farm of UNESP, Botucatu, São Paulo. This farm just had a rabies outbreak 1 month before sampling involving seven cattle and horses. A second collection of five bats was performed 30 days after in this same spot. The second site was Bofete, where eight bats were captured and the nearby farms had reported rabies among livestock in 2013 and 2016. After 30 days, 16 bats were captured in the same farms. The third site of capture was Anhembi where six bats were captured and rabies has been reported on livestock in 2015 for the last time. After 30 days, six more bats were captured in the same place.

Serum Sampling and Rabies Neutralizing Antibody Testing

From the second capture, six bats from Anhembi, four bats from Botucatu, and six from Bofete were aleatorily separated and kept in captivity to study their rabies antibodies for 4 months. The animals were quarantined under observation in isolated cages in a controlled environment in the animal facility of the infectious diseases sector—School of Veterinary Medicine and Animal Science-UNESP/Botucatu (approved by the Ethical Committee of Animals Uses in Veterinary Medicine and Animal Production of São Paulo State University "Julio de Mesquita Filho," number 85/2015, and SISBIO (Biodiversity Authorization and Information System) license number 51231). Animals were fed with defibrinated bovine blood negative for anti-rabies antibodies using automatic drinking bottles, in addition to daily cleaning of the cages. In all bats captured in nature and not kept in captivity, blood samples were followed by euthanasia using halothane anesthetic induction and deepening of the anesthetic plane with 0.1 ml intraperitoneal ketamine. Bats kept in captivity were subjected to monthly blood collection with anesthetic induction. Blood was collected from the saphenous vein using needles 25 G ½ (0.45 × 13 mm) and capillaries (microhematocrit) with prior asepsis with 70% alcohol. Sera were stored at −20°C until antirabies neutralizing antibodies titer evaluation by the rapid fluorescent focus inhibition test (RFFIT) (17). For the RFFIT (18), a constant dose of a previously titrated (calibrated to give 80% fluorescent focus infected cells) cell culture-adapted RABV challenge virus (CVS) was incubated with 3-fold dilutions of the sera. After incubation of the serum-virus mixtures, a suspension of a clone of BHK-21 (BSR) cells was added. After 20 h of incubation, the cell monolayer was acetone-fixed and labeled with anti-rabies virus antibody conjugated to FITC (19). The optimal challenge dose (the dilution giving 80% infected cells for each virus production) was calculated. Titers of sera were calculated by comparison with a reference serum calibrated to the WHO reference serum. A minimal threshold of 0.5 IU/ml was considered for protection.

Animals that died during observation period (7) and those euthanized on the day of capture (or at the end of the 4 month period) were subjected to the same protocols of cardiac puncture for collection of blood used for RFFIT, and brain was submitted to qRT-PCR for rabies diagnosis as previously described (20), resulting all negatives to rabies.

Given the low number of samples from each type of colony, we used a Pearson's chi-squared test using the R function prop test to compare the proportion of seropositive individuals between day 0 and day 30 of sampling and between sampling locations.

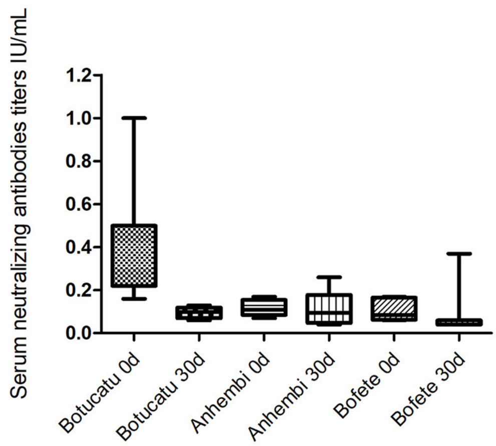

FIGURE 2 | Geometric mean titers (GMT) of virus-neutralizing antibodies in the bats from the studied municipalities. Municipalities with different scenarios evaluated at 0 and 30 days.

RESULTS

Virus Neutralizing Antibodies (VNA) During and After the Outbreak

The highest rabies antibodies titers were obtained from bats captured in Botucatu (0.45 ± 0.05), which had the most recent outbreaks. In this area, 13 animals out of 23 had protective antibody titers (0.64 ± 0.05). Average titer concentrations in the bats captured 30 days after (0.09 ± 0.01) decreased significantly ($p = 0.0075$), and none of those five bats had protective titers. Bats from Anhembi and Bofete had a similar average concentration of titers (0.11 ± 0.01 and 0.10 ± 0.01; $p = 0.51$), respectively, that were lower than in Botucatu (0.45 ± 0.05) at the first capture ($p < 0.0001$). However, bats from Anhembi maintained the same level of titer 30 days later (0.11 ± 0.03), whereas Bofete presented a titer >10 IU/ml, which increased the overall average concentration (0.70 ± 0.63) (**Figure 2**). Considering that this bat could interfere with the biological interpretation of our results, we run another statistics test excluding its titer (>10 IU/ml) to the analysis. The statistical analysis showed a significant difference of the VNA titers from bats captured in Bofete in both moments ($p = 0.0129$). Overall, the proportion of seropositive animals, on day 0, was higher in Botucatu compared to either Anhembi and Bofete (Pearson's test, $p < 0.01$), and there was no significant difference in the proportion of seropositive individuals between day 0 and 30 in neither of three locations (Pearson's test, $p > 0.05$). All captured bats were apparently in a good sanitary condition, and no clinical

TABLE 1 | Antirabies neutralizing antibodies titers in *Desmodus rotundus* bats captured, from December 2015 to june 2016, in geographic areas with different epidemiological situation for rabies among livestock.

***Desmodus rotundus* bats**	**Botucatu 0 d**	**Botucatu 30 d**	**Anhembi 0 d**	**Anhembi 30 d**	**Bofete 0 d**	**Bofete 30 d**
1	0.50	0.13	0.17	0.13	0.06	0.06
2	1.00	0.06	0.07	0.26	0.15	0.04
3	0.22	0.11	0.12	0.15	0.17	0.04
4	0.66	0.08	0.09	0.04	0.07	0.12
5	0.25	0.10	0.10	0.06	0.08	0.04
6	0.33		0.15	0.05	0.06	0.37
7	0.66				0.09	0.04
8	1.00				0,17	0.04
9	0.50					0.06
10	0.50					0.04
11	1.00					0.04
12	0.22					0.04
13	0.16					0.04
14	0.16					0.05
15	0.50					0.04
16	0.22					10.22
17	0.50					
18	0.50					
19	0.50					
20	0.22					
21	0.50					
22	0.16					
23	0.16					
Median ± sd	0.45 ± 0.05	0.09 ± 0.01	0.11 ± 0.01	0.11 ± 0.03	0.10 ± 0.01	*0.07 ± 0.02
Seropositive/total bats captured	**13/23**	**0/5**	**0/6**	**0/6**	**0/8**	**1/16**
Cattle outbreaks in nearby farms	**Recent outbreak of rabies (1 month before)**		**Rabies reported in 2015**		**Rabies reported in 2013 and 2016**	

**Median ± sd excluding the bat with titer 10.22. Bold values represents collection not performed due to technical difficulty or death of animals.*

signs were observed. Details on specific VNA are given in **Table 1**, **Figure 2**.

Virus Neutralizing Antibodies (VNA) Profile of Bats Kept in Captivity

During the observation period, seven bats died and resulted negative by rabies virus qRT-PCR test, so it was possible to have the serological profile from nine bats during the 120 days, one bat from Botucatu, and four bats from Bofete and Anhembi, respectively. The serological profile of bats kept in captivity showed a constant decrease in the titer levels in all animals (**Table 2**, **Figure 3**) up to days 120, where all titers were lower than 0.10 ($p < 0.0001$). The statistical analysis of the VNA titers from day 0 compared to 60 days showed a significant difference ($p = 0.0009$), and the same result was obtained when comparing to 90 days ($p = 0.0017$) and 120 days ($p = 0.0004$). Statistical significance was also evidenced comparing 30–60 days ($p = 0.0017$), 90 days ($p = 0.0090$), and 120 days ($p = 0.0004$), demonstrating a decrease in the levels of VNA in bats in captivity. Although two bats showed an increase in the VNA at 30 days, it is not possible to make any conclusion due to the low number of animals studied.

DISCUSSION

Serological studies of VBR comparing populations of bats with different scenarios of rabies outbreaks in cattle are rare but crucial to understand within-bat dynamics of rabies. We found that vampire bats from the region of Botucatu, which had experienced a recent outbreak of rabies in cattle 30 days prior to sampling, showed a high proportion of seropositive individuals compared to two other sampling sites where the latest outbreak in cattle was reported at least 3 years prior to sampling. In Botucatu, 30 days after the outbreak, half of the bats had antibodies considered to be protective against rabies, which decreased in proportion 60 days after the outbreak in the region. Seropositive animals kept in captivity showed a variable decreased in titer after 4 months, suggesting a rapid loss of antibodies to non-protective levels. Our study suggests that rabies serology in bats can be an accurate indicator to identify if rabies had circulated in bats sampled.

TABLE 2 | Antirabies antibodies titers from bats captured in regions with different epidemiological situation.

Bat	Origin	0 d	30 d	60 d	90 d	120 d
1	Botucatu	0.13	0.09	0.04	0.04	0.04
2	Anhembi	0.17	0.07	0.05	0.08	0.04
3	Anhembi	0.12	0,11	0,04	0,1	0,04
4	Anhembi	0.1	0.26	0.04	0.04	0.04
5	Anhembi	0.15	0.08	0.04	0.04	0.04
6	Bofete	0.15	0.37	0.1	0.09	0.06
7	Bofete	0.17	0.15	0.04	0.04	0.04
8	Bofete	0.08	0.07	0.04	0.04	0.04
9	Bofete	0.09	0.1	0.04	0.06	0.04
Median ± sd		0,12 ± 0.01	0.14 ± 0.02	0.04 ± 0.019	0.05 ± 0.02	0.04 ± 0.02

Botucatu: Recent outbreak of rabies (1 month before); Anhembi: Rabies reported in 2015; Bofete: Rabies reported in 2013 and 2016.

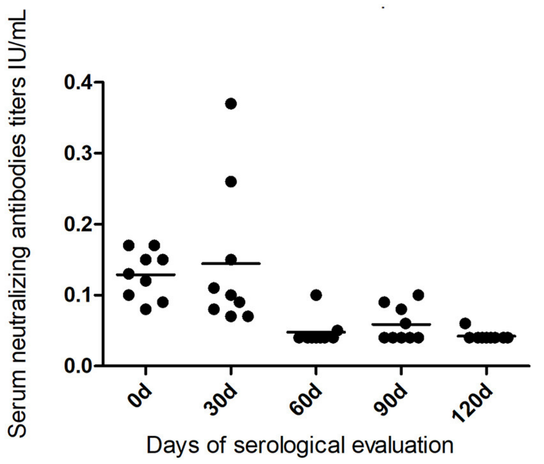

FIGURE 3 | Serological profile of virus-neutralizing antibodies in the bats kept in captivity for 120 days from the studied municipalities.

The high proportion of antibodies considered as protective for rabies among bats sampled in Botucatu suggests the presence and circulation of the virus (8, 11) and corroborates cases reported among livestock in nearby farms. Although the second collection of bats 30 days later was much smaller ($N = 5$), there was a decrease on the proportion of seropositive animals. This decrease suggests that not all bats in a colony are exposed to the virus and that rabies does not necessarily persist within a colony after an outbreak (16).

Although outbreaks from cattle in Bofete and Anhembi were not reported for at least 3 years, antibodies were detected among bats in the first capture in both colonies, but not high enough to be considered protective (<0.5 IU/ml). This could reflect recent exposure to low levels of the virus that does not result in disease symptoms including exposure shortly after birth, subclinical or asymptomatic infections, sublethal infection, carrier state, or latent virus activated by stress (11, 21–23). In fact, Blackwood et al. (11) suggested that the probability of developing a lethal infection upon exposure to rabies is low in vampire bats (∼10%), which enables viral persistence in the slowly reproducing bat colonies. This is also observed in other bats species (14, 24).

Low levels of antibodies within a bat colony without recent exposure could also result from long-term persistence of antibodies if rabies exposure happened several years in that colony (8, 25). However, the duration of rabies antibodies after exposure without re-exposure remains poorly understood for vampire bats. In this study, anti-rabies antibodies decreased to low levels 60 days after capture of seropositive individuals but fluctuations and sudden increases were also observed in some individuals. Antibodies fluctuation has been reported in free bats but explained by re-exposure to the virus, which was not possible in our laboratory settings (26). This result suggests possible rabies virus persistence in the bat tissue with periodic antigenic stimulation. This calls for future studies on the mechanisms behind anti-rabies antibody production and on the immunological and protective consequences of having low levels of antibodies in healthy bats.

The increase of anti-rabies antibodies titers on the second capture observed in Bofete suggests that rabies circulation can be detected in the bat population despite cases not been reported among livestock. This highlights the possibility of using bat serology surveillance to predict risk in that population and therefore affects livestock in the region too. Therefore, cost-effective analysis and more understanding on the serological behavior of bat population are needed to evaluate whether rabies serological surveillance among bats can be added as a tool to improve our understanding and prevention of rabies risk in both livestock and humans.

Overall, our study shows that anti-rabies antibodies among vampire bats could reflect the current risk of rabies outbreaks among nearby cattle. Furthermore, we observed a decrease in antibody titers 2 month after animals had high titers, although titers fluctuated in time and between individual bats. Future studies are required to assess whether the observed titer levels >0.5 IU/ml are indeed protective against rabies on bats and whether low-titers can also reflect rabies circulation among bats and subsequent risk to livestock and humans.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was reviewed and approved by CEUA FMVZ.

AUTHOR CONTRIBUTIONS

FC, LB, BR, CA, and JM Bats capture, blood collection and work execution. JB, JM, and LB Foreground research, writing and editing. IK, KS, SS, AR, GB, and LA Serology. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2021.681423/full#supplementary-material

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Immunogenicity of the Oral Rabies Vaccine Strain SPBN GASGAS in Dogs Under Field Settings in Namibia

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Dog-mediated rabies is endemic throughout Africa. While free-roaming dogs that play a crucial role in rabies transmission are often inaccessible for parenteral vaccination during mass dog vaccination campaigns, oral rabies vaccination (ORV) is considered to be a promising alternative to increase vaccination coverage in these hard-to-reach dogs. The acceptance of ORV as an efficient supplementary tool is still low, not least because of limited immunogenicity and field trial data in local dogs. In this study, the immunogenicity of the highly attenuated 3rd-generation oral rabies vaccine strain SPBN GASGAS in local free-roaming dogs from Namibia was assessed by determining the immune response in terms of seroconversion for up to 56 days post-vaccination. At two study sites, free-roaming dogs were vaccinated by administering the vaccine either by direct oral administration or *via* a vaccine-loaded egg bait. Pre- and post-vaccination blood samples were tested for rabies virus neutralizing as well as binding antibodies using standard serological assays. A multiple logistic regression (MLR) analysis was performed to determine a possible influence of study area, vaccination method, and vaccine dose on the seroconversion rate obtained. About 78% of the dogs vaccinated by the oral route seroconverted (enzyme-linked immunosorbent assay, ELISA), though the seroconversion as determined by a rapid fluorescence focus inhibition test (RFFIT) was much lower. None of the factors examined had a significant effect on the seroconversion rate. This study confirms the immunogenicity of the vaccine strain SPBN GASGAS and the potential utility of ORV for the control of dog-mediated rabies in African dogs.

Keywords: Africa, dogs, rabies, oral vaccination, SPBN GASGAS, neutralizing antibodies, binding antibodies

INTRODUCTION

Rabies is one of the zoonotic tropical diseases that remains neglected until today, with tens of thousands of humans still dying of rabies, mostly infected by rabid dogs (1). With highly efficacious rabies diagnostics as well as biologicals available, i.e., pre- and post-exposure prophylaxis, human rabies is clearly preventable. Also, broad-scale canine rabies elimination is both epidemiologically and operationally feasible and could be achieved across a wide range of settings in Africa and Asia (2). As a consequence, a global concept to eliminate dog-mediated human rabies by 2030 was initiated by the international rabies community and its stakeholders (3, 4). Using a true One Health concept, the Global Strategic Plan considers to control the disease at its animal source, the dog. It may appear straightforward to the outside world to vaccinate dogs. However, considering the differing socio-cultural and environmental conditions across canine rabies endemic regions in the world, it remains a challenge to achieve herd immunity in the population so that transmission stops. Mass dog vaccinations (MDV) using parenteral inactivated vaccines have been the primary means of rabies control in dogs. However, outside of North America (5, 6), Europe (7), and Latin America where this approach has been used successfully (7, 8), there are only a few examples of success for Asian countries including Japan, South Korea, Singapore, and Taiwan (9–12) where the same applies. In contrast, in Africa, sustained success of MDV using parenteral vaccines is still scarce.

The central problem in Asia and Africa are huge numbers of free-roaming dogs that are often inaccessible for parenteral vaccination during MDV campaigns (13). To increase vaccination coverage in these hard-to-reach dogs, the concept of oral rabies vaccination (ORV) of dogs as a complementary tool to mass parenteral dog vaccination has been proposed (14). Unfortunately, it is still the most underused of all tools in the fight against dog-mediated rabies (15), however, the concept has gained reviving interest in recent years (16–20). In fact, by targeting free-roaming and partly inaccessible dogs, ORV does not only increase the vaccination coverage of the overall dog population (16, 21) but also reaches those animals that are considered to play a key role in the transmission of rabies (16, 22, 23). While there are numerous oral rabies vaccines available (24), only vaccines with the highest safety profile that have been licensed for wildlife in accordance with international standards (25) prequalify for use in dogs. Next to safety assessments, immunogenicity studies and field trials are considered an essential part for either conditional or full-fledged licensure of oral rabies vaccines for dogs (15). However, there are still very limited experimental and field data for local free-roaming dogs available.

Therefore, the objective of this study was to assess the immunogenicity of SPBN GASGAS, a 3rd-generation vaccine licensed for wildlife (26, 27), in free-roaming dogs under African field settings. We aimed at determining the immune response post-vaccination after administration of the vaccine *via* an egg-flavored bait or direct oral administration. Also, we wanted to elucidate the impact of study area, vaccination method, and vaccine dose on the seroconversion obtained.

MATERIALS AND METHODS

Study Design

The study sites were located in two different rural communities, Groot Aub and Ongombo West, about 56 km south and 42 km northeast of Windhoek, Namibia, respectively. Vaccinations were carried out in Groot Aub and Ongombo West at the end of February and end of September 2020, respectively. Owners were asked to bring their dogs to a central vaccination point established in both of the study sites for vaccination at a predetermined date that had been previously announced. All dogs were identified by morphological features, owner details, and two photographs and assigned a study number.

TABLE 1 | Seroconversion of local Namibian dogs after oral (bait and d.o.a.) and parenteral vaccination according to RFFIT (≥0.5 IU/ml) and ELISA (≥40% PB).

Route of administration	rVNA (RFFIT)			rVBA (ELISA)		
	n	***N***	**%**	***n***	***N***	**%**
Oral	18	34	52.94	26	33	78.79
Bait	13	26	50.00	20	26	76.92
d.o.a.	5	8	62.50	6	7	85.71
Parenteral	2	2	100.00	2	2	100.00

Only animals that tested seronegative prior to vaccination are included (n, number of animals that tested seropositive; N, number of animals tested; ELISA, enzyme-linked immunosorbent assay; rVBA, rabies-specific binding antibodies; RFFIT, rapid fluorescence focus inhibition test; rVNA, rabies virus-neutralizing antibody; d.o.a., direct oral administration).

After the general health conditions were checked by a veterinarian, dogs were vaccinated with the oral rabies vaccine strain SPBN GASGAS (26–28) with a titer of 10∧7.5 or 10∧7.1 FFU/ml, using an egg-flavored bait with the vaccine filled in a soft blister (3.0 ml) (**Tables 1**, **2**). The egg bait was essentially the same as used in field studies in local dogs from other areas (29–31) or large-scale oral rabies field trials in dogs from Thailand (32). For comparison, a few dogs were vaccinated by direct oral application (d.o.a., 3.0 ml), when they refused to consume a bait, or parenterally using a commercial inactivated vaccine (RABISIN® ad us. vet., Boehringer Ingelheim, Germany), with the latter serving as positive controls. Bait uptake and blister perforation were closely monitored.

Blood samples were collected -14 (B0) days pre-vaccination and 28 (B1) days post-vaccination (dpv), while at the time point of vaccination (V), no blood samples were taken. The dogs were manually restrained for blood collection from the cephalic or the jugular vein. Subsequently, blood samples were labeled with the dogs' study number and placed in a cooling box for transportation. After arrival in the laboratory, all blood samples were centrifuged at 1,000 *g* for 10 min within 24 h of collection, aliquoted, and the serum stored at −20°C until serological testing.

In order to obtain an acceptable sample size per study area, dog owners in Ongombo West willing to participate with their dogs in the study were paid an incentive for following the study protocol. Because of unforeseen circumstances due to a sudden implementation of COVID-19 restrictions, blood sampling at 28 dpv was impaired, so study sites were revisited 56 dpv in an attempt to obtain a complete set of samples from all vaccinated dogs.

Diagnostic Assays

Prior to testing, sera were inactivated at 56°C for 30 min. Sera were tested for rabies-specific binding antibodies (rVBA) in a commercial blocking enzyme-linked immunosorbent assay (ELISA) (BioPro Rabies ELISA, Czech Republic) essentially as

TABLE 2 | Parameter estimates from the multiple logistic regression model (linear without interactions) indicating factors associated with seroconversion (ELISA and RFFIT) in local dogs in Namibia.

	rVNA (RFFIT)			rVBA (ELISA)		
	Coefficient	**Odds ratio**	**95% CI**	**Coefficient**	**Odds ratio**	**95% CI**
Factors						
Intercept	1.216	3.373	1.029–15.100	1.699	5.466	1.455–35.500
Study area						
Ongombo West	−2.850	0.058	0.006–0.347	−1.119	0.327	0.037–2.150
Route						
d.o.a.	1.115	3.175	0.456–31.570	0.856	2.353	0.285–51.840
Dose						
High	1.453	4.274	0.500–47.020	0.919	2.506	0.259–57.940

Groot Aub, bait, and low dose were set as reference in MLR; 95% CI, 95% confidence interval of odds ratio.

described (33, 34) by using positive (PC) and negative controls (NC) provided by the manufacturer and following validity parameters and characteristics as stated in the kit insert.

Additionally, sera were tested for the presence of rabies virus-neutralizing antibodies (rVNAs) in a modified rapid fluorescence focus inhibition test (RFFIT) using RABV (CVS-11) as test virus and BHK21-BSR/5 (Collection of Cell Lines in Veterinary Medicine—catalog N° CCLV-RIE 0194/260) cells with VNA titers expressed in international units per milliliter (IU/ml) as described (35). The calibrated WHO international standard immunoglobulin (2nd human rabies immunoglobulin preparation, National Institute for Standards and Control, Potters Bar, UK) adjusted to 0.5 IU and a naive dog serum served as PC and NC, respectively.

Sera were considered seropositive for rVBA if sera showed a percentage of inhibition compared to the negative serum >40% in ELISA or for rVNAs if titers were $\geq$0.5 IU/ml (25, 36).

Statistical Analysis

A multiple logistic regression (MLR) analysis was performed for seroconversion in the orally vaccinated dogs. It was determined if the study area (Groot Aub or Ongombo West), vaccination method (bait or d.o.a.), and vaccine dose (low or high) had an influence on seroconversion as determined by ELISA and RFFIT. For the MLR analysis, GraphPad Prism 9.0 (GraphPad Software Inc., San Diego, CA, USA) was used.

Ethical Considerations

This preliminary study was part of a larger approved project on oral vaccination of dogs in Namibia sponsored by the German Ministry of Health under the Global Health Protection Programs (Project number Ri-0755) and implemented as an agreed activity in the frame of the Official National Dog Rabies Control Program issued by the Namibian Directorate of Veterinary Services (DVS) at the Ministry of Agriculture, Water, Forestry and Land Reform (MoA). In this respect, the study was regarded a disease control trial, and ethical committee approval was not warranted. The study was approved by the appropriate authority within the MoA, Namibia. Manipulations such as vaccination and blood sampling of dogs were only conducted after elaborate explanatory meetings and under the premise that the dog owner had previously given his/her written consent. Institutional biosecurity and safety procedures for handling vaccines, samples, reagents, and virus were followed.

RESULTS

A total of 85 local Namibian dogs were initially included and bled (B0) from both study sites; Groot Aub ($N = 51$) and Ongombo West ($N = 34$). However, on the days of vaccination, 28 dogs were not brought to the vaccination sites. Hence, a total of 57 dogs were vaccinated; 36 dogs were offered a bait, 12 dogs received the oral rabies vaccine by d.o.a., and 9 dogs were vaccinated by the parenteral route. For 10 dogs (27.78%) offered a bait, an impaired uptake was observed. These dogs either initially refused to take a bait and it needed two or three attempts before they accepted it or hastily consumed the bait offered before swallowing the sachet during bait consumption; the other 26 dogs discarded the perforated sachet.

Unfortunately, from 17 (29.82%) of the 57 treated dogs, no blood sample could be collected after vaccination. Of the 40 remaining dogs, four dogs each tested positive for rVBA and rVNA prior to vaccination (B0) and were omitted from statistical analysis. Furthermore, no blood sample was collected from three of 36 remaining dogs on 28 dpv, and therefore, the results of the blood sample collected on 56 dpv from these three dogs were used.

The seroconversion rate of the dogs vaccinated is shown in **Table 1**. Almost 80% of the dogs vaccinated by the oral route seroconverted (ELISA), and the seroconversion rate using the 0.5 IU/ml cut-off for the RFFIT was considerably lower at 53% (**Figure 1**). Six of the eight dogs that swallowed the sachet during bait consumption after offering them a bait and were bled at 28 dpv showed rVBA in ELISA, while only two had rVNA titers $\geq$0.5 IU/ml. As we only have two data sets for dogs vaccinated by the parenteral

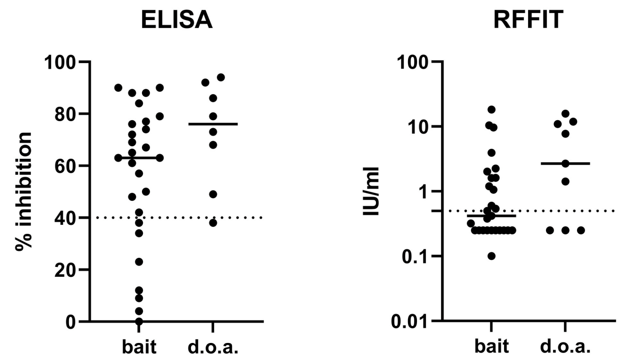

FIGURE 1 | The immune response measured by ELISA (rVBA) and RFFIT (rVNA) after oral bait or direct oral administration (d.o.a.). Of several samples that tested negative in RFFIT, no exact value could be estimated (<0.5 IU/ml); thus, an average value of 0.25 IU/ml (range 0–0.5 IU/ml) was set for these samples. The solid lines indicate the means of the measured values in ELISA and RFFIT, while the cut-off levels for both serological tests are indicated by the dashed lines. ELISA, enzyme-linked immunosorbent assay; rVBA, rabies-specific binding antibodies; RFFIT, rapid fluorescence focus inhibition test; rVNA, rabies virus-neutralizing antibody.

TABLE 3 | Seroconversion rate of dogs after oral [bait and direct oral administration (d.o.a.)] vaccination according to RFFIT ($\geq$0.5 IU/ml) and ELISA ($\geq$40% PB) according to study site.

Study area	rVNA (RFFIT)			rVBA (ELISA)		
	n	*N*	%	*n*	*N*	%
Groot Aub	12	15	80.00	12	14	85.71
Ongombo West	6	19	31.58	14	19	73.68

Only animals that tested seronegative prior to vaccination are included (n, number of animals that tested seropositive; N, number of animals tested).

route, they are not included in the statistical analysis; both dogs seroconverted.

The results of the MLR showed that none of the factors examined (study area, route, and dose) had a significant effect on seroconversion as determined in ELISA (**Table 2**). However, for rVNAs as measured by RFFIT, the seroconversion rate was much lower in Ongombo West than in Groot Aub. Only 31.58% of the dogs seroconverted ($\geq$0.5 IU/ml) in Ongombo West, compared to 80% in Groot Aub (**Table 3**).

DISCUSSION

This is the first study assessing the immune response of the 3rd-generation oral rabies vaccine strain SPBN GASGAS in local, free-roaming dogs under Southern African conditions. In settings where traditional parenteral methods have failed to reach adequate vaccination coverages against rabies, oral rabies vaccines can be very helpful in reaching adequate herd immunity (15), particularly in free-roaming dogs, which are considered to play a key role in the transmission of rabies (16, 22, 23). In great parts of Africa, actually, most free-roaming dogs are owned and are deemed accessible for parenteral vaccination at MDV campaigns (37–39). However, experience shows that many of these MDV campaigns are not able to reach a sufficient number of dogs (40).

Several studies examined the immunogenicity and/or efficacy of oral rabies vaccines in dogs. Most of these studies were conducted under (semi-) controlled laboratory conditions using laboratory dogs or local dogs adapted to dog kennel conditions (41–48).

Generally, the immune response in the target population of free-roaming dogs under field conditions has rarely been investigated both after vaccination with inactivated rabies vaccines (49, 50) and oral rabies vaccines (51). Often, these animals are in poor condition compared to dogs well-looked after. Free-roaming dogs are generally malnourished as their diet is of low quality and/or quantity, having a negative impact on immunity (52, 53). Also, these animals are usually infected by endo- and ectoparasites or other immunosuppressive infectious agents (54). For example, canine distemper virus (CDV) can infect and modulate the antigen presenting properties of dendritic cells and thus influence the immune response against CDV and other concomitant infections/vaccinations (55). Hence, the possibility of immunosuppression through various stressors may reduce seroconversion in subpopulations of free-roaming dogs after rabies vaccination and the duration of immunity of seroconverted dogs and result in a decline in the vaccination coverage (50). Consequently, investigating the effectiveness of oral vaccination in free-roaming dogs under field conditions prior to large-scale implementation is critical.

Unfortunately, the sample size particularly in the first study area of Groot Aub continuously decreased during the course of the project. Initially, it was planned to obtain blood samples after 4 weeks, as day 28 post-vaccination seroconversion appears to be a good predictor for protection (35). However, due to the emerging SARS-CoV-2 pandemic in February 2020 and associated restrictions, subsequent sampling in Groot Aub was delayed and compliance was heavily reduced. To cope with the situation, it was decided to return to the study sites 56 dpv. However, only a few more blood samples could be collected and included in the analysis. In order not to jeopardize the outcome of the study by a similar situation occurring in Ongombo West half a year later, an incentive to the owners was provided. This ensured full compliance with study protocol.

Nevertheless, independent of study area, route, and dose, 78.8% of the free-roaming Namibian dogs vaccinated orally (either offered a bait or d.o.a.) developed a detectable immune response post-vaccination as measured by ELISA (rVBA), while the seroconversion rate in terms of rVNAs as measured by RFFIT was lower (**Tables 1**, **3**). The discrepancy is due to the fact that methods for measuring rabies immunity vary with regard to the humoral component measured (i.e., the Ig subclass or functional activity) and performance characteristics (i.e., specificity or sensitivity). When results of testing sera by both RFFIT and ELISA kit are compared for a particular target species, similar measures of sensitivity and specificity are obtained only when using different cut-off values for both assays (56). However, in our study, we followed international standards and recommendations of the manufacturer regarding cut-off values. As to why only 31.58% of the dogs in Ongombo West compared to 80% in Groot Aub had positive rVNA remains elusive (**Table 3**). However, while the level of 0.5 IU/ml was established as an indication of adequate vaccination rather than protection in humans, when the RFFIT is employed for other species (not humans), the accepted level of 0.5 IU/ml may not apply (56). So, the seroconversion rate based on rVNAs might even be higher; however, if the cut-off level is set too low, it gets more difficult to distinguish specific from non-specific neutralizing activity of a test serum. In any case, data from experimental studies clearly showed that rVBAs are a better predictor for protection than rVNAs (35).

The seroconversion rate obtained in our study is consistent to those found in local dogs vaccinated by the oral route under field settings in Haiti using the same SPBN GASGAS vaccine and ELISA (51). In a recent immunogenicity study with local Thai dogs kept in a dog shelter, 100% of the dogs vaccinated by the oral route had detectable levels of antibodies 28 dpv, again using the same vaccine and ELISA. These dogs in Thailand, however, had received vaccination against some common infectious diseases (canine distemper, parvovirus infection, adenovirus infection, bronchitis, and leptospirosis). They also received helminthic treatment when they were around 3 months old (48). Finally, the animals were fed on a daily basis, receiving commercially available high-quality pet food, hence superior conditions compared to the dogs used in the Namibian and the Haitian study.

However, the difference in seroconversion after oral vaccination with SPBN GASGAS between the study in Thai shelter dogs and the two studies in free-roaming dogs from Haiti and Namibia most likely cannot only be explained just by the physical condition of the dogs involved. It has been shown, for example, that the development of antibodies is slower after oral vaccination compared to parenteral vaccination (48). In the Thai dog study, the highest levels of antibodies were not reached before 4 weeks post-vaccination in dogs orally vaccinated with SPBN GASGAS (48). Therefore, the short interval between vaccination and sampling (17–20 dpv) may explain the relatively low seroconversion rate for the Haiti study (51). Another factor that most likely affected the seroconversion rate in the latter study was the study design; the baseline blood sample was collected immediately after bait consumption (51). Hence, the free-roaming dogs were surrounded by the vaccination team to prevent it from wandering off after bait consumption. This made the dogs anxious, negatively influencing bait handling and consumption and, consequently, vaccine virus release in the oral cavity. If the vaccine bait is swallowed completely without chewing, the vaccine is not discharged in the oral cavity, or if such a cornered animal chews half-hearted on the bait, most vaccine is often spilled on the floor instead of being absorbed in the oral cavity.

Essential for a successful vaccination attempt is therefore not only an efficacious vaccine and attractive bait but also the circumstances under which baits are offered to the dogs. In this respect, a likely stressful situation occurred in the present study in Namibia. Although blood samples were collected several weeks prior to vaccination, for logistical reasons, most animals were not offered a bait on their own premises as would be done under normal circumstances. Instead, the normally free-roaming dogs were brought to a central vaccination point in the study area where they were offered a bait. Hence, differing environmental conditions may have had a negative effect on bait consumption (handling) of individual dogs. Obviously, many of them were stressed due to the fact that they were on a leash to which they are usually not accustomed with many other strange dogs and humans around them in unfamiliar territory. Although the bait acceptance rate equaled those of other studies with the same bait (29–31), bait uptake and handling were impaired during the present study, as vaccine baits were often initially refused or hastily consumed as documented for at least 10 dogs. These animals hardly chewed on the baits before swallowing it completely, including the most likely unperforated sachet. Hence, under such suboptimal conditions, it can be expected that in some dogs, an inadequate immune response after bait consumption was induced, as the vaccine was not sufficiently or not released at all in the oral cavity. This might help to explain the lower seroconversion obtained in RFFIT as six of eight of these dogs sampled 28 dpv seroconverted in ELISA, while only two showed rVNA in RFFIT. However, a recent field study in India and Thailand showed that when free-roaming dogs are offered a bait directly when encountered in their familiar environment, most animals will accept the bait readily and perforate the sachet (31, 32). Hence, it can be assumed that under real-scenario conditions, bait uptake most likely will not be impacted, resulting in an even higher seroconversion rate post-vaccination. Also, when data sets of rabies-binding and -neutralizing antibodies from animals experimentally immunized with both live attenuated or recombinant oral rabies vaccines were examined, an analysis suggested that, though rVNA are expected to reflect *in vivo* protection, rVBA (ELISA) obtained at 28 dpv were a better predictor of protection against lethal rabies infection (35).

CONCLUSIONS

The results demonstrated that the great majority of orally vaccinated free-roaming Namibian dogs mount an immune response. The seroconversion rate in this study was slightly lower as compared to other immunogenicity studies under field and laboratory conditions, which we speculate is most likely associated with the central-point-vaccination (CPV) protocol used. Nevertheless, the study confirmed that local African dogs like any other local dogs across the world are very likely to develop an adequate immune response after oral vaccination with the 3rd-generation oral rabies vaccine strain SPBN GASGAS and are protected against rabies. These results underline the potential of ORV as an important tool for targeting hard-to-reach free-roaming dogs in mass dog vaccination campaigns under African settings. Rapid implementation of field trials is needed to see how ORV can be effectively and cost-efficiently integrated into African mass dog vaccination strategies at large scale.

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 animal study because this preliminary study was part of a larger approved project on oral vaccination of dogs in Namibia sponsored by the German Ministry of Health under the Global Health Protection Programs (Project number Ri-0755) and implemented as an agreed activity in the frame of the Official National Dog Rabies Control Program issued by the Namibian Directorate of Veterinary Services (DVS) at the Ministry of Agriculture, Water, Forestry and Land Use (MoA). In this respect, the study was regarded a disease control trial and Ethical Committee approval was not warranted. The study was approved by the appropriate authority within the MoA, Namibia. Manipulations such as vaccination and blood sampling of dogs were only conducted after elaborate explanatory meetings and under the premise that the dog owner had previously given his/her written consent. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

UM, RH, ML, and SO planned and conducted the vaccination campaigns in the two rural communities, collected blood samples, and were responsible for data curation. AS was responsible for resource allocation and overall supervision of the study. SO developed and produced the egg-flavored baits. TM, UM, and CF were responsible for serological testing of sera, analyzed data, wrote, reviewed, and edited the manuscript. AV conceived the ideas, conducted the statistical analysis, and contributed to writing and reviewing of the manuscript. All authors contributed to the article and approved the submitted version.

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Spatial Distribution and Population Estimation of Dogs in Thailand: Implications for Rabies Prevention and Control

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Poor management of dog populations causes many problems in different countries, including rabies. To strategically design a dog population management, certain sets of data are required, such as the population size and spatial distribution of dogs. However, these data are rarely available or incomplete. Hence, this study aimed to describe the characteristics of dog populations in Thailand, explore their spatial distribution and relevant factors, and estimate the number of dogs in the whole country. First, four districts were selected as representatives of each region. Each district was partitioned into grids with a 300-m resolution. The selected grids were then surveyed, and the number of dogs and related data were collected. Random forest models with a two-part approach were used to quantify the association between the surveyed dog population and predictor variables. The spatial distribution of dog populations was then predicted. A total of 1,750 grids were surveyed (945 grids with dog presence and 805 grids with dog absence). Among the surveyed dogs, 86.6% (12,027/13,895) were owned. Of these, 51% were classified as independent, followed by confined (25%), semi-independent (21%), and unidentified dogs (3%). Seventy-two percent (1,348/1,868) of the ownerless dogs were feral, and the rest were community dogs. The spatial pattern of the dog populations was highly distributed in big cities such as Bangkok and its suburbs. In owned dogs, it was linked to household demographics, whereas it was related to community factors in ownerless dogs. The number of estimated dogs in the entire country was 12.8 million heads including 11.2 million owned dogs (21.7 heads/km^2) and 1.6 million ownerless dogs (3.2 heads/km^2). The methods developed here are extrapolatable to a larger area and use much less budget and manpower compared to the present practices. Our results are helpful for canine rabies prevention and control programs, such as dog population management and control and rabies vaccine allocation.

Keywords: dog survey, rabies, random forest model, stray dogs, Thailand

INTRODUCTION

The poor management of dog populations, especially stray dogs, may result in different problems that exist in many countries. These problems are complexly related to human and animal health, welfare, socio-economics, politics, and religion. According to the World Organization for Animal Health (OIE), a "stray dog" was defined as "any dog not under direct control by a person or not prevented from roaming" (1). The sources of stray dogs may come from the following: (i) owned dogs that roam freely; (ii) abandoned owned dogs, including their puppies born from uncontrolled breeding; and (iii) ownerless dogs that reproduce successfully (1). Stray dogs are associated with the transmission of a number of zoonotic pathogens, dog bite injuries, and road traffic accidents (2). Many of these dogs may be packed and claim a street, which they beg for food, and are a general nuisance to local people and tourists (3). Dog bites are a serious public health problem in many countries. Many studies suggest that dog bites account for tens of millions of injuries annually (4). In Thailand, no <1 million people are bitten by animals annually, and 97% of animal bite injuries are dog bites (5). The majority of dog bites are stray dogs (6). Most bites are delivered by male dogs (7). In addition, bitches with puppies may be aggressive and bite people who approach their litter (8). Other negative impacts caused by stray dogs living in the city environment may include noise pollution, fecal garbage, and traffic accidents. These have been previously observed in different cities in the USA (9). Moreover, previous reports have shown that stray dogs have become a serious public administration problem in many Asian countries, such as China (10), India (11), Bangladesh (12), Indonesia (13), and Cambodia (14). Thailand has been facing stray dog problems for a long time. The rising number of stray dogs has raised concerns regarding human hygiene and public health. Similar problems have also been found, as some pets have been abandoned and become strays (3). In Thailand, dog-keeping practices and duties of responsible ownership vary depending on the cultural setting (15). In urban areas, the majority of owned dogs were frequently confined, but were sometimes allowed to roam. In rural areas, dogs were allowed to roam freely in the village and reproduce with other dogs. People will abandon unwanted dogs and puppy litters in public places, such as temples and universities. Most stray dogs are being fed and looked after by the surrounding community or dog caretakers; however, they have limited capabilities to capture or restrain these dogs (16). Therefore, these dogs cannot be vaccinated or sterilized, and are responsible for sustaining endemic rabies.

One of the most life-threatening issues arising from poor dog population management is dog-mediated rabies. Rabies is a bullet-shaped virus belonging to the genus *Lyssavirus* of the family *Rhabdoviridae* (17). Rabies is a fatal disease once individuals are symptomatic. In Thailand, rabies is endemic and has been listed as an important zoonotic disease (18, 19). Forty-six people died from rabies between 2010 and 2015, and more than 600,000 post-exposure prophylaxis treatments are provided annually (20).

In developed countries, wild animals have been identified as important maintenance hosts for rabies. For example, raccoons, skunks, and bats accounted for 92.4% of animal rabies reported in the USA in 1998 (21). Foxes and raccoons were responsible for 72.1% of animal rabies reported in Europe in 2000 (21). Nonetheless, the scenarios are completely different in developing countries. In Africa and Asia, dog is identified as the main reservoir for rabies (22–24). It was estimated that rabies causes over 59,000 deaths every year in these countries (25) and most of the cases were dog-mediated (26). In Thailand, dog rabies cases have been continuously reported in all regions. From the surveillance system of the Department of Livestock Development between 2013 and 2020, from a total of 58,651 samples, 4,239 tested positive for rabies. The highest number of animal rabies reported was in 2018 (15.3%; 1,476/9,643), and most of the rabies-positive animal samples (87.2%; 1,287/1,476) were retrieved from dogs (27). Thus, dogs are important maintenance hosts of animal rabies in Thailand that continuously spread the virus to humans and other animals. A more comprehensive understanding on the different aspects of dogs living in these areas is crucially needed for a better dog population management. Furthermore, WHO, OIE, FAO and the Global Alliance for Rabies Control have set a global target of "Zero by 30" to end human deaths from dog-mediated rabies by 2030, in line with Thailand's strategic plan under "Saving Animals and Human Lives from Rabies Project" to make Thailand rabies-free through various approaches. A better understanding of the dog population will ultimately help in tailoring strategic plans for rabies prevention and control in a more sustainable direction and encourage the achievement of our 2030 rabies elimination goal.

Nonetheless, a wide variety of data are required, such as the number of dogs and the distribution and ecology of dog populations in a particular area. These data are helpful for dog population management, rabies control, and intervention adaptation (1). For a precise analysis, dog populations should be classified according to ownership status and levels of movement restriction. In general, the number of dogs is derived from different sources, such as household surveys and mark-recapture techniques (28, 29). However, household surveys are labor-intensive, and mark-recapture techniques are not applicable to very large areas (1). Moreover, other factors affecting the living conditions of dogs, including food, shelter, water, and human activities should be considered in dog population estimation and distribution (30–32). Therefore, this study aimed to describe the characteristics of dog populations in Thailand, explore the spatial distribution and relevant ecological factors, and estimate the number of dogs in different population types across the country.

MATERIALS AND METHODS

Study Areas

We purposively selected four districts representing the main region of Thailand (**Figure 1**), including Kampangsan district, Nakhon Pathom province (central region); Mueang Nong Khai district, Nong Khai province (northeastern region); Mueang Nan district, Nan province (northern region); and Mueang Trang

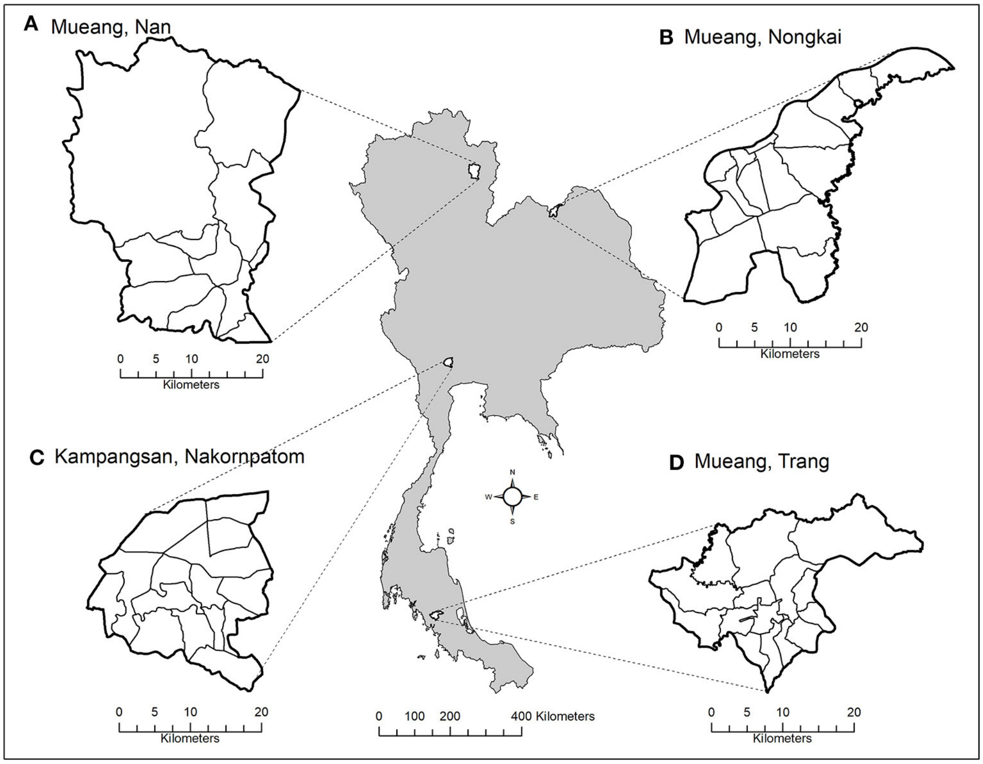

FIGURE 1 | Study areas. The study areas include Mueang Nan district, Nan province **(A)**, Mueang Nong Khai district, Nong Khai province **(B)**, Kampangsan district, Nakhon Pathom province **(C)**, and Mueang Trang district, Trang province **(D)**.

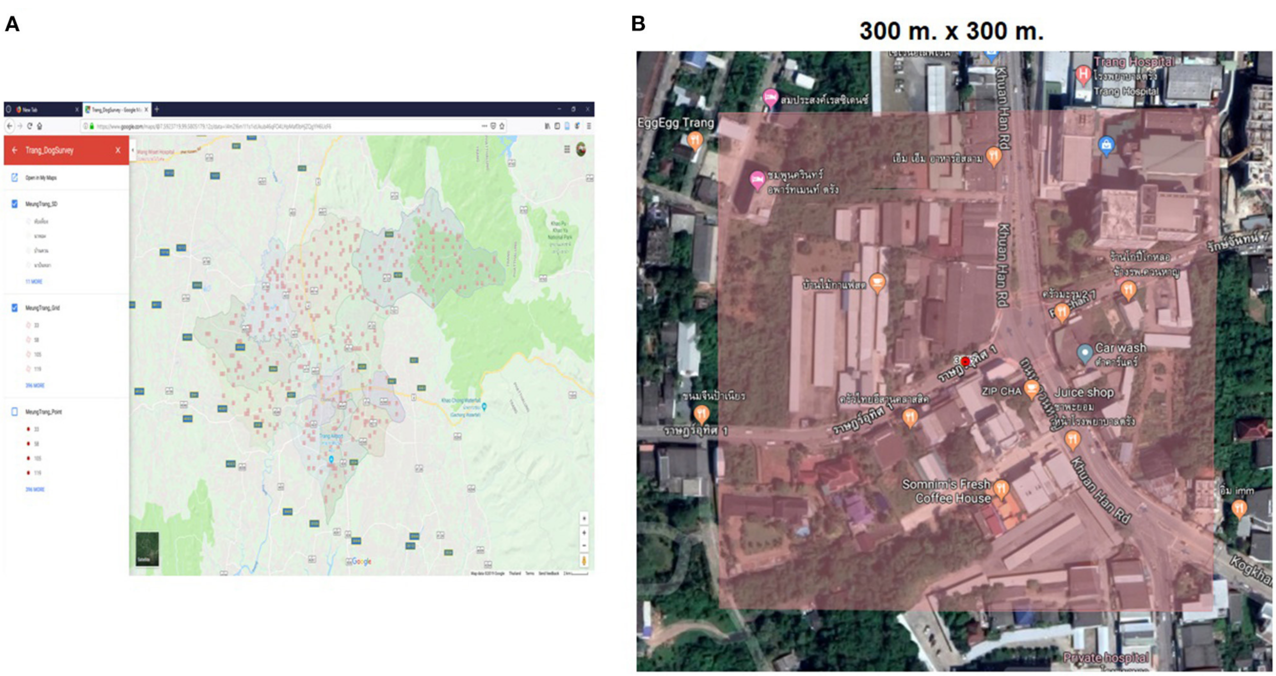

FIGURE 2 | Surveyed grids. Using a Google map to navigate the selected grids **(A)**, which buildings, and areas within a grid (red color) were surveyed **(B)** (33).

TABLE 1 | Datasets for the random forest model.

Model	Training site	Test site
I	Northeast, North and South	Central
II	Central, North and South	Northeast
III	Central, Northeast and South	North
IV	Central, Northeast and North	South
V	Central, Northeast, North and South	No test set

district, Trang province (southern region). In each district, the area was divided into grids with 300-m resolution, and at least 10% of the grids (approximately 400 grids) in each district were selected by simple random sampling for dog surveys.

Dog Surveys

The dog survey was conducted between May 2018 and September 2019. In each survey team, a navigator and an observer were recruited. The navigator used Google Map application (Google LLC, California, United States) equipped on their mobile phone to locate the area within the selected grids (**Figure 2**). Meanwhile, the observer counted the number of dogs and asked owners, neighbors, or witnesses on the ownership status and movement restriction of each dog. Ownership was divided into owned dogs (usually cared for) and ownerless dogs (not cared for or unusually cared for). The movement restriction of owned dogs was characterized as confined (raised only inside the house), independent (lived independently all the time), semi-independent (raised inside and independently), and unidentified (the observer found dogs in the household but no one informed data). The ownerless dogs were characterized into community (fed but not usual) and feral dogs (not fed by anyone).

Data Analysis

The collected data obtained from the surveys were then used to describe the characteristics of the dog populations in Thailand, including ownership patterns and movement restrictions. Subsequently, these data were used to model the spatial distribution of the dog populations and to predict the number of dogs in each area and the entire country.

We used a random forest (RF) model to quantify the association between the dependent variables (owned and ownerless dogs) and predictor variables. The predictor variables included demographic data (human population), land use (aquaculture, community, residential areas, field crop, and horticulture), and proximity to food sources (Buddhist temples, schools, and roads). A human population density raster map at 100-m resolution was obtained from the WorldPop project (34). Land use data was provided by the Land Development Department (35). We defined "residential areas" as a housing space where people and their companion animals live, whereas "community area" is where public places such as markets, temples, schools, and commercial buildings are located. All predictor layers were transformed into raster maps with a resolution of 300 m. All predictor layers were extracted and divided into five datasets, as listed in **Table 1**.

We modeled the datasets in two separate parts: count data and binary data (presence/absence). This approach is called "a two-part model" or "a zero-altered model" or "a hurdle model" (36, 37). First, we selected grids with dog presence and modeled them as a quantitative RF (with count data). We then evaluated the predictive power of the models using two statistical metrics, the correlation coefficient (COR), and the root mean square error (RMSE) to quantify the goodness of fit (GOF) between the observed values of the model sets and predicted values. Second, we defined grids with dog presence as 1 and 0 as otherwise. A binary RF is then applied. Two other statistical metrics, the area under the curve (AUC) of the receiver operating characteristic plots, and COR were used to evaluate the predictive power of the binary RF models. Finally, we combined the predictive values of both approaches to produce a final map of predictive values and evaluated them with the test sets using the COR and RMSE. The packages "randomForest" (38) and "hydroGOF" (39) equipped in program R (R Core Team, Vienna, Austria) were employed for the RF model and goodness of fit estimation, respectively. The total numbers of dogs in the whole country were calculated using ArcGIS 10.2 (Esri, California, United States), separated by the five models.

RESULTS

A total of 1,750 grids were surveyed in this study (945 grids with dog presence and 805 grids with dog absence). Among the observed dogs, 12,027 (86.6%) were owned and 1,868 (13.4%) were ownerless. The ratio between owned and ownerless dogs was 6.4:1. Among the owned dogs, 25% were confined, 51% were independent, 21% were semi-independent dogs, and the rest were unidentified. Among the ownerless dogs, 72% were feral, and the remaining were community dogs. Details of the surveyed dogs are presented in **Table 2**.

The variable importance of the predictors is shown in **Figure 3** and **Table 3**. The three predictors that mostly influenced the distribution of owned dogs in the count models were the resident area, followed by the human population density and proximity to the road. Concerning the ownerless dogs, the community was the strongest variable, followed by human population density and proximity to temples. In contrast to owned dogs, residential area was a less important factor influencing the distribution of ownerless dogs.

The association between the fitted function and the predictor variables in owned dogs is shown in **Supplementary Figure 1** (quantitative RF) and **Supplementary Figure 2** (binary RF). These plots demonstrate that aquaculture, community, residential area, field crop, and horticulture had a positive association with the predicted values. Interestingly, human population density showed different associations at different densities. First, a negative association with the fitted function was found until reaching approximately 100 persons/km^2. The association then returned to a positive trend, which rose to the highest point at 1,000 persons/km^2. Finally, it turned back down again when the density exceeded that peak. Three proximity variables, including proximity to school, Buddhist temple, and

TABLE 2 | Descriptive analysis of surveyed dogs.

Study areas	Grids (P/A)*	Owned dogs					Ownerless dogs			Total
		Confined	Independent	Semi	Unidentified	Total	feral	community	Total	
Kampangsan district (Nakhon Pathom province)	550 (459/91)	1,572	3,309	1,690	263	6,834	1,052	134	1,186	8,020
Mueang Nong Kai district (Nong Kai province)	400 (206/194)	743	1,467	461	26	2,697	87	42	129	2,826
Mueang Nan district (Nan province)	400 (44/356)	90	213	129	2	434	0	4	4	438
Mueang Trang district (Trang province)	400 (236/164)	553	1,175	264	70	2,062	209	340	549	2,611
Total	1,750 (945/805)	2,958	6,164	2,544	361	12,027	1,348	520	1,868	13,895

**P stands for the number of grids with dog presence and A stands for the number of grids with dog absence.*

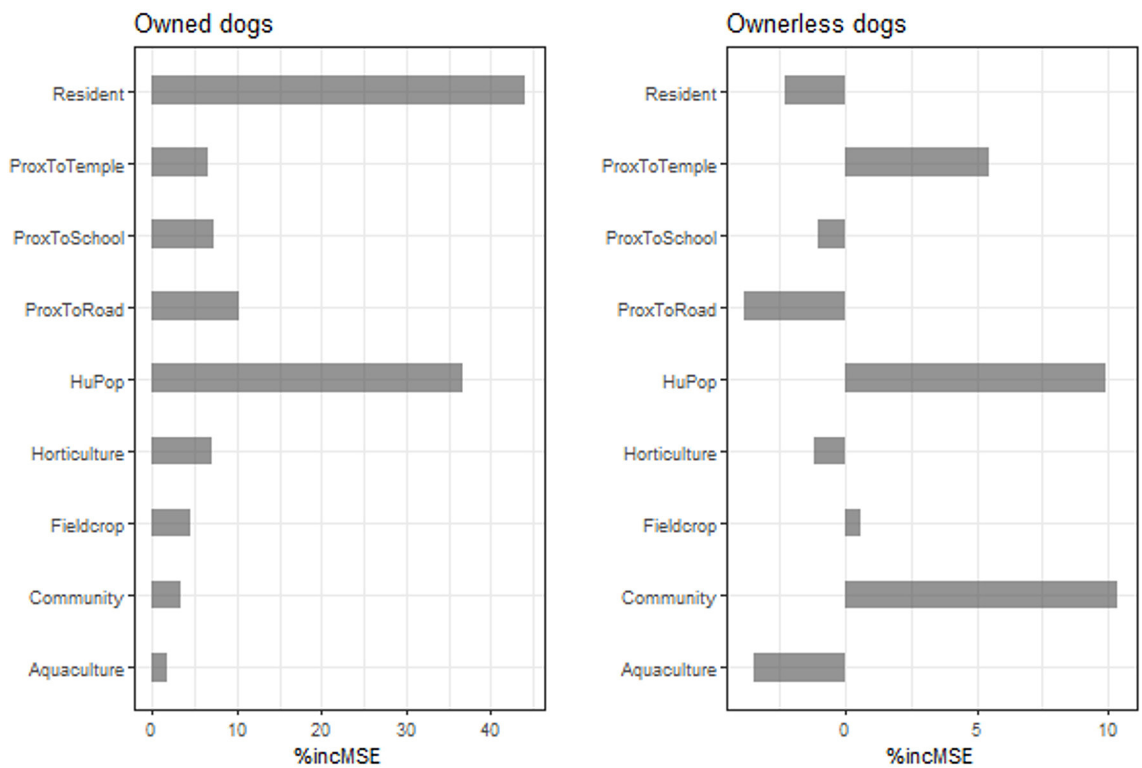

FIGURE 3 | Graphs showed the importance of variable. The percent increase of MSE of the factors influencing the distribution of owned dogs **(left)** and ownerless dogs **(right)**.

road, showed a similar trend with two different association patterns, starting with a negative association to a certain level and turning back to a positive direction for a higher distance.

The association between the fitted function and the predictor variables for the ownerless dogs is shown in **Supplementary Figure 3** (with quantitative RF) and **Supplementary Figure 4** (with binary RF). Four variables, including community, resident area, field crop, and horticulture, showed a positive association. Aquaculture showed a negative association, while proximity to school, Buddhist temple, and road first showed a negative association and then turned back to be positive at a certain level. In contrast, the human population density initially showed a positive association and became negative when the density reached 1,000 persons/km^2.

All the count and binary models showed a high predictive power for the training sets, whereas the evaluation of the final outputs by quantifying the GOF between the observed values of test sets and predictive values showed medium to low accuracy (**Table 4**). Regarding the count models of the training sets, the correlation varied in the range of 0.910–0.936, while the RMSE varied from 0.16 0.19. The binary models of the training sets also showed a high predictive power with an AUC ranging between

TABLE 3 | The variable importance obtained from the combined set of all study sites.

Predictor variables	Owned dogs			Ownerless dogs		
	Count model		Binary model	Count model		Binary model
	%IncMSE*	IncNodePurity**	IncNodePurity	%IncMSE	IncNodePurity	IncNodePurity
Aquaculture	1.792	1.305	6.940	−3.458	0.067	1.579
Community	3.360	1.329	3.420	10.375	1.907	4.043
Proximity to school	7.348	20.163	52.344	−1.053	5.851	32.046
Proximity to temple	6.516	22.242	50.707	5.479	7.531	29.308
Proximity to road	10.174	10.745	66.909	−3.799	2.469	16.997
Human population	36.581	47.463	149.117	9.933	10.867	68.335
Resident area	44.142	14.542	12.762	−2.258	0.892	5.012
Field crop	4.462	2.519	5.971	0.605	0.657	3.961
Horticulture	7.028	1.207	2.541	−1.188	0.419	2.043

**%IncMSE is the increase in the mean square error of prediction, which the higher number, the more important variable is. It is the most robust and informative measure.*
***IncNodePurity relates to the loss function which by best splits are chosen, which more useful variables achieve higher increases in node purities.*

TABLE 4 | Goodness of fit of the models.

Model*	Response variables	Training sites				Test site	
		Count model		Binary model		Correlation	RMSE*
		Correlation	RMSE**	AUC***	Correlation		
I	Owned dogs	0.933	0.17	0.992	0.887	0.516	0.51
	Ownerless dogs	0.910	0.16	0.992	0.888	0.319	0.38
II	Owned dogs	0.936	0.17	0.997	0.909	0.580	0.52
	Ownerless dogs	0.919	0.19	0.997	0.909	0.335	0.34
III	Owned dogs	0.936	0.17	0.993	0.884	0.634	0.36
	Ownerless dogs	0.914	0.18	0.993	0.885	0.101	0.16
IV	Owned dogs	0.934	0.17	0.998	0.913	0.451	0.51
	Ownerless dogs	0.921	0.17	0.997	0.911	0.472	0.30
V	Owned dogs	0.934	0.17	0.994	0.896	-	-
	Ownerless dogs	0.927	0.18	0.996	0.868	-	-

Details of each model are described in **Table 1.*
***Root mean square error (RMSE).*
****Areas under the curve (AUC).*

0.992 and 0.998, and the correlation ranged from 0.868 to 0.913. With regard to the evaluation of the final outputs, the accuracy of the predictive values in owned dogs was higher than that of ownerless dogs. The correlation ranged between 0.451 and 0.634 for owned dogs and from 0.101 to 0.471 for ownerless dogs. For the RMSE, the values ranged between 0.36 and 0.52 for the owned and from 0.16 to 0.38 for the ownerless dogs.

The total number of dogs in the entire country was calculated using our models. The lowest value was observed in Model I, whereas Model III showed the highest values (**Table 5**). On average, the total number of dogs was 12,840,452 (10,251,591–15,836,093 heads). Of these, 11,196,042 heads (ranging from 9,176,028 to 13,676,025) were owned with the density of 21.7 heads/km^2 (ranging from 0 to 251.6 heads/km^2). The estimated number of ownerless dogs was 1,644,419 heads (ranging from 1,075,563 to 2,256,904) with the density of 3.2 heads/km^2 (ranging from 0 to 5.6 heads/km^2).

TABLE 5 | Predicted number of dogs in Thailand.

Response variables	Predicted number of dogs				
	Model I	Model II	Model III	Model IV	Model V
Owned dogs	9,176,028	12,137,153	13,676,025	11,266,308	11,196,042
Ownerless dogs	1,075,563	2,256,904	2,160,068	1,431,072	1,644,419
Total dogs	10,251,591	14,394,057	15,836,093	12,697,380	12,840,452

The spatial distribution of owned and ownerless dog populations in Thailand, separated by the types of modeling approaches, are shown in **Figure 4**. The distribution maps of

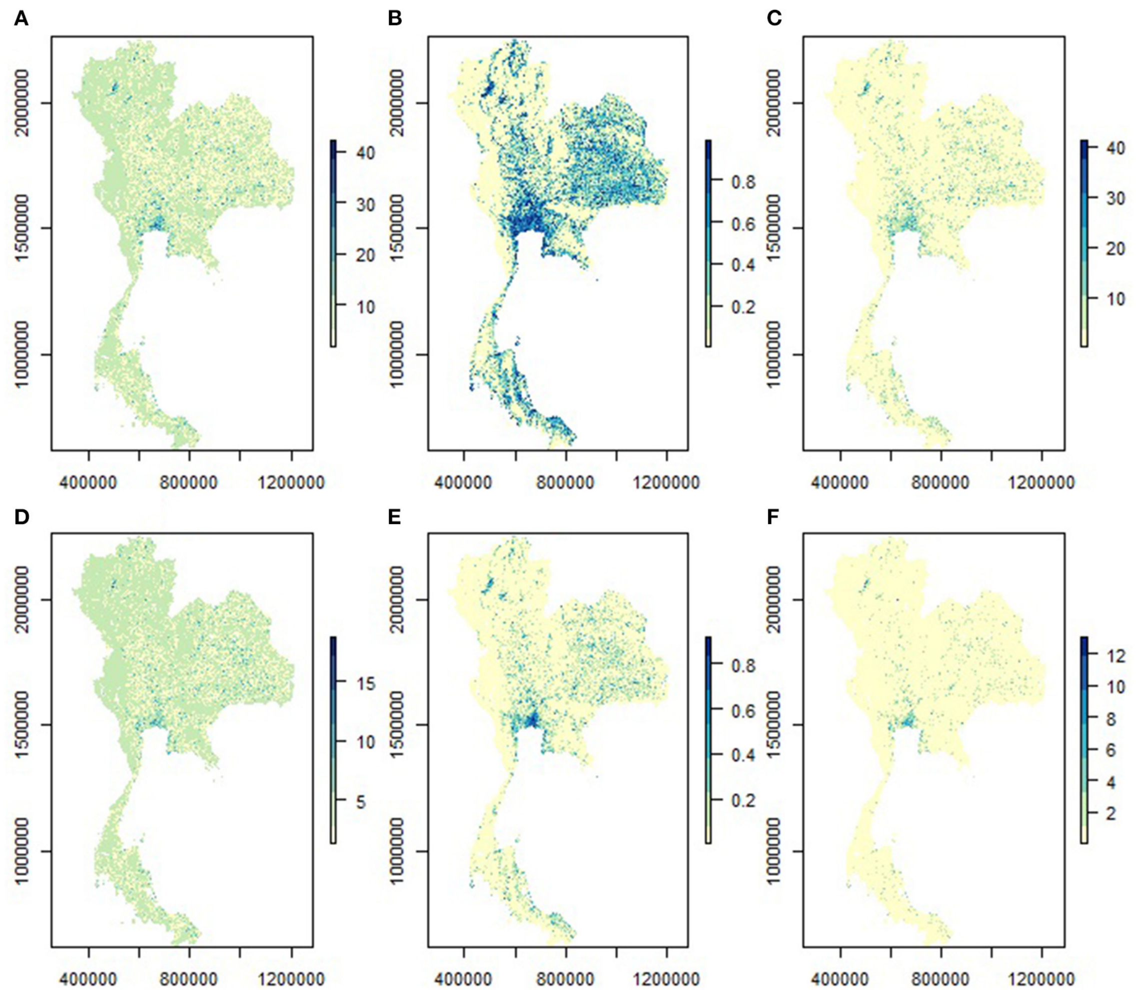

FIGURE 4 | The predicted distribution of dog population in Thailand (300-m resolution). **(A)** Owned dogs with count model, **(B)** owned dogs with a binary model, **(C)** owned dogs with combining the two models **(A,B)**, **(D)** Ownerless dogs with count model, **(E)** Ownerless dogs with a binary model, **(F)** Ownerless dogs with combining the two models **(D,E)**.

the owned and ownerless dog populations predicted by the combined models are shown in **Figures 5**, **6**, respectively. It appears that the spatial distribution of owned and ownerless dogs had a similar pattern where the high-density areas were mostly distributed in the big cities, namely Bangkok (148 heads/km^2 owned dogs and 42 heads/km^2 ownerless dogs), Nonthaburi (136 heads/km^2 of owned dogs and 32 heads/km^2 of ownerless dogs), Samut Prakan (101 heads/km^2 owned dogs and 22 heads/km^2 ownerless dogs), and Pathumthani (95 heads/km^2 of owned dogs and 16 heads/km^2 of ownerless dogs). Focusing on the studied districts (**Figures 5**, **6**), it was found that the urban areas showed a higher density of dogs compared to the rural areas.

DISCUSSION

This study integrated two techniques to estimate the number of dog populations in Thailand, including dog surveys and spatial modeling. Dog surveys throughout the country have been routinely conducted by local government organizations twice a year and reported to a web-based reporting system called "ThaiRabies.net" (27). However, such surveys face different challenges, such as technical problems within the system and incomplete data entry, resulting in unreliable data for dog populations. In this study, we selected the study areas at the district level, partitioned the selected districts into grids with a 300-m resolution, and surveyed the number of dogs in these chosen grids considering ownership statuses and movement restriction. A spatial modeling technique (40–42) was then used to predict the number of dogs using data from the surveys. This method also determined the factors influencing the spatial distribution of these dogs. Mark-recapture techniques were used in a previous study (29). Nonetheless, such methods are relatively labor-intensive and not extrapolatable to large areas (1). Therefore, what we developed here, which is applicable to the whole country, uses much less budget as well as manpower

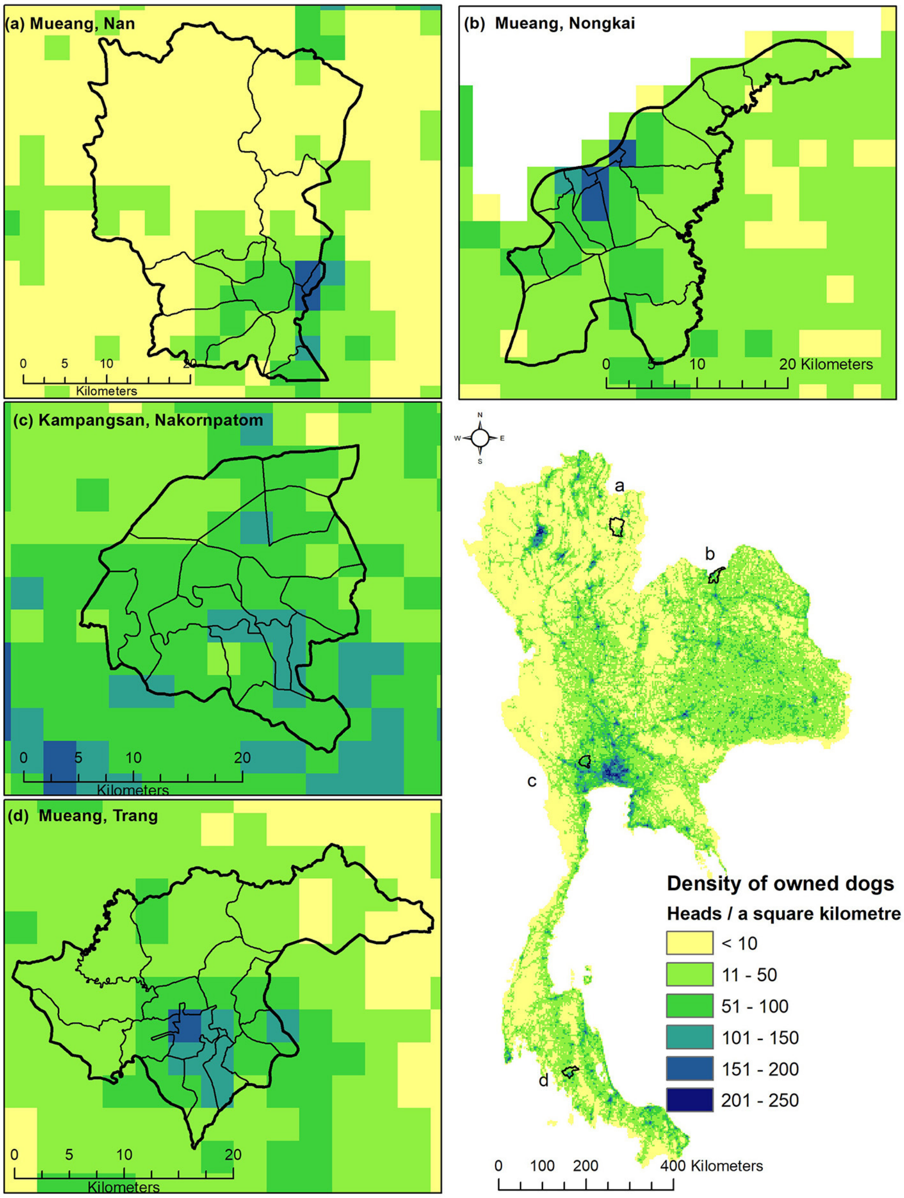

FIGURE 5 | The distribution map of owned dog population in Thailand (1,000-m resolution). The distribution maps of the owned dog population in Muang Nan district, Nan province **(A)**, in Muang Nong Khai district, Nong Khai province **(B)**, in Kampangsan district, Nakhon Pathom province **(C)**, Muang Trang district, Trang province **(D)**, and the whole country (bottom right).

compared to manual dog surveys. This should be considered an alternative nationwide survey method.

Effective dog population management can mitigate the adverse impacts caused by dogs, particularly stray ones (1, 3, 8, 9, 21). This study found that the ratio of owned and ownerless dogs was 6.4:1. Apart from ownerless dogs, most owned dogs (∼75%) also roamed freely. Concerning rabies, if these stray dogs are not vaccinated for any reason, the chance for these dogs to become infected and circulate the virus in the areas is higher (26, 43). Our findings are in line with a previous rabies investigation report from endemic areas in Thailand, in which most of the affected animals were unvaccinated-owned dogs (70%) with the characteristics of independent or semi-independent roaming, while the rest were ownerless dogs without vaccination (44). Therefore, the dog population control program should be emphasized to promote responsible dog ownership to reduce the number of strays, especially ownerless dogs. The size of dog populations and their distribution estimated from our study are helpful in strategic planning for dog population control programs, such as surgical sterilization. It was well-documented in a previous study that a proper dog population control can reduce the prevalence of rabies in dogs (45). In

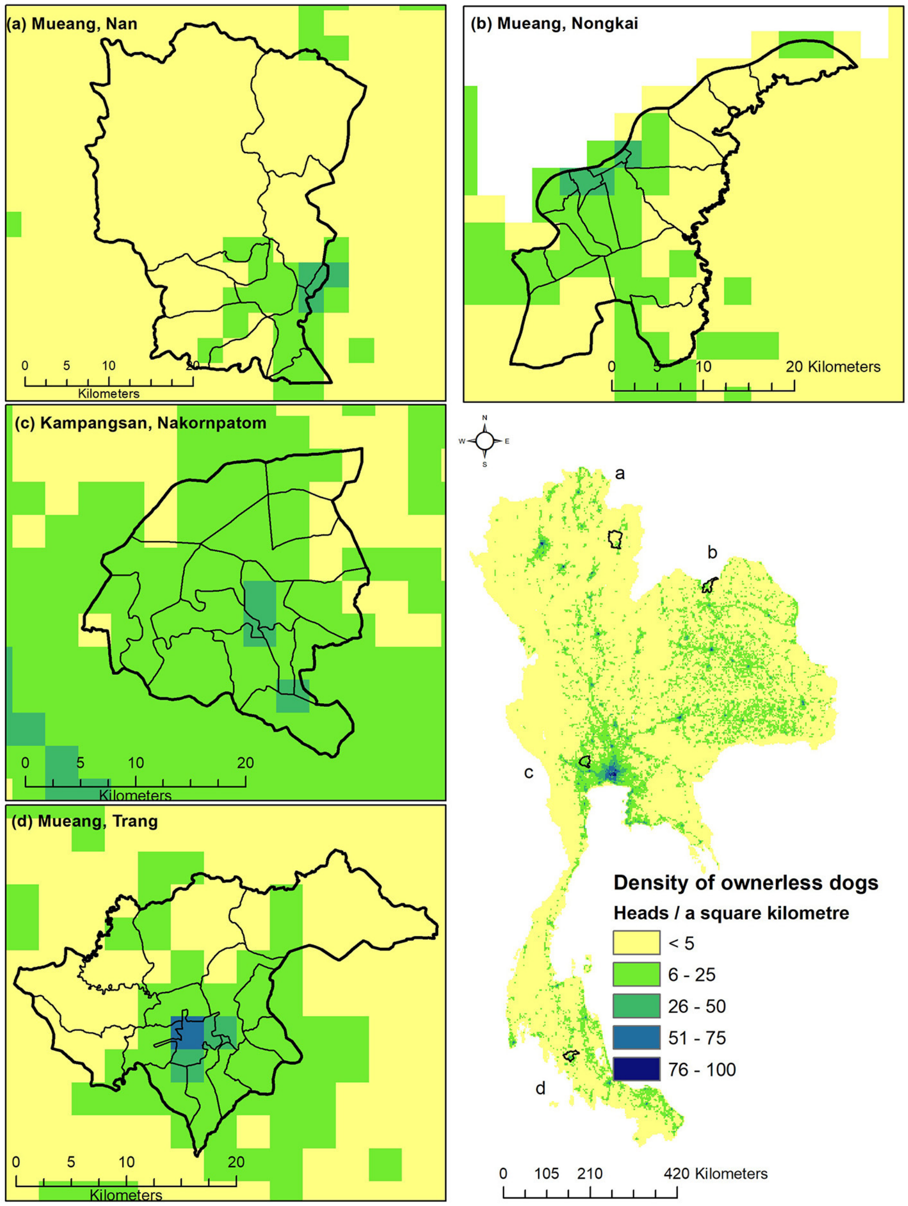

FIGURE 6 | The distribution map of the ownerless dog population in Thailand (1,000-m resolution). The distribution maps of the ownerless dog population in Muang Nan district, Nan province **(A)**, in Muang Nong Khai district, Nong Khai province **(B)**, in Kampangsan district, Nakhon Pathom province **(C)**, Muang Trang district, Trang province **(D)**, and the whole country (bottom right).

addition, our findings can also improve canine rabies vaccine allocation as per dog heads estimated in each area. In a previous study, herd immunity derived from vaccination coverage of 60% was proven effective in the prevention and control of rabies (46). However, an annual vaccination coverage of at least 70% of the dog population is recommended by the WHO to prevent ongoing transmission and eradicate rabies (47). To achieve the herd immunity threshold, the dog population size must be precisely estimated. This study provides an initial technical approach to do so. Future studies may modify our technique or include more data to improve our model.

Dog habitats are related to sources of food, water, and shelter, which are essential factors for dog living. Our study indicates that the habitats of owned dogs were linked to household demographics (resident areas, human population, and proximity to the road, respectively), which can be the sources of those factors. In ownerless dogs, however, the habitats were related to the community (premises in the community, human population, and Buddhist temple, respectively). A community is composed of markets, garbage dumps, restaurants, and other premises related to human activities. These scenarios have also been observed in other developing countries where the dog population is not well managed (13, 48–50). In Thailand, Buddhist temples are important sources of food for stray dogs and, in many cases, temples are also shelters for abandoned dogs (3). A study conducted in Bali, Indonesia also reported that dogs were mostly found around temples (13).

Based on our estimation, dog populations were highly distributed in large cities across the country, such as the Bangkok Metropolitan Region and surrounding provinces, Chiang Mai (Northern), Chonburi (Eastern), and Songkhla (Southern). Focusing on our studied districts, it was obvious that the urban areas showed a higher density of dogs compared to the rural areas. For example, in Chiang Mai province in northern Thailand, the estimated dog density of Mueang Chiang Mai district as the urban area was 184 heads/km^2 (143 heads/km^2 of owned dogs and 41 heads/km^2 of ownerless dogs), while the estimated dog density of Fang district in rural areas was 29 heads/km^2 (25 heads/km^2 of owned dogs and 4 heads/km^2 of ownerless dogs). This was associated with household demographics, which has been reported in other studies (51, 52). The population distribution map we produced can be further used to predict the number of dogs by involving other factors such as population structures and dynamics, predict the occurrence of diseases such as rabies in dog populations, and use as baseline data for dog population management plans.

In this study, we used the random forest model with a two-part approach because of its high performance and the ability to handle zero inflation of the dataset. The RF model is a machine learning method with a non-parametric approach. The algorithms combine the prediction of a high number of classification trees in an ensemble (53). Compared to other methods, RF has a high capability to model complex interactions among predictor variables (54) and has recently provided highly accurate results in modeling livestock (41, 55) and human populations (56, 57). A two-part approach (36, 37, 58) was used to deal with many of the surveyed grids with dog absence (zero inflation), where the presence/absence was modeled separately from abundance (presence only). The absence of dogs in the census grids may have occurred due to unsatisfactory conditions for dog living (structural unsuitability); satisfactory conditions but dogs were temporarily absent at the time of survey (design error); satisfactory conditions and dogs were present, but the observers misidentified or missed their presence (observer error); and satisfactory conditions but with no attendance (human error). The zeros due to design, observer, and human errors are also called false zeros or false negatives, and the structural unsuitability is called positive zeros, true zeros, or true negatives (59). The limitation of the two-part models applied in this study is that the four different types of zeros are not distinguishable (59). However, based on a previous comparative study on five regression models (Poisson, negative binomial, quasi-Poisson, two-part model, and zero-inflated Poisson), it was found that the two-part model is still the most promising method (36).

This study has some potential limitations. First, we may not have reached an appropriate sample size. The 400 sample grids with 300-m resolution located in four districts across the country might be too small compared to the entire territory. Nonetheless, the manual survey at the national level is not practical in the long run, as it is labor-intensive, costly, and time-consuming. Our spatial modeling is an alternative to solve this problem, even though the results may not perfectly reflect the reality. A greater number of sample grids is required in future studies to improve the model performance. However, it is still not practical to conduct manual surveys. Pattern recognition or biometric technology is suggested for automatically identifying and distinguishing individual dogs in the survey. With such a technological approach, the number of survey grids would be dramatically increased with less effort, and a more accurate estimation would be generated.

We estimated approximately 12.8 million dogs across Thailand. This estimation seems plausible. A previous study conducted in 1994 reported approximately 7.3 million dogs nationwide (60), and given that the population growth rate is 1–2% per year (61, 62), there should be approximately 9.5–12.5 million heads in 2020. However, direct model validation and verification are not possible because of the lack of actual field data. A nationwide survey using the aforementioned technology is needed at least once to assess model accuracy.

Dog population management is a keystone in addressing dog-related problems, especially in stray dogs. Responsible dog ownership should be rigorously promoted as well as the control of stray dog populations to an acceptable level to improve their health and welfare simultaneously. The community should be involved in promoting responsible dog ownership, which can help reduce the number of ownerless dogs. Better veterinary care should be employed by improving the health of individual animals and increasing dog vaccination coverage, which could reduce rabies transmission (45). With these approaches, the elimination of rabies in dog populations is possible. Moreover, knowledge of the population size and spatial distribution of dogs can facilitate the implementation of mass dog vaccination campaigns and stray dog population control programs to control canine rabies, which will greatly contribute to the elimination of rabies in Thailand by 2030.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Department of Livestock Development by restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the Department of Livestock Development. Requests to access the datasets should be directed to weeraden@yahoo.com.

ETHICS STATEMENT

Ethical review and written informed consent for participation were not required for the animal study because this research is on spatial modeling and population estimation of dogs, that cannot link to individual animal subjects.

AUTHOR CONTRIBUTIONS

WT, SK, and KL conceived and designed the study. SK, VW, KB, TP, KL, and WT conducted dog surveys and generated the raw data. WT performed statistical analysis with contributions from MG. WT drafted the paper which was meticulously revised by AW and KL. WT, KL, and AW contributed to writing, reviewed, and editing the final manuscript. All authors read and approved the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2021.790701/full#supplementary-material

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Capacity Building Efforts for Rabies Diagnosis in Resource-Limited Countries in Sub-Saharan Africa: A Case Report of the Central Veterinary Laboratory in Benin (Parakou)

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Rabies has been listed as a priority zoonotic disease in many African countries and the countdown to reach the goal of eliminating dog-mediated human rabies deaths by 2030 means that disease control measures need to be applied fast. In this context, an essential pillar of any national plan to control rabies is the implementation of reliable diagnostic techniques to ensure the success of field surveillance systems. Although many African countries have received international support for the control of rabies–some countries, like Benin, have not received a similar level of support. Indeed, until 2018, Benin was not able to diagnose rabies and rabies diagnosis in animals as well as humans relied solely on observed clinical symptoms. Although the Central Veterinary Laboratory (CVL) of Parakou had the equipment to implement two recommended tests, the lack of specific reagents and skills prevented the implementation of a rabies diagnostic service. Here we present the joint efforts of the national authorities in Benin, intergovernmental agencies, and non-governmental organizations to assess the strengths and weaknesses of the government's rabies control efforts. We have applied the Stepwise Approach toward Rabies Elimination (SARE) analysis, implemented rabies diagnostic capacities at the CVL of Parakou, characterized strains of rabies virus circulating in Benin, and finally integrated an inter-laboratory comparison program.

Keywords: rabies diagnostic, capacity building, surveillance, rabies elimination, phylogenetic analysis (phylogeny)

INTRODUCTION

The most recent predictive disease burden models suggest that rabies, caused primarily by the *Rabies Lyssavirus* (RABV), causes an estimated 59,000 human deaths every year, which is likely an underestimation (1). As rabies has a higher burden on resource-limited countries in Africa—where disease surveillance and data reporting are often lacking or non-existent—it is often caught up in the cycle of neglect where the under-reporting of the disease results in rabies being given a lower priority by stakeholders and decision makers. This cycle of neglect can, however, be broken with the generation of empirical burden data that is generated through laboratory diagnosis and confirmation. In this context, improving the laboratory capacity of a country or a region ensures that animal rabies cases can be diagnosed efficiently, which in-turn ensures that (i) accurate data on the incidence of the disease is collected, (ii) control efforts can be monitored, and (iii) potentially exposed patients can be managed clinically. To this end, the network of centralized and decentralized diagnostic facilities that implement rabies diagnostic techniques that are in line with the recommendations of the World Organization for Animal Health (OIE) constitutes one of the main pillars of any rabies control plan and should thus be fostered where possible.

Through the contributions of the global community over the years, the rabies surveillance networks in various sub-Saharan countries have been supported, while other countries had yet to receive a similar level of support (2, 3). One of these countries in West Africa is Benin, which shares political borders with Burkina Faso, Niger, Nigeria, and Togo. Benin is a small country (∼115,000 km^2) with 11.5 million inhabitants that are spread out over 12 counties. Of those, ∼265,000 people live in Porto Novo, which is the capital city located in the county of Ouémé. Although Benin is considered rabies-endemic and predictive burden models estimate that ∼178 people die of preventable dog-mediated rabies every year, there is still very little data available regarding the actual epidemiological situation of rabies in the country (2). Indeed, data collected by the Public Health and Veterinary services between 2016 and 2019 indicated that 45 human rabies cases were diagnosed clinically. Furthermore, 1,978 dog bites and 77 canine rabies cases (diagnosed clinically) were officially reported between 2008 and 2018. While these cases were identified in nine of the twelve counties, the majority occurred in the Borgou County where the third largest city of the country, Parakou, is located.

Until 2018, the laboratory confirmation of rabies in either animals or humans was not available in Benin and diagnostic confirmation relied on the identification of clinical diagnosis, which is known to be misleading and inaccurate (4, 5). The Central Veterinary Laboratory (CVL) of Parakou, the Laboratoire de Diagnostic Vétérinaire et de Sérosurveillance (LADISERO), was built in 1985 thanks to the European Development Fund (EDF). However, due to a lack of further funding, diagnostic activities for diseases of animals (not rabies) only started in 1993 under the financial support of the Economic Community of West African States.

Here we present a collaborative effort between National Agencies in Benin and several International Organizations, leading to the advancement of Benin toward the overarching objective of the Zero-by-30 goal at a global level (6). First, a situational analysis of rabies in Benin was undertaken, after which the country's diagnostic capacity at the CVL was strengthened in various manners in response to the outcome of the landscape analysis. More specifically, in addition to establishing diagnostic confirmation for rabies at the CVL, we characterized recent canine strains of rabies virus circulating in Benin to gain an improved understanding of the epidemiology of rabies in the country. As pointed out in the study by Mbilo et al. (2), no scientific data on the rabies situation in Benin has been available to date. As such, to our knowledge, this is the first study on animal rabies in Benin presenting both a report of the actions undertaken to assist the government of Benin in eventually reaching the goal of zero dog-mediated human rabies by 2030 goal and a phylogenetic analysis of the canine rabies virus variants circulating in the country.

MATERIALS AND METHODS

Stepwise Approach Toward Rabies Elimination Workshop

In January 2018 the Global Alliance for Rabies Control (GARC) undertook an in-country Stepwise Approach toward Rabies Elimination (SARE) workshop in collaboration with the Rabies Advisory Group of Benin as well as representatives from both the Ministries of Agriculture and Health. The SARE is a One Health oriented monitoring and evaluation tool that allows countries to undertake a comprehensive situational analysis of the rabies situation in their country. As a primary outcome, the SARE serves to highlight completed and pending activities that align with key components that make up a successful rabies elimination strategy, and provides an empirical score out of five, which is calculated based on the activities that have been completed according to the workshop participants (7–10). In addition to the SARE tool, the GARC Educational Platform (GEP) and the Rabies Epidemiological Bulletin (REB) was introduced during the workshop.

Implementation of Multiple Diagnostic Tests at LADISERO

The implementation of diagnostic tests at LADISERO was guided by the availability of essential equipment and by the established previous experience of laboratory staff. Following the SARE assessment, the direct, rapid immunohistochemical test (DRIT) was implemented in Benin through a training workshop undertaken in 2018 at the Ministry of Health's National Service of Public Health Laboratories in Cotonou, Benin, relying on an established training methodology that had been used in various laboratories across Africa. Five local diagnosticians were trained in the implementation of the test, including two individuals from LADISERO in Parakou, one individual from the Veterinary Laboratory (LABOVET) in Bohicon, one private veterinarian from Cotonou, and one individual from the National Service of Public Health Laboratories in Cotonou. Over the course of the 3-day workshop, trainees were introduced to the DRIT assay and its use in a diagnostic setting by applying the assay to a cohort of brain samples that had been collected and stored specifically for the purpose of the workshop. In addition to the DRIT training course, the workshop also included a training on the safe and effective collection of brain samples by sampling through the occipital foramen to ensure an adapted sample collection methodology for diagnostic confirmation (11, 12) and the establishment of a rabies diagnosis database. Thereafter, any suspect rabies samples were delivered to the CVL for diagnostic confirmation using the DRIT assay as described elsewhere (13). During the routine implementation of the DRIT, each of the brain samples were blindly tested by treating the homogenized tissue impression smears with a biotinylated anti-ribonucleoprotein polyclonal antibody (PAb) preparation (ARC-OVI, Rabies Unit). Two experienced diagnosticians interpreted the results, and a consensus agreement was required before confirming the diagnostic outcome of any given sample.

Between March 2018 and June 2021, samples of central nervous system (CNS) tissues were collected from animals suspected to be infected with RABV. The samples were collected using the foramen magnum sampling procedure under strict biosafety conditions and procedures that aligned with the necropsy of potentially rabid carcasses. From the point of sample collection, the cold chain was maintained, and the samples were stored at −20°C at the CVL after being subjected to diagnostic confirmation (11, 14).

Due to a limited access of the laboratory to real-time RT-PCR technology, a conventional RT-PCR (15) was subsequently implemented at the CVL in Parakou through remote assistance in 2019. A French version of the detailed protocol was provided to the CVL via email (**Supplementary Data**). Briefly, each of the brain samples submitted at the CVL for rabies diagnosis were blindly tested by homogenization directly in lysis buffer and RNA was extracted using the RNeasy Mini kit (Qiagen). Analysis for the presence of lyssavirus viral RNA was performed (15) and amplicons were visualized using 1.5% agarose gel electrophoresis.

Finally, in February 2021, fifty Rapid Immunochromatographic Diagnostic Test (RIDT) kits [Rapid Rabies Ag Test Kit Bionote, Inc. (Hwaseong-si, Korea)] were also provided to the CVL in Parakou. Briefly, the cotton swabs provided in the kit were inserted directly into the brain samples to collect a pea-sized amount of brain tissue. The brain sample was placed into the buffer solution (provided in the kit) followed by thorough homogenization directly in the tube using the swab. Four drops of the buffer solution were then added to the sample inlet using a disposable dropper provided in the kit. The readout was made 10 min afterwards, as recommended by the manufacturers (16).

Diagnostic Confirmation and Strain Characterization at an International Reference Center

A cohort of DRIT-tested samples ($n = 10$) were sent to the IZSVe for diagnostic confirmation. Of the ten samples, seven (7) were rabies-positive and three (3) rabies-negative by means of the DRIT assay as applied in Benin. Once delivered to the FAO RC for rabies, the samples were subjected to diagnostic confirmation by means of the DFA test and a conventional RT-PCR reaction as described elsewhere (15). Slides for the DFA test were prepared by smear technique, fixed, and then stained using a commercial anti-rabies nucleocapsid conjugate (Bio-Rad, 3572112) to which was also added some Evans blue counterstain (final concentration = 1%).

The products of RT-PCR were further characterized by Sanger sequencing before phylogenetic analyses were undertaken. Maximum likelihood (ML) nucleotide phylogenetic trees were inferred using PhyML (version 3.0) (17), employing the GTR+Γ4 substitution model, a heuristic SPR branch-swapping algorithm and 100 bootstrap replicates; obtained tree was edited online for graphical display using iTOL (18).

Participation to an International Inter-laboratory Comparison

In the fall of 2020, the LADISERO, and sixteen other African laboratories, were invited to participate in an inter-laboratory comparison (ILC) test for the diagnosis of rabies. The sample panel to be tested included 12 freeze-dried blind coded samples and a starter kit composed of a positive and a negative control. Samples were prepared as previously described (19) following a well-established procedure. Prior to sending the samples, the vaccination status of the staff was controlled via the collection of a vaccination datasheet.

Ethical Statement

The brain specimens were collected with the consent of pet owners or collectivities for stray animals. Animal health professionals are mandated to collect samples from non-endangered suspect rabid animals without requiring ethical permission since it is done under the framework of passive surveillance for rabies. The Animal Ethics Committee (AEC) of the University of Pretoria (South Africa) provided ethical approval for the implementation of the DRIT as described here (Approval numbers: EC027-16).

The samples prepared for the ILCs panel included (i) the use of laboratory animals and (ii) the use of samples collected and submitted to the laboratory and tested within the diagnostic activities carried out at the FAO Reference Center (RC) for rabies. (i) All experimental procedures involving laboratory animals were performed in strict accordance with the relevant national and local animal welfare bodies [Convention of the European Council no. 123 and National guidelines (Legislative Decree 26/2014)]. The Committee on the Ethics of Animal Experiments of the IZSVe approved the protocol (CE.IZSVe20/2014) which was then authorized by the Italian Ministry of Health (Decree 505/2015-PR). (ii) The brain tissue obtained from archived CNS samples of non-endangered mammals was collected in the framework of the national passive surveillance for rabies. All animals were found dead or legally euthanized by a competent veterinarian. According to the national legislation regulating animal experimentation, no ethical approval or permit was required for collecting and processing this type of specimen.

RESULTS

In-country SARE Assessment in Benin

After completing the SARE assessment, Benin's SARE score was determined to be 1.5 out of 5. This broadly signified that Benin had small-scale rabies control programs in place and that the government was working toward developing a national rabies control program. In addition to the score itself, the

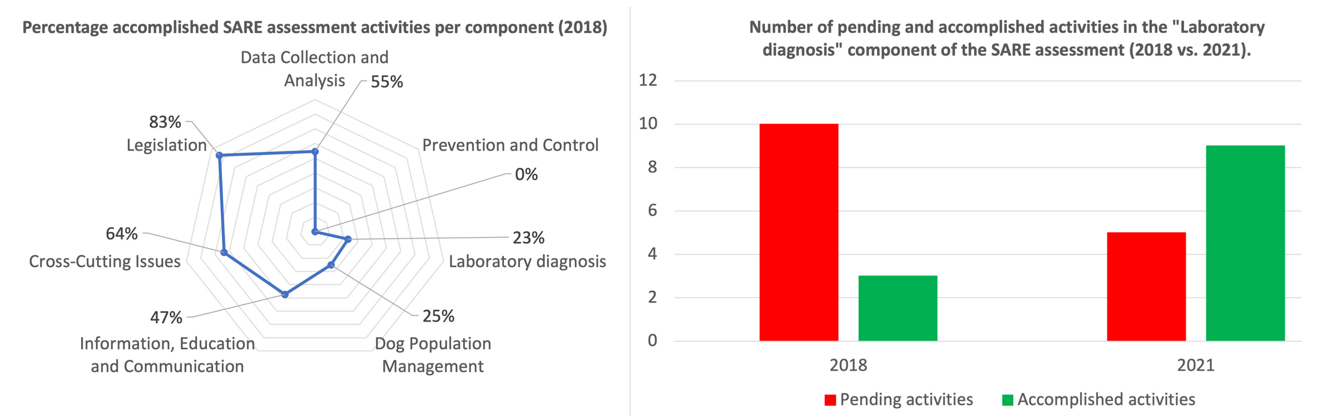

FIGURE 1 | Summary of results for the SARE assessment of the national rabies control program, disaggregated by the seven components (left), and impact of the development of Benin's laboratory capacity (right).

outputs generated by the SARE indicated that various SARE activities forming part of the different SARE components were still "pending" in Benin (**Figure 1**). Most significant in the scope of the work described here were the activities that aligned with laboratory capacity in Benin. More specifically, the participants indicated the following: (i) there was no laboratory testing of suspect rabid samples in the country (although diagnostic capacity was to be established after the workshop), (ii) there was no secondary diagnostic assay that could be used for diagnostic confirmation of suspect rabid samples, (iii) there was no participation in any proficiency testing initiatives with an internationally recognized laboratory and, (iv) there was no molecular characterization of RABV variants circulating in Benin (completed SARE output provided in **Supplementary File 1**). In support of the government's efforts to control rabies, these specific activities were addressed through various collaborative efforts described here (**Figure 1**).

Rabies Diagnostic Flowchart Implemented at LADISERO Allows the Accurate Identification of Positive Samples

Between March 2018 and June 2021, 61 suspect rabid samples were subjected to diagnostic confirmation at LADISERO using the DRIT assay. The samples had all been collected from domestic animals, i.e., two (2) cats (2/61, 3.3%) and fifty-nine (59) dogs (59/61, 96.7%) (**Table 1**). Of these samples, 34.4% (21/61) were initially found to be rabies-positive (**Table 1**).

The results obtained by nucleic acid amplification at the laboratory by means of the conventional RT-PCR showed that all 34 samples collected in 2018 matched those provided by the DRIT (**Table 1**). However, out of the 13 samples collected in 2019, 4 discrepant results were observed between the two techniques (DRIT and RT-PCR). As seen in **Table 1**, four samples (samples 35, 37, 41, and 42) were found rabies-negative by the DRIT, but rabies-positive when tested using RT-PCR. These four samples were blindly retested by means of the DRIT and results from the second test were rabies-positive. Therefore, twenty-five samples (25/61, 41%) tested positive for rabies after diagnostic confirmation was established by means of two diagnostic assays. A map presenting the geographical repartition of the collected samples is available in **Figure 2**.

To ensure that the diagnostic outcomes produced by the LADISERO were correct, ten samples (3 negatives and 7 positives) were shipped to the IZSVe for additional diagnostic confirmation in March 2019. Here, the samples were blindly re-tested by means of the DFA test as well as the same conventional RT-PCR protocol implemented at the CVL. All the results generated at the RC agreed with those from LADISERO, providing further evidence in support of the CVL correctly implementing the protocols (**Table 1**).

Molecular Epidemiology of Rabies in Benin

To further enhance the resolution of the surveillance data, a phylogenetic analysis was performed on the seven (7) positive samples collected from dogs in Benin, i.e., Parakou (4/7), Tchaourou (1/7), N'Dali (1/7), Ouessé (1/7), and sent at the IZSVe for confirmatory analysis. The analysis of the seven (7) partial N gene segments showed that the RABV sequences circulating in 2019 in Benin belonged to the Africa 2 lineage, which is known to be circulating within terrestrial animal population in Central and Western African countries (20). More specifically, the new sequences clustered together with other viruses from the "F group," circulating in neighboring countries such as Nigeria, Niger, and Burkina Faso (**Figure 3**) (20). The sequences were deposited in GenBank on the 23rd of April 2020 (Accession Numbers MT370501/MT370507).

Implementation of Post-mortem Rabies Rapid Immunochromatographic Diagnostic Assays

All the positive samples ($n = 25$), and a small panel of negative samples ($n = 4$), received at the CVL were tested using the LFD after being subjected to diagnostic confirmation with the DRIT assay. The results showed 100% agreement in the detection

TABLE 1 | Description of the samples analyzed throughout this study.

ID number	Date of laboratory submission	Specie	Symptoms	Anamnesis (including putative origin/owner/history of vaccination)	Results dRIT test 1	Results DFA	Results RT-PCR at IZSVe	Results RT-PCR at CVL Parakou	Results dRIT test 2	Results LFD
1	07/03/2018	Dog	Agitation, Salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
2	29/03/2018	Dog	Agitation, Salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
3	02/04/2018	Cat	Agitation	Biting animal/killed	Negative	Negative	Negative	Negative	N/A	Negative
4	04/04/2018	Dog	Hypersalivation	Slaughtered	Negative	N/A	N/A	Negative	N/A	Negative
5	11/04/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
6	21/04/2018	Dog	Agitation, salivation	Biting stray dog slaughtered immediately	Positive	N/A	N/A	Positive	N/A	Positive
7	19/05/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
8	09/05/2018	Dog	Abatement, agitation	Biting stray dog slaughtered immediately. No boost for vaccination	Positive	Positive	Positive	Positive	N/A	Positive
9	15/05/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
10	23/05/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
11	19/06/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
12	12/06/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
13	27/06/2018	Cat	Salivation	Biting cat immediatly slaughered. Non vaccinated	Positive	Positive	Positive	Positive	N/A	Positive
14	04/07/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
15	18/07/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
16	01/08/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
17	08/08/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
18	14/08/2018	Dog	Agitation	Biting animal slaughtered	Negative	N/A	N/A	Negative	N/A	N/A
19	23/08/2018	Dog	Agitation, salivation	Biting stray dog slaughtered immediately. Non vaccinated	Positive	Positive	Positive	Positive	N/A	Positive
20	28/08/2018	Dog	Salivation	Biting stray dog slaughtered immediately	Positive	Positive	Positive	Positive	N/A	Positive
21	11/09/2018	Dog	Salivation, agitation	Slaughered	Negative	N/A	N/A	Negative	N/A	N/A
22	20/09/2018	Dog	Prostation, apathy	Slaughered	Negative	N/A	N/A	Negative	N/A	N/A
23	15/10/2018	Dog	Agitation, hypersalivation	Biting animal slaughtered immediately	Negative	N/A	N/A	Negative	N/A	N/A
24	23/10/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
25	30/10/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
26	30/10/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
27	04/11/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A

(Continued)

TABLE 1 | Continued

ID number	Date of laboratory submission	Specie	Symptoms	Anamnesis (including putative origin/owner/history of vaccination)	Results dRIT test 1	Results DFA	Results RT-PCR at IZSVe	Results RT-PCR at CVL Parakou	Results dRIT test 2	Results LFD
28	04/11/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
29	09/11/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
30	21/11/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
31	26/11/2018	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
32	27/11/2018	Dog	Abatement, agitation	Biting stray dog slaughtered immediately. Non vaccinated	Positive	Positive	Positive	Positive	N/A	Positive
33	29/11/2108	Dog	Salivation	Slaughtered for consumption at the discretion of the owner	Positive	Positive	Positive	Positive	N/A	Positive
34	30/11/2018	Dog	Agitation, salivation	Biting stray dog slaughtered immediately. Non vaccinated	Positive	Positive	Positive	Positive	N/A	Positive
35	01/03/2019	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Positive	Positive	Positive
36	01/03/2019	Dog	Agitation	Biting animal slaughtered	Negative	Negative	Negative	Negative	Negative	Negative
37	18/07/2019	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Positive	Positive	Positive
38	18/07/2019	Dog	Salivation	Biting stray dog slaughtered immediately	Negative	N/A	N/A	Negative	N/A	N/A
39	19/07/2019	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
40	27/07/2019	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
41	27/07/2019	Dog	Hypersalivation	Biting animal slaughtered	Negative	N/A	N/A	Positive	Positive	Positive
42	27/07/2019	Dog	Hypersalivation	Biting animal slaughtered	Negative	N/A	N/A	Weak positive	Positive	Positive
43	27/07/2019	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
44	27/07/2019	Dog	Agitation, salivation	Biting animal slaughtered	Negative	Negative	Negative	Negative	Negative	N/A
45	17/09/2019	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
46	01/10/2019	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
47	30/10/2019	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Negative	N/A	N/A	Negative	N/A	N/A
48	17/03/2020	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Positive	N/A	N/A	Positive	N/A	Positive
49	17/03/2020	Dog	Agitation	Biting animal slaughtered	Positive	N/A	N/A	Positive	N/A	Positive
50	17/03/2020	Dog	Agitation, salivation	Slaughtered for consumption at the discretion of the owner	Positive	N/A	N/A	Positive	N/A	Positive
51	17/03/2020	Dog	Salivation	Slaughtered for consumption at the discretion of the owner	Positive	N/A	N/A	Positive	N/A	Positive
52	28/05/2020	Dog	Agitation, salivation	Biting stray dog slaughtered immediately	Positive	N/A	N/A	Positive	N/A	Positive
53	28/06/2020	Dog	Anorexia, aggressiveness, and paralysis	Biting stray dog slaughtered immediately	Positive	N/A	N/A	Positive	N/A	Positive
54	28/06/2020	Dog	Aggressiveness, incoordination, salivation	Biting stray dog slaughtered immediately	Positive	N/A	N/A	Positive	N/A	Dubbious (EXCLUDED)

(Continued)

TABLE 1 | Continued

ID number	Date of laboratory submission	Specie	Symptoms	Anamnesis (including putative origin/owner/history of vaccination)	Results dRIT test 1	Results DFA	Results RT-PCR at IZSVe	Results RT-PCR at CVL Parakou	Results dRIT test 2	Results LFD
55	28/08/2020	dog	Aggressiveness, incoordination, salivation	Biting stray dog slaughtered immediately	Positive	N/A	N/A	Positive	N/A	Positive
56	29/08/2020	Dog	Salivation	Biting stray dog slaughtered immediately	Negative	N/A	N/A	Negative	N/A	Negative
57	19/09/2020	Dog	Anorexia, aggressiveness, and paralysis	Biting stray dog slaughtered immediately	Positive	N/A	N/A	Positive	N/A	Positive
58	19/09/2020	Dog	Aggressiveness, incoordination, salivation	Biting stray dog slaughtered immediately	Positive	N/A	N/A	Positive	N/A	Positive
59	21/09/2020	Dog	Anorexia, aggressiveness, and paralysis	Biting stray dog slaughtered immediately	Positive	N/A	N/A	Positive	N/A	Positive
60	24/12/2020	Dog	Aggressiveness, incoordination, salivation	biting dog	Positive	N/A	N/A	Positive	N/A	Positive
61	04/06/2021	Dog	Agitation, salivation	Biting animal slaughtered	Positive	N/A	N/A	Positive	N/A	Positive
Total negative samples					(40/61), 65.6%	/	/	(36/61), 59%	(36/61), 59%	
Total positive samples					(21/61), 34.4%	/	/	(25/61), 41%	(25/61), 41%	

In red, the samples found negative by DRIT (test 1) but positive by RT-PCR. In blue, the samples sent to the IZSVe for confirmatory test analysis and submitted to phylogenetic analysis.

of negative samples but very importantly, 100% agreement in the detection of the positive samples compared to the results obtained by the DRIT and the conventional RT-PCR (**Table 1**). One sample, sample ID 54, had to be excluded from the analysis as the device control line did not appear on two consecutive tests, despite the device's test line indicating a positive result in both instances.

First Participation in an Inter-laboratory Comparison Test Confirmed the Appropriate Testing Procedure

Prior to receiving the panel of samples in November 2020, the vaccination status of the staff that were in contact with rabies suspected samples was checked and the three technicians in charge for rabies diagnostic activities at the CVL (Parakou) were found to be fully vaccinated against rabies. The CVL in Parakou tested the ILC panel by means of the DRIT and the conventional RT-PCR. In agreement with the acceptance criteria, the analysis of results demonstrated that the laboratory successfully passed this ILC and was able to correctly identify all the samples of the panel, thus demonstrating a high level of technical skills for the diagnosis of rabies. For confidentiality purposes, a detailed presentation of the results cannot be provided in this study.

DISCUSSION

The newly developed Global Strategic Plan for rabies elimination (GSP) (6) relies on a country-centric approach whereby governments need to take ownership of their national rabies programs; with the global community then identifying possible areas to assist and support national governments. In the case of Benin specifically, the Beninese government undertook an in-country SARE assessment in 2018 and identified various activities that would need to form part of their own national rabies control program. In so doing, the government outlined various activities that members from the global community identified as specific activities where support could be provided in furtherance of the national government's own efforts.

Prior to the work reported here, Benin was not able to diagnose rabies in either animals or humans due to a lack of specific reagents and/or skills required to implement any assays. This hindered the government's ability to showcase the burden of the disease and thus break the prevailing cycle of neglect. By establishing rabies diagnosis by means of the OIE-recommended DRIT assay [which involved the collaboration between the government, Swiss Tropical and Public Health Institute (Swiss TPH) and GARC] and confirmatory testing by means of a conventional RT-PCR (which involved the collaboration between the government and the IZSVe) in Benin, the government can begin to understand the disease burden on their population. In fact, the CVL had received and tested 61 samples in about 3 years—an incredible increase for a small and low populated country that had no capacity prior to the work presented here and out of which more than 40% were tested positive for rabies. Sadly, the number of samples received since 2018 has decreased significantly–thirty-four samples in 2018 and one sample in 2021.

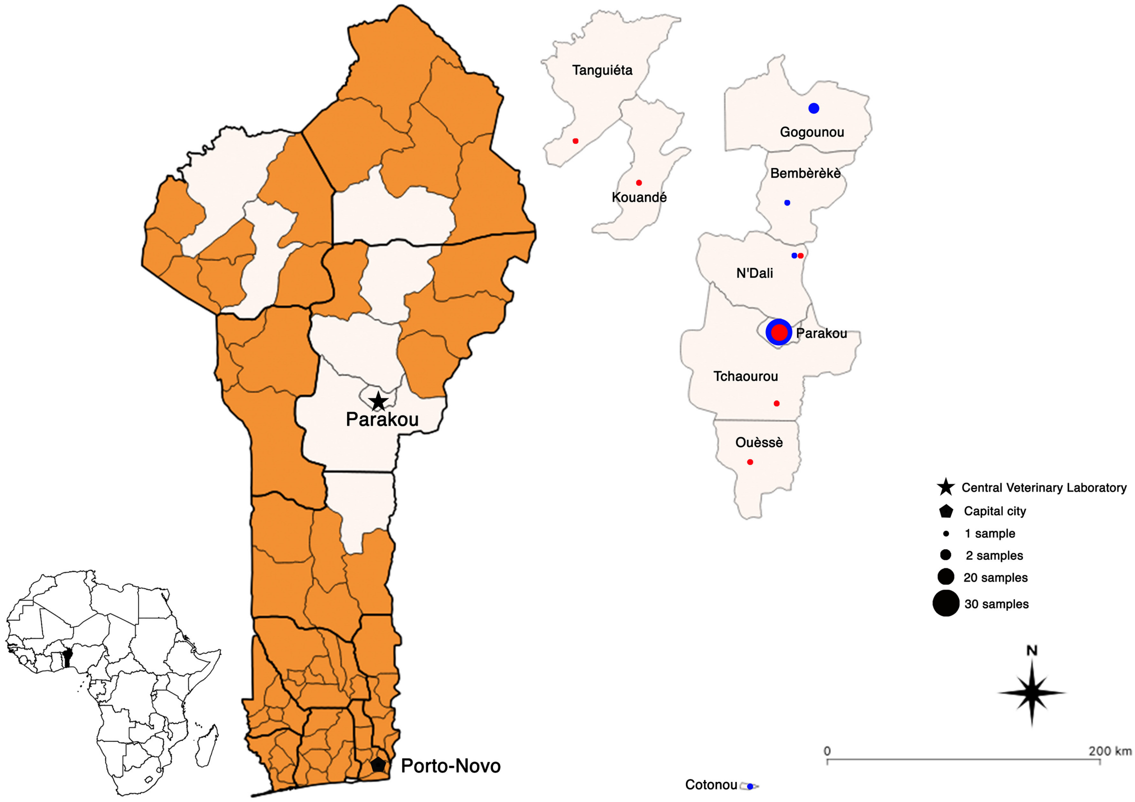

FIGURE 2 | Geographical maps of Benin showing the location of all the rabies-positive (red dots) and -negative (blue dots) samples collected between 2018 and 2021. Map created using QGIS software (version 3.10).

The reason for this is mainly attributable to the weakness of the surveillance system infrastructures currently in place in Benin, infrastructures that must be urgently strengthened along with the vaccination campaigns to achieve the final goal of Zero by 30. Indeed, the entire surveillance system was based on the CVL in Parakou field agents actively collecting samples in the field. Those agents who were specifically trained in the collection of field samples for the diagnosis of rabies, have been only partially included in a wider system and trained other staff, in order to lay the foundation of a solid and sustainable rabies surveillance. Recruiting and training field agents in Africa is not always an easy task, given many perceived or real competing disease priorities. In any case, the CVL in Parakou is also working to address this issue in the shortest delay and is currently establishing some collaborations with another division within the Veterinary Services. Although great achievement has been reached in Benin so far, the country's rabies epidemiology must be fed by continuous surveillance from the field. In this context, the role of operational veterinary services in collaboration with diagnostic laboratories is pivotal to ensure an efficient rabies control and prevention (21).

Furthermore, the CVL now has the capability to detect both the viral antigen and the viral nucleic acids, which dramatically decreases the chance of misdiagnosing samples. The importance of this ability to undertake diagnostic confirmation was highlighted in this study where the presence of four false negative results (two collected from biting dogs and two collected from dogs slaughtered for meat consumption) using the DRIT would have gone undetected had it not been for the use of the conventional RT-PCR. Importantly, these findings do not suggest that the DRIT is an inferior test or that it should be replaced by RT-PCR as both tests have pro and cons. The presence of false negative results by means of antigen detection can be due to various factors such as a weak viral load in the brain specimen collected for analysis, an inadequate quality of the brain specimen or a misinterpretation by the slide readers (22). Similarly, while the RT-PCR is more sensitive than antigen detection methods (both the DFA and DRIT), the risk of cross-contamination of the molecular approach is significantly higher in resource-limited laboratories (23). These results, as mentioned elsewhere (24), suggest that diagnostic confirmation by means of a second assay that is applied in parallel is of the utmost importance when confirming the presence or absence of rabies. Given the public health threat that animal rabies constitutes, the parallel analysis of high-risk suspect samples, principally those collected from biting animals or animals slaughtered for meat consumption, for the presence of the viral antigen and the viral nucleic acids should never be seen as a wasted effort and/or investment. Indeed, an ideal scenario for a laboratory possessing antigen and molecular detection platforms is to test any high-risk suspected samples

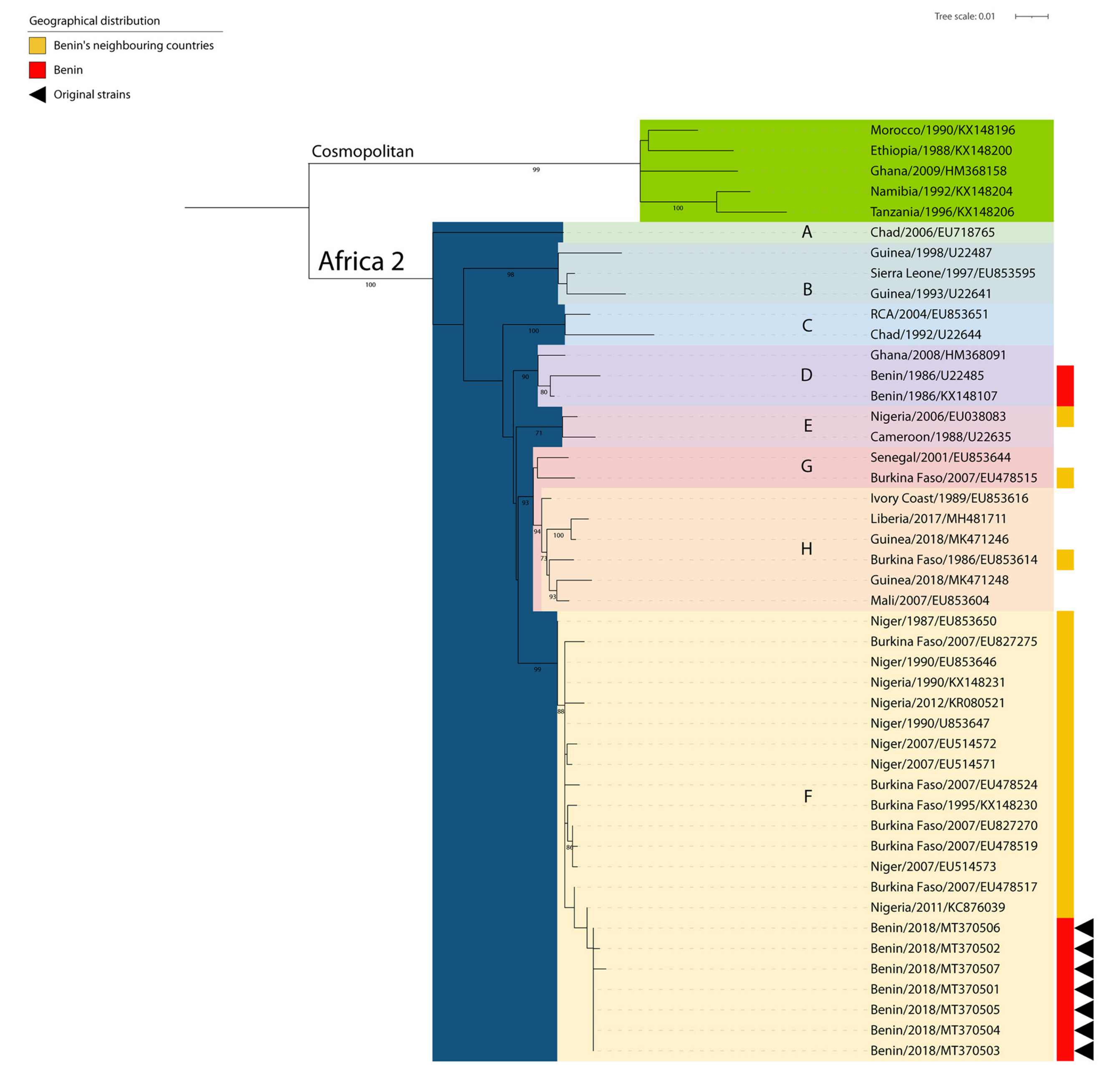

FIGURE 3 | Phylogenetic tree constructed by Maximum Likelihood of the N gene. The rabies viruses from Benin are indicated with a red bar (right side) and those arisen from this study further identified with black triangles (right side). The rabies viruses collected from neighboring countries are indicated with a yellow bar (right side). SH-like supports higher than 70% are indicated next to the nodes.

either by DRIT or DFA test and to then confirm the results, both negative and positive, by means of RT-PCR.

Based on the collected data, 32 out of the 61 dogs (52.5%) were consumed after culling upon suspicion of rabies infection. Of those, 6 (18.8%) tested positive for rabies, including two dogs (sample 35 and 37) which were first tested as negative using the DRIT. Based on the information collected in the field, these animals were consumed before the diagnostic outcomes. Although the consumption of dog meat can be seen as taboo in some industrialized countries, it is a common practice in Sub-Saharan African countries with notably Nigeria often described as the largest consumer (25, 26). Given the prevalence of rabies in Africa, the risk associated for butchers, meat handlers, and consumers are high and should not be underestimated. As a matter of fact, studies which all took place in Nigeria showed that whilst butchers are not vaccinated against rabies, they frequently handle contaminated meat (25, 26). The level of positive attitude and practice related to a potential contact with rabies positive animals correlates with the level of education pointing out the need to raise awareness about the disease (27).

Overall, the samples collected near Parakou were sampled either at a meat market or at the Parakou veterinary clinic close to the CVL. Due to yearly awareness campaigns during World Rabies Day (WRD) which is celebrated on the 28th of September, the population of Parakou has adopted the appropriate behavior in case of animal rabies suspicion, i.e., to inform the local animal health services or to directly bring animals to the clinic. This further demonstrates the importance of correctly educating the population on the impact of the disease and the actions to take upon suspicion of animal rabies. Samples collected from other counties were processed by local animal health services and then sent to the CVL. Indeed, the lack of decentralized testing facilities implies that all samples must travel to the CVL for analysis using the DRIT and the RT-PCR. Although RIDT is not yet a recommended test for the detection of rabies antigens, it still constitutes a low technology diagnostic alternative which is also rapid, reader-friendly, and applicable to diagnostic screening under in-field condition. Within that framework, further evaluation of the performance of the RIDT could be highly beneficial in a country like Benin. The LADISERO received enough kits to test all the positive samples as well as a handful of negative samples. As a matter of fact, whilst the sensitivity of this type of kit is still under debate, their specificity has not been criticized. An updated protocol has been developed and so far, has proven to be highly performant in several studies (16, 28–31). In our hands, the results obtained using the RIDT were in total agreement with the ones obtained using the two gold standard techniques available at the LADISERO (**Table 1**). However, one sample had to be excluded, this sample resulted twice in being positive in the result test line but with the device control line not appearing. The exact reason behind this observation is unknown as this sample did not appear to be of a different texture or freshness of other tested samples. Importantly, GARC has recently developed the Rapid In-field Diagnosis and Epidemiology of Rabies (RAIDER) protocol which aims to facilitate the diagnosis of rabies in remote place with limited access to diagnostic facilities. The RAIDER protocol should further validate the use of the updated protocol for the RIDT (16) as a useful field screening approach thus facilitating fast responses when PEP is necessary of potentially exposed human cases. This protocol aims to promote the use of the RIDT as a screening method of samples directly on the field coupled with the shipment of samples to a laboratory applying a gold standard technique for final confirmation of rabies-status.

International travel of rabies suspected specimens is costly and requires the use of a dedicated courier under the Cat B UN2900 as defined by the IATA regulation. Thanks to the support of the Joint FAO/IAEA Center, the CVL was able to send 10 samples for diagnostic confirmation and further strain characterization to the IZSVe. Firstly, this collaboration established that sample analysis using the DRIT had been applied correctly by the CVL. Importantly, the appropriate application of the protocols and correct analysis of rabies suspected samples was further demonstrated by a successful participation in an inter-laboratory comparison (ILC) test in the fall of 2020. Secondly, the phylogenetic analysis of the seven (7) positive samples demonstrated that all of them belonged to the Africa 2 lineage/group F. This lineage of RABV is known to be circulating in neighboring countries (Nigeria, Niger, and Burkina Faso) (20), but this is the first time that a phylogenetic analysis focused on viruses collected from Benin is made publicly available. This finding, although expected, underlines the importance of monitoring the epidemiology of canine rabies circulation through a constant characterization of samples representative of the entire geographical area. Before our study, only one isolate collected from a cat in Benin in 1986 without any precise location (Accession Numbers U22485 and KX148107) was shown to cluster within the Africa 2 lineage/group D (20, 32). To this date, no information from Togo is available yet and overall sequencing from the entire region is occasional, thus jeopardizing any efforts in characterization. The conventional approach followed by partial sequencing selected herein provides a proof of principle that relevant epidemiological information can be obtained by the CVL in Parakou with a little additional funds. In this context, amplicons obtained at the CVL might be further sequenced making use of outsource facilities, thus enabling local authorities in assessing the origin of the circulating strains.

From the initial SARE assessment in January 2018 to the implementation of the DRIT as well as RT-PCR, the characterization of the strains circulating in Benin and the participation to an ILC, several milestones were achieved for the diagnosis of animal rabies in the country (**Figure 1**). Although partially supported by international partners (*per se* committed to capacity building in developing countries), all achievements were obtained thanks to the firm engagement of local staff. A summary of the costs related to the activities undertaken within the framework of the implementation of a rabies diagnostic service at the CVL of Parakou is described in this study and showed that about 16,000 euros (**Supplementary Table 1**) equivalent to about 19,000 US dollars was invested. This proves that even small investment can lead to great achievement, and this should be forwarded to decision makers and national authorities to improve the budget access.

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.ncbi.nlm.nih.gov/genbank/, MT370501/MT370507.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Ethics Committee (AEC) of the University of Pretoria (South Africa). Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

FD, MG, and PD conception and design of the study. MG and AC were involved in the manuscript drafting. FD, RA, RT, YA, and CG were all involved in the field study and collection of data. SL was involved in analysis of data. SM and LN were involved in the field study and implementation of methodology. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2021.769114/full#supplementary-material

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A One Medicine Mission for an Effective Rabies Therapy

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Despite the disease's long history, little progress has been made toward a treatment for rabies. The prognosis for patient recovery remains dire. For any prospect of survival, patients require aggressive critical care, which physicians in rabies endemic areas may be reluctant or unable to provide given the cost, clinical expertise required, and uncertain outcome. Systematic clinical research into combination therapies is further hampered by sporadic occurrence of cases. In this Perspective, we examine the case for a One Medicine approach to accelerate development of an effective therapy for rabies through the veterinary care and investigational treatment of naturally infected dogs in appropriate circumstances. We review the pathogenesis of rabies virus in humans and dogs, including recent advances in our understanding of the molecular basis for the severe neurological dysfunction. We propose that four categories of disease process need to be managed in patients: viral propagation, neuronal degeneration, inflammation and systemic compromise. Compassionate critical care and investigational treatment of naturally infected dogs receiving supportive therapy that mimics the human clinical scenario could increase opportunities to study combination therapies that address these processes, and to identify biomarkers for prognosis and therapeutic response. We discuss the safety and ethics of this approach, and introduce the Canine Rabies Treatment Initiative, a non-profit organization with the mission to apply a One Medicine approach to the investigation of diagnostic, prognostic, and therapeutic options for rabies in naturally infected dogs, to accelerate transformation of rabies into a treatable disease for all patients.

Keywords: rabies, treatment, pathogenesis, prognosis, canine, neurodegeneration, immunotherapy, blood-brain barrier

INTRODUCTION

Rabies is one of the oldest described infectious diseases of humans and dogs (1). An entry in the *Susruta Samhita*, a text from around 600–1,000 years BCE, states that if a person bitten by a rabid dog shows signs of hydrophobia—a feature characteristic of rabies in humans—the person is considered "doomed" (2). Despite considerable advances in knowledge of the etiology and pathogenesis of this neglected tropical disease (NTD) over the intervening millennia, the prognosis for human (and canine) patients with clinical manifestations of rabies remains largely unchanged today. This zoonosis has the highest case fatality of any infectious disease, with death typically occurring within 14 days of onset of clinical signs. Since the 1970s, survival has been well documented in only around 30 of the estimated 3 million human cases that have occurred in that time (3–5). Of these survivors, most suffered severe neurological sequelae (3, 4).

A report of the recovery, with only minor neurological deficits, of a previously unvaccinated rabies patient following induction of a therapeutic coma in Milwaukee, WI, USA in 2004 provided hope of an effective therapeutic protocol (6). Unfortunately, multiple efforts to replicate this Milwaukee protocol have been unsuccessful, with at least 53 documented failures (3). Although there has been an increase in the number of reports of survival from rabies in more recent years, particularly from India, this is likely due to improvements in critical care and more attempts at aggressive management rather than development of specific therapies. Survivors frequently have severe neurological sequelae, with a poor quality of life (3, 7, 8). With abandonment of the Milwaukee protocol, and with poor patient prognoses even with aggressive management, physicians are presented with few therapeutic options beyond palliative care (4, 9).

Worldwide, most human rabies deaths are caused by infection with rabies virus (RABV) transmitted from domestic dogs. Accurately determining the number of people who die from dog-transmitted rabies is hampered by lack of reliable surveillance data from many of those countries where the disease is most prevalent, but estimates place this number at ∼59,000 each year (95% confidence intervals 29,000–159,000) (5). The burden of this NTD falls predominantly on populations in resource-poor communities in Africa and Asia. The number of dogs that die from rabies each year is unknown, but extrapolation of figures used to estimate the burden of human disease would place this number in the millions, if not tens of millions. This number does not include those dogs that are not rabid but are killed on suspicion of being infected or in response to rabies outbreaks.

The fatal outcome of rabies and lack of therapeutic options have focused efforts on prevention of human cases by reducing or eliminating transmission of RABV among dogs through mass vaccination, and through the timely provision of post-exposure prophylaxis (PEP) to exposed people. These efforts are culminating in the goal to eliminate human deaths from dog-transmitted rabies by 2030 (10). Alongside this, efforts to develop a treatment for this NTD should continue. Rabies is not a candidate for eradication. The disease will continue to pose a threat in circumstances in which there is failure to recognize exposure or to seek PEP; when there is lack of access to PEP or deviation from recommended PEP protocols; or in rare cases when PEP fails or is refused (11, 12). Transmission of RABV from wildlife hosts will continue to pose a risk, including for the re-emergence of dog rabies through spillover events and host switching (13). Lyssaviruses against which PEP is not effective continue to emerge and pose a threat to public and animal health (14, 15).

Thus, there remains a need to develop an effective therapy for rabies. This paper examines the case for a One Medicine approach to research and development of a combination therapy in naturally infected canine patients, to supplement research in experimental animal models and human cases. We conclude with a call to action for veterinarians to play a more active role in development of an effective therapy for this NTD.

RABIES VIRUS PATHOGENESIS

The pathophysiology of rabies has been reviewed in detail (16–18). Many of the clinical features of rabies are similar in human and canine cases, although not all elements are well described in both species. The main mode of transmission of RABV in both hosts is through exposure of mucosal membranes or broken skin to the saliva of an infected dog, typically through a bite, scratch, or lick. Clinical signs develop after a variable and occasionally prolonged incubation period, and are often non-specific, particularly in the early stages of disease. As the disease progresses to the acute neurological phase, one of two clinical forms usually become apparent. Most human and canine patients develop an encephalitic or "furious" form, while the remainder develop a paralytic or "dumb" form (18, 19). The pathogenesis of the two forms is unclear, although the host immune response is thought to play an important role, with a more prominent inflammatory response evident in paralytic cases (20, 21). Both forms of the disease typically progress to coma and death within days, although the time to death may be influenced by critical care interventions.

A typical feature of rabies is that despite the consistent and catastrophic clinical outcomes, histopathological changes observed in the central nervous system (CNS) after death following acute disease are relatively mild. Preservation of the neuronal network is essential for this strictly neurotropic virus to complete its cycle within a host, from site of entry, transit, and propagation through the nervous system, and exit through the salivary glands. As such, RABV has evolved mechanisms to promote survival of infected neurons by inhibiting apoptotic death and by avoiding or inhibiting activation of inflammatory responses that would lead to death of infected cells (22–25). Rabies virus neutralizing antibodies (RVNAs) are often detectable only late in the course of disease, if at all (7, 18, 26). There is evidence that RABV infection of the CNS maintains the blood-brain barrier (BBB), thereby minimizing viral exposure to circulating RVNAs (27, 28). Permeability of the BBB may be affected by the animal model and RABV isolate used. Given the importance of the BBB for effective drug delivery (see

Therapeutic Approaches), its permeability in naturally infected cases warrants investigation.

Recent findings have advanced our understanding of the pathological basis for the severe neurological dysfunction that occurs in rabies and may open new avenues for treatment. Although conventional histopathology reveals few changes, ultrastructural studies in a mouse model showed severe degeneration of neuronal processes, characterized by swelling and/or beading of axons in particular (29). These changes are considered sufficient to explain the severe clinical disease and fatal outcome in rabies (29, 30). Changes are associated with oxidative stress resulting from mitochondrial dysfunction (30–32). Sundaramoorthy et al. (33), using an *ex vivo* neuronal model, identified the enzyme SARM1 as the central mediator of this axonal degeneration in RABV-infected neurons. When activated, the TIR domain of SARM1 breaks down nicotinamide adenine dinucleotide (NAD^+). The SARM1 enzyme, normally maintained in an inhibited state, is activated by axon injury or disease (34). This results in a rapid loss of NAD^+ that is sufficient to drive axon degeneration (35), through an ordered process of loss of cellular ATP, mitochondrial dysfunction, calcium influx and loss of membrane permeability (36). In RABV infection, SARM1-mediated axonal degeneration impedes the spread of virus among interconnected neurons, but also results in the pathological loss of axons and dendrites that could explain the severe neurological dysfunction in late-stage rabies (33). Future studies should determine whether this pathway of axonal degeneration occurs in naturally infected hosts and investigate upstream initiators of SARM1 activation. One candidate for the latter is the mitochondrial dysfunction seen in RABV infection that results from interaction of the viral phosphoprotein with mitochondrial complex 1 in infected neurons (37). Notably, while mitochondrial dysfunction is a consequence of programmed axon death following SARM1 activation, it has also been identified as an important upstream initiator of the process (38).

THERAPEUTIC APPROACHES

Given the complexity of the pathological processes following RABV infection of the CNS and the severity of the outcome, it is likely that combination therapies will be needed for successful treatment, targeting different aspects of the disease process (3, 39, 40). We propose that the following four broad categories of disease process need to be managed to ensure the best patient outcome:

Viral Propagation

Inhibition of viral propagation using specific antiviral drugs or immunotherapeutics will be essential. This could target different steps of cell infection including receptor recognition, endosomal pathway, virus replication, intra-neuronal transport or cell budding. Antiviral drugs for use against RABV have been reviewed (41–44). Despite promising *in vitro* and occasionally *in vivo* results, no drugs have shown clinical benefits in patients. Development of an effective antiviral agent for therapy of rabies remains an important goal for the future.

Immunotherapeutics, in the form of monoclonal antibodies (mAbs) targeting specific RABV proteins, offer some promise. Single (SII RMab) and cocktail (docaravimab and miromavimab) mAb products against the RABV glycoprotein are safe and effective for rabies PEP given shortly after exposure (45, 46). Access of antibodies to sites of viral replication in the clinical phase of the disease, when RABV is widely distributed in the CNS, is however problematic. Recently, de Melo et al. (47) showed that continuous intracerebroventricular infusion for up to 20 days of two mAbs (RVC20 and RVC58) against the viral glycoprotein led to 56% survival of RABV-infected mice when initiated in the prodromal phase of the disease. Survival decreased to 33% when treatment was initiated one day later, in the early acute neurological phase. Direct administration of antibodies into the cerebrospinal fluid (CSF) is intended to bypass the BBB, but results in limited drug penetration into the brain parenchyma (48). The BBB remains a significant obstacle to the effective delivery of antivirals and immunotherapeutics for the treatment of rabies. Strategies to overcome this should be a part of drug discovery processes or added to the therapeutic regimen.

Neuronal Degeneration

The molecular mechanisms that induce neuronal degeneration need to be managed in rabies patients. These mechanisms may be beneficial in early infection by impeding viral spread, but destructive once infection is more widespread. Deletion of the SARM1 gene significantly delayed axonal degeneration in RABV-infected neurons, from 24 h in the presence of the gene to beyond 7 days in SARM1 knockout neurons (33). In an *ex vivo* model of axonal damage using the mitochondrial respiratory chain inhibitor rotenone, which produces SARM1-dependent axonal degeneration, treatment with a SARM1 inhibitor not only prevented further axonal degeneration but also allowed recovery of axons that had already entered an intermediate stage of damage (49). These findings raise the possibility that modification of SARM1 function may be a novel ancillary therapeutic approach in rabies, in combination with other therapies. More broadly, enhanced understanding of rabies as a neurodegenerative disease may lead to application of neuroprotectants developed for other, more prevalent neurodegenerative diseases.

Inflammation

Host immune responses are directed toward RABV and degenerate cellular material. These inflammatory processes may be lifesaving in early infection through the elimination of RABV, but damaging in later stages if widely activated in the CNS (50). A therapeutic benefit of immunomodulators in late RABV infection is supported by experimental studies in mice. When corticosteroid treatment was initiated early (0–24 h after inoculation) in mice inoculated with a bat RABV variant, mortality rates were 16% higher than in non-treated groups. However, if corticosteroid treatment was initiated later (72–96 h after inoculation), mortality rates were 14% lower in treated groups compared to controls, although this difference was not statistically significant, possibly due to the small sample size (51).

More recently, Smreczak et al. (52) tested the effect of selected inhibitors of pro-inflammatory processes in RABV-infected mice, with treatment initiated from 5 days post-inoculation. Onset of clinical signs and time to death were delayed in mice treated with infliximab, an inhibitor of the pro-inflammatory cytokine TNF-α, or with sorafenib tosylate, a multi-kinase inhibitor. Treatment with tocilizumab, an IL-6 receptor blocker, also increased time to death, but this did not reach statistical significance. Conversely, Jackson et al. (53) showed that treatment with minocycline, a tetracycline derivative with anti-inflammatory properties, was not beneficial in adult mice infected with highly virulent RABV and was in fact detrimental in neonatal mice infected with an attenuated strain. In addition to differences due to RABV type (pathogenic vs. attenuated), host species may also influence immune responses to RABV infection. A recent study demonstrated that the inflammatory response in the mouse brain following RABV infection differed substantially from that in the human brain, with a more pronounced inflammatory response in the former (54), suggesting that the effect of immunomodulatory therapy in mice may not translate to humans. Rabid dogs naturally infected with wild-type RABV may have more similar CNS inflammatory responses to humans (55). Further studies should substantiate this as a basis to address the hypothesis of therapeutic benefit of immunomodulators in investigational protocols in rabid dogs.

Systemic Compromise

An indispensable component of any therapeutic protocol for rabies will be aggressive critical care to manage the severe systemic compromise that occurs in patients in late-stage disease. Respiratory and cardiac complications are the most common (56). Critical care facilities will be needed to manage these complications, including mechanical ventilation and administration of supportive drugs (3, 4). Complications may result from the widespread CNS infection, involvement of the autonomic nervous system, or from extraneural organ involvement due to centrifugal spread of RABV from the CNS (57).

Timing of initiation of therapy for the four categories of disease process will be important, with expected improved outcomes from early initiation of therapy. Therapy should be tailored according to the stage of disease, clinical form (encephalitic vs. paralytic), immunological response, and patient prognosis. Tools for rapid, minimally-invasive bedside diagnostic and prognostic indicators are needed, and initiation of therapy prior to diagnostic confirmation will need to be considered in order to achieve optimal clinical outcomes. Neurofilament proteins from damaged neurons released into CSF and blood have emerged as sensitive biomarkers of neurodegeneration in several diseases (58, 59). Their utility in the diagnosis, prognosis and monitoring of response to therapy in rabies should be explored. Detection of microRNA signatures associated with RABV infection could also offer a novel diagnostic route and their value as markers of neuronal health and disease during rabies infection should be investigated (60).

INNOVATION IN THERAPEUTIC APPROACHES

Preclinical studies of RABV infection are important for research and development of individual therapeutic components, particularly viral inhibitors and neuroprotectant drugs. However, preclinical animal models will not always replicate important aspects of the disease process in humans. Furthermore, rodent models do not easily allow for study of the effects of combination therapies that incorporate aggressive critical care, as this is not usually feasible in small animal models. Individual therapies that may only extend time to death in preclinical studies may have more substantial beneficial effects when combined and supported by aggressive critical care. Innovation and refinement of combination therapies for rabies could potentially be accelerated by systematic application in a patient population, but several factors reasonably preclude this investigational approach in human patients. The incidence of human cases is typically low in any given geographic area and may be unpredictable over time. Prediction of locations where outbreaks may occur is challenging, as these depend on external factors like incidence and vaccination rates in reservoir animal populations, and availability and uptake of human PEP. Higher numbers of human cases are generally restricted to resource-constrained areas, where dog vaccination may be limited and access to affordable PEP may not be readily available. In these circumstances, aggressive management of patients with investigational protocols, even if feasible, poses substantial ethical dilemmas for physicians, given the uncertainty of patient outcomes (7, 8).

We propose that development of a successful combination therapy for rabies could be accelerated by implementation of investigational protocols in naturally infected dogs, using a One Medicine approach. "One Medicine" is a term that captures the similarities between human and veterinary medicine and the benefit of collaboration in the study of pathogenesis and treatment of diseases that affect both humans and animals (https://www.cdc.gov/onehealth/basics/history/index.html). The etiology and pathogenesis of rabies are similar in dogs and humans, but the incidence is far higher and arguably more predictable in the former. Veterinary treatment of canine patients can, in many ways, mimic critical care and supportive therapy in human clinical scenarios. In endemic areas with robust veterinary surveillance and response systems, suspect rabid dogs are caught and killed by the veterinary services, thereby providing a potential point of access of canine patients to specialized critical care and investigational treatment on a compassionate use basis. In some of these areas, facilities and veterinary expertise in the critical care of dogs are well developed and available at a fraction of the cost for human intensive care patients. These factors create opportunities to initiate restraint, sedation, support, and critical care of canine rabies cases while investigating and refining therapeutic protocols, with dogs themselves standing to benefit through the compassionate use of investigational drugs. Even in the event of therapeutic failure, dogs would receive appropriate palliative care and euthanasia.

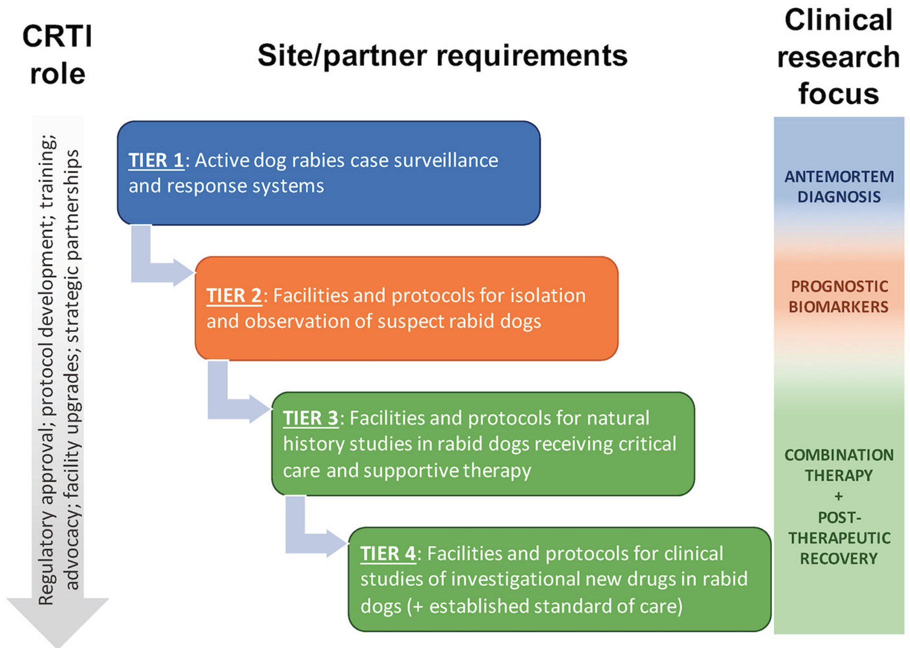

FIGURE 1 | The Canine Rabies Treatment Initiative (CRTI) will implement a tiered approach to clinical research on rabies in naturally infected dogs, progressing to clinical studies of investigational new drugs in dogs receiving critical care and supportive therapy that mimic the human clinical scenario. Naturally infected dogs provide a higher and more predictable caseload than human patients, providing opportunities for additional clinical research to supplement preclinical and human clinical research.

SAFETY AND ETHICS OF A ONE MEDICINE APPROACH TO RABIES TREATMENT

Clinical care and investigational treatment of rabid canine patients requires careful consideration of risks and benefits to patients, clinical staff, and the public. Humane euthanasia and testing of brain specimens from suspect rabid animals reduce risk of RABV transmission and ideally provide timely information for the appropriate management of exposed persons and animals. Current antemortem diagnostic methods for rabies carry a relatively high probability of false negative results (61). Single negative antemortem test results cannot be relied on for decisions on whether to initiate or continue human PEP. Therefore, initiation of care and treatment of suspect rabid animals should be limited to those cases with no potential exposure of unvaccinated persons or should be coupled with timely provision of PEP to all potentially exposed persons regardless of case outcome.

Canine patients should be treated in specialized facilities that meet Animal Biosafety Level 2 (ABSL2) criteria (62). Protocols should include appropriate use of sedation and anesthesia to allow safe handling and enhance patient welfare. Routine precautions should be taken by clinical staff to prevent exposure to infectious material, particularly during procedures such as airway intubation, suctioning, and esophageal feeding tube placement. All staff should be fully vaccinated and undergo regular serological testing for RVNA levels. Clinical criteria such as euthanasia for therapeutic futility or discontinuation of ABSL2-level isolation should be established beforehand. Facilities and plans should be in place for post-therapeutic monitoring and rehabilitation of recovered patients, including monitoring for viral shedding over a predefined period.

THE CANINE RABIES TREATMENT INITIATIVE

The Canine Rabies Treatment Initiative (CRTI; www.treatrabies.org) is a non-profit organization established to attain the vision of rabies as a treatable disease for all patients. While we fully support efforts to eliminate human deaths from dog-mediated rabies through dog vaccination and human PEP, we believe there remains a need for an effective rabies therapy. The mission of the CRTI is to apply a One Medicine approach to clinical research on rabies in naturally infected dogs, as a model of the disease in humans and as patients in their own right, with the goal of developing an effective combination therapy for successful treatment of the disease. CRTI is implementing this mission through a tiered approach in canine rabies endemic areas (**Figure 1**), in collaboration with governmental agencies, non-governmental organizations and academic partners. This approach allows for phased progression toward treatment while conducting translational research on pathogenesis, diagnosis and prognosis; developing an ethical framework and clinical and biosafety protocols; providing training for veterinary personnel; obtaining regulatory approval; and strengthening strategic partnerships. Implementation of a One Medicine approach, with active involvement of veterinary clinician-scientists in the provision of compassionate care and investigational treatment to canine cases, will accelerate development of an effective combination therapy for rabies, ending the years of neglect of a treatment for this disease and providing hope for patients.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

DK wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Continued Failure of Rabies Elimination—Consideration of Challenges in Applying the One Health Approach

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INTRODUCTION

Rabies is a fatal, yet vaccine-preventable neglected zoonotic disease (1); however, control continues to evade public health measures and researchers, whether due to neglect, poor awareness of the disease, limited economic impact, minimal societal pressure to eliminate it, or lack of programmatic funds (2). Rabies infects mammalian species through infected saliva, but dogs are the main reservoirs of rabies. Cost-effective control includes vaccination and population control of the canid reservoir, i.e., prevention at the source. Many parts of the Western Hemisphere were successful in eliminating canine rabies, likely due to mass culling in 19th century and perhaps early 20th century, followed by robust legislation, however rabies continues to be endemic in numerous countries in Asia and Africa (3, 4). As noted by many in the past, human rabies deaths is only an estimate as it remains underreported (5).

One Health is a collaborative, cross-disciplinary effort between public, environmental, and animal health sectors, recognizing that disease outbreaks are multifactorial (6). Integration of disease epidemiology enables a holistic and enduring approach to disease prevention and control in humans. "One Health" was first mentioned during the Severe Acute Respiratory Syndrome (SARS) outbreak in 2003–2004, as a concept that "clearly recognized the link between human and animal health and the threats that diseases pose to food supplies and economies" (7). However, the concept of One Health is not new, and can be traced back at least 200 years (previously known as One Medicine, then One World, One Health) (8).

It is undeniable that rabies elimination can be more effectively achieved by engaging multiple sectors in a One Health collaboration due to the zoonotic and bio-social nature of the disease (9, 10). In addition to canine vaccination campaigns, microchipping programmes, and population control, it is important for countries to coordinate multi-sectorally, and integrate their approaches to eliminating rabies. From the in-country research teams' decades-long experiences in rabies and progress tracking, the authors strongly believe that countries need to increase their accountability, directly impacting policymaking and prioritization, in order to unlock access to required funding. Hospitals and clinics need to be equipped (stock and correctly store) and trained to calculate and administer post-exposure prophylactics and immune globulins, as required. Surveillance activities need to continue as it identifies action points, and feeds back on how successful the control measures have been. Rabies, a neglected and underreported disease, should become a notifiable disease, tying in with country ownership and prioritization.

As OIE leads the collective "United Against Rabies" to drive progress toward "Zero human deaths from dog-mediated rabies by 2030" (11), what are the additional considerations to ensure that One Health, and the goal, is achieved? Rabies has indisputable animal origins, with association with dogs since antiquity (12), therefore the human health sector cannot work in solitary. In the One Health approach defined by the WHO Expert Committee on Rabies since 1991, in addition to diagnostics, vaccination campaigns, and post-exposure treatments in both humans and canines, epidemiological surveillance and community participation have been encouraged, as well as legislative actions (13). If neither the elimination approach nor the disease is new, then why is rabies continuing to kill more than 59,000 humans every year? (14). This paper aims to serve as a reminder of the additional challenges, from the author's experiences, that countries need to overcome or address in order to successfully achieve the WHO's goal by 2030.

COLLABORATION FAILURES

Collaboration, although vital, is not easy. Though the issues outlined below are not specific to rabies, it does apply to global rabies elimination effort. Increased globalization, economic and population growth, and technological advancements resulted in increased competition and reduced research funding and resources. Stakeholders continue to be pitted against one another to compete for limited resources rather than encouraged to pool resources toward a common goal (3, 15). The world is used to living in a competitive culture and are worried that pooling resources and taking risks together will result in loss of rewards that they are working to gain. Additionally, they worry that others will take advantage of them, use them, steal credit, or leave them behind. Therefore, it is important to take the time to learn to work together cooperatively, establish trust, and clearly define roles and responsibilities when collaborating (16).

Despite shared aspirations, each organization has a radically different method and approach. It is not always straightforward to identify the root of the problem. For example, the specific facilitating factors for the transmission of rabies in one country often does not apply to another (3). Different sectors bring in different expertise and perspectives. Therefore, it is crucial to spend time identifying the general problems and specific issues, which can help to clarify a shared common goal and develop a mission statement to define direction for the collaboration (16, 17). It is important to frame problems at a higher level, which is relevant to all sectors and for the public good (18), while acknowledging competing interests. By establishing procedural ground rules from the beginning of the collaboration such as how the decisions will be made, who will speak to the media, publications rights, etc., trust is grown and opportunities for mistakes and misunderstandings are reduced (16, 19). Increased trust leads to increased commitment, accommodates the various points of views, as partners grow more comfortable in compromising their own interests and giving up previous organizational requirements. It is also important for partners to accept the loss of autonomy and recognition and to speak with a single voice, inherent to collaboration (17). Additionally, to address the societal issue, cultural and civic norms should be integrated in the approach to engender collaborations unique to each nation's strategy for lasting impact.

DATA SHARING FAILURES

To effectively identify the specific obstructions in combating rabies in a country, it is vital to coordinate and share data among the human, animal, plant, and environmental health sectors (20), such as systematic data collection and management of dog population surveys, number of suspected and confirmed dog and human rabies cases, human-dog population densities, and vaccination statistics. Data shared may be used for variety of analytical purposes such as holistic examination of the annual epidemiological trends in humans, wild and domestic animals, to better allocate resources to rabies prevention and control activities (21). However, data sharing raises important cultural, ethical, financial, and technical challenges. It is not easy to strike a balance between data accessibility while safeguarding privacy, determining authorship, and protecting intellectual property. Lack of clarity regarding intellectual property and ownership rights often hinders sharing of data, or obscures who has the authority to decide. Technical issues or necessary resources required to manage and transform data for compatibility purposes are also cited as barriers to data sharing, especially in low- and middle- income countries (22). With clearly defined roles and responsibilities for each organization, it is additionally important for the collaborative working group to further define how authorship is determined and how intellectual property will be protected from the very beginning. Without data sharing, One Health approach to eliminating rabies fails at the core.

PROOF OF BURDEN

As many countries are seeing the profits of their investment in the decrease of human rabies cases and deaths to single digits per year, it becomes harder for these countries to justify the required resources to eliminate rabies once and for all, which can be visualized with an asymptote curve. This "asymptotic phenomenon" makes it harder for countries approaching zero human rabies deaths to achieve this milestone. It is even more difficult for the countries to maintain this milestone. One of the contributing factors to the asymptotic phenomena is identified by Miao and team, where the diminution of human rabies deaths is mistakenly labeled as "progress" by the political sector. This leads to the relaxation of subsequent control efforts. Intermittent "relaxation" and commitment of these efforts leads to human rabies "peaks" or "epidemic waves," as was observed in China, and is currently observed globally with the COVID-19 pandemic (23). The only way to combat this is to maintain pressure and preserve the priority status of the disease at the country level. This has proved to be beneficial in many countries in the past (20). Most recently, India, which accounts for 36% of the world's rabies cases, launched a new National Action Plan which gives priority by raising awareness of the importance of action against rabies and has now declared rabies a notifiable disease. The plan aims to operationalize One Health through better coordination and communication between the animal- and human health and other relevant sectors. If successful, India's plan can help set an example for other rabies endemic countries (24).

POLICY AND LEGISLATIVE FAILURES

Rabies elimination requires mass vaccination campaigns, increased vaccination coverage and improved efficiency of vaccination. It requires country ownership, regulatory might, policy changes, and capacity building (25, 26). Political will for eliminating rabies needs to be well-established, despite the lack of direct disease burden or due to the aforementioned "asymptotic phenomena." There needs to be a recognition that no solid quantitative data is available because most human deaths occur at home as the disease is neglected. Additionally, poor sanitary conditions in rural and urban areas favor an increase in roaming dog populations (20). Thus, without prioritization from the country's political sector to implement proper legal framework and allocate funds and resources to regulate vaccination and sterilization of stray and community dogs, and to raise awareness among dog-owners and public on the importance of pre- and post-exposure vaccinations for both dogs and humans and parameters of dos and don'ts, epidemic waves of rabies will continue. Neglecting these epidemic waves could mean expanding geography, because rabies knows no borders, or host-shift, for example new cases have been recorded in previously rabies-free or low-incidence provinces in China (23). In-country teams need to brainstorm innovative ways of seizing their national political sector's attention toward advocating for the need to eliminate rabies. This could be simply by ensuring that politicians are aware of the connection between canine management and human lives (27), engaging local and political leaders to raise awareness of rabies preventive measures and risky behaviors, underlining the unseen burdens, highlighting the impact of previously successful programmes (28), and even using social media's influence to raise awareness.

COVID-19 PANDEMIC

COVID-19 has disrupted the lives and economies of most countries all over the world, whether directly or indirectly (29). As public health professionals are diverted to working on the pandemic, it has disrupted vaccination campaigns in countries (30), including rabies vaccination, and field training. Field staff were overloaded with COVID-19 related duties, and rabies was once again neglected (31). Additionally, due to prioritization failure and supply chain disruption, many remote provinces, where rabies is often widespread (5, 12), faced vaccine shortage (31, 32). Due to lockdowns, fewer people were leaving home, and increased difficulty in travel to and from hospitals or clinics led to decrease in identification of suspected rabid dogs, slower dispatch of rabies response teams, and delayed removal of suspected rabid dogs (30). Lockdowns also deterred people from seeking medical attention due to fear of contracting COVID-19 or because hospitals were overflowing. The pandemic exacerbated the tendencies to neglect post-exposure treatment, underscored by lack of awareness of the threats and dangers of rabies. Lastly, sick COVID-19 patients and those economically affected by the pandemic are unable to care for their pets, which has led to an increase in stray dogs. As the world continues to battle the pandemic, true data on the preventable human-rabies cases and deaths will take some time to emerge. As COVID-19 continues to challenge the public health sector, it is important to develop strategies for rabies prevention and surveillance activities which can accommodate social distancing (30), such as oral rabies vaccine for dogs (33), survive lockdowns, and account for and manage abandoned pets.

DISCUSSION

The goal and approaches to eliminate rabies, a disease with clear transmission dynamics, although well-established, have not been successful to date. It is time to target the political sector, to ensure that temporary disease burden reduction is not misconstrued as progress, to ensure that a legal framework is in place, and that the strategies account for the restrictions imposed by the COVID-19 pandemic. It is crucial that countries maintain pressure and preserve the priority status of the disease at the country level, so that rabies can be eliminated once and for all.

AUTHOR CONTRIBUTIONS

SG drafted the manuscript. TH critically reviewed the manuscript. Both authors contributed to the article and approved the submitted version.

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One Health Surveillance for Rabies: A Case Study of Integrated Bite Case Management in Albay Province, Philippines

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Canine rabies is a significant public health concern and economic burden in the Philippines. Animal Bite Treatment Centers (ABTCs) that provide post-exposure prophylaxis (PEP) to bite patients have been established across the country, but the incidence of bite patient presentations has grown unsustainably, whilst rabies transmission in domestic dogs has not been controlled. Moreover, weak surveillance leads to low case detection and late outbreak responses. Here we investigated the potential for Integrated Bite Case Management (IBCM) to improve rabies detection in Albay province. Using information obtained from animal bite histories combined with phone follow-ups and field investigations, we demonstrated that IBCM resulted in a fourfold increase in case detection over 13 months of study compared to the prior period. Bite patient incidence across Albay was very high (>600/100,000 persons/year) with PEP administered mostly indiscriminately. Clinic attendance reflected availability of PEP and proximity to ABTCs rather than rabies incidence (<3% of patient presentations were from "probable" or confirmed rabies exposures) and is therefore not a suitable indicator of rabies burden. Further analysis of the IBCM data suggests that rabies transmission is mostly localized with focal cases from the previous month and current cases in neighbouring villages being most predictive of future rabies occurrence. We conclude that investigations of suspicious biting incidents identified through IBCM have potential to foster intersectoral relationships, and collaborative investments between public health and veterinary services, enabling the One Health ethos to be applied in a more sustainable and equitable way. Triage of patients and investigations of suspect dogs offer an effective tool for improved PEP provisioning and reduction of unnecessary expenditure, whilst targeted field investigations should lead to increased and earlier detection of rabid dogs. Given the enduring risk of re-introductions from neighbouring populations, enhanced surveillance is critical to achieving and maintaining rabies freedom.

Keywords: rabies surveillance, one health, IBCM, intersectoral collaboration, rabies management

INTRODUCTION

Canine rabies has been eliminated across the Global North, but throughout the majority of low- and middle-income countries (LMICs), the disease remains a serious public health concern and economic burden. Every year, thousands of people die, and billions of dollars are lost due to rabies spread by domestic dogs (1). Although fatal once symptoms appear, rabies is preventable through prompt administration of post-exposure prophylaxis (PEP) after a person is bitten by a rabid animal, and the virus can be eliminated from source populations through mass dog vaccination (2). PEP is highly effective in preventing human deaths, but shortages, high costs and limited awareness of rabies in communities still leaves many people at risk. Elimination of rabies through mass dog vaccination can negate these risks and is ultimately a more effective solution. However, to date there are only a few LMICs where dog vaccination has been sufficiently scaled up and sustained to achieve this goal (3).

In the Philippines, rabies control and prevention efforts are being implemented, but only a few islands and provinces are on track for elimination of both human and dog rabies (4, 5). Despite ongoing initiatives by the national government to interrupt transmission and prevent the disease, canine rabies remains a major public health challenge, with 200-300 people dying from rabies every year (6). Though there is generally high rabies awareness in the Philippines, elevated through anti-rabies initiatives, human rabies deaths are still occurring and consequently the demand for PEP is continuing to increase. As a result, local and national healthcare budgets are under strain from the expensive administration of often unnecessary PEP, which in turn results in frequent vaccine shortages and redirects focus away from mass dog vaccination campaigns.

The lack of effective and integrated cross-sectoral surveillance is a major impediment to understanding local rabies dynamics and effectively controlling the disease, with relatively sparse detection of rabies cases in dogs (even where human cases are diagnosed). Estimates suggest that less than 10% of canine rabies cases are detected in many endemic settings where routine surveillance is rudimentary (7, 8). Current routine surveillance guidelines in the Philippines recommend that an investigation is only carried out when a human rabies case is detected (9; personal communications with Dr Florencio Adonay and Dr Rona Bernales). However, by this time it is too late to detect the rabid dog responsible for the case, perhaps explaining why so few dog cases are diagnosed in the country. Conversely, active case-finding by tracing dog-bite patients attending Animal Bite Treatment Centers (ABTCs) can improve surveillance (6). Histories of biting animals provide valuable and oftentimes the only epidemiological information to inform rabies management. Where animal rabies surveillance is very weak, information on the history of the biting animal from bite-patient presentations may provide a better measure of disease incidence than laboratory confirmed cases and can be used to guide investigations of rabies suspect incidents. Moreover, contact-tracing of transmission events linked to bite-patients presenting at ABTCs allows reconstruction of rabies transmission pathways that are in turn critical to identification of areas where dog vaccination requires improvement. Ultimately, integrated approaches for rabies surveillance using information collected by interviewing dog-bite patients to initiate investigations of biting animals – Integrated Bite Case Management (IBCM) – has potential to increase case detection and expedite outbreak response, while strengthening intersectoral relationships between public health and veterinary services, including trust in data sharing and prospects for integrated surveillance systems (10).

Here we present a descriptive epidemiological study of an integrated intersectoral surveillance approach aiming to link human and animal health sectors to guide investigations in response to suspicious animal bite incidents. Specifically, we developed detailed epidemiological questionnaires, recorded using a mobile phone application at three ABTCs in Albay province, Philippines to document bite incidents, and to inform and trigger field investigations for timely detection and containment of rabies in dogs. Our objectives were 1) to examine the relationship between bite patient presentations and rabies cases; 2) to determine whether the histories of bite incidents can be a reliable predictor of rabies risk and therefore 3) to compare our approach to the existing routine surveillance and assess whether IBCM can improve detection of rabies in the dog population.

MATERIALS AND METHODS

Study Site

We established a 13-month longitudinal study of dog bite-injury patients beginning in March 2018 in Albay province, in central Bicol peninsula (Region V, Philippines; **Figure 3A**). The province comprises 19 municipalities and a human and dog population of 1,314,826 (11) and 161,478 (Albay Provincial Veterinary Services) in 2015 respectively. Rabies in the province likely persists at a low-level of endemicity with frequent incursions from adjacent provinces and nearby islands, reflecting dynamics from across the Philippines archipelago (4, 12). Dog vaccination has been conducted intermittently in the region since 2013. Between 2015 and 2016 annual dog vaccination campaigns were strengthened through the OIE STANDZ (World Organisation for Animal Health Stop Transboundary Animal Diseases and Zoonoses) project (5, 13), but have since become less comprehensive and more spatially heterogeneous, with some barangays (villages) receiving no vaccines in certain years. The average cost per dog vaccination in the Philippines using parenteral vaccination is ~2 USD (4). Data on mass dog vaccination were provided by Albay Provincial Veterinary Services (PVS) office and data on the human population were taken from the Philippine Statistics Authority official website (11).

PEP Provision

Both rabies post-exposure vaccines and immunoglobulins (RIG) are provided to bite patients free-of-charge from government-run ABTCs located within hospitals and can also be bought privately (at a cost of 600-1000 PHP/12-20 USD and 1300-2000 PHP/25-40 USD per dose of Rabipur and Verorab respectively) from Animal Bite Clinics (ABCs). ABTCs administer vaccines intradermally (ID) following the updated Thai Red Cross regimen (TRC) with two 0.1ml doses (to deltoids) delivered on day 0, 3, 7 and 28 (14); however, patients are typically charged for the fourth dose (~650 PHP/13 USD per dose). Following the WHO guidelines (15), RIG, if available, should be provided to all Category III bite patients, directly into and around the wound and as soon as possible after the exposure; however, delays are common as clinics usually store only small amounts of RIG if any at all. The exact dose of RIG depends on the weight of the patient, with approximately 2 ml (equal to one vial) required per 10 kg (16) resulting in a typical cost per patient of around 5400 PHP/ 106.5 USD for RIG. There are three government-run ABTCs in Albay province located at Bicol Regional Training and Teaching Hospital (BRTTH) in Legazpi City, Josefina Belmonte Duran Memorial District Hospital (JBDMDH) in Ligao City and Ziga Memorial District Hospital (ZMDH) in Tabaco City (**Figure 3A**). The only private ABC in Albay is located in Daraga, a short distance from Legazpi City, typically visited less frequently by patients than ABTCs due to the additional costs of purchasing PEP.

Bite Patient Interviews and Follow-Up

From 1 March 2018 to 31 March 2019, one data collector was stationed at each of the three ABTCs. The ABC in Daraga was excluded due to negligible patient throughput. At each ABTC nurses recorded bite patient information including their name, address, and phone number within standardized animal bite registers, which were shared with the data collectors who conducted a short interview with patients after PEP was administered. During the interviews the data collectors recorded details of the bite incident, including the history and health condition of the biting animal and of the exposure and treatment (Interview Forms **Figure S1**).

All patients were requested to ensure that the biting dog would be put in quarantine and observed for 14 days, either by managing their own dogs directly or by contacting the dog owner to follow the same protocol. Additionally, bite patients/dog owners responsible for their dogs were urged to immediately contact the data collector should any behavioural/symptomatic changes occur during the quarantine period. Otherwise, when telephone numbers were provided, bite victims were called after 14 days, and a follow-up questionnaire was completed over the phone to identify whether the biting animal was rabid or healthy. Criteria used for this assessment were as follows: if the animal was either sick, exhibited unusual aggression, had bitten multiple people/animals, had been killed, died or was untraceable during the 14 days following the bite. If any or any combination of these criteria were reported during the 14-day period a field investigation was prompted by contacting a corresponding Local Governmental Unit (LGU) officer (Investigation Guidelines **Figure S2**). Specifically, a field investigation performed by an LGU officer was immediately prompted for: unvaccinated dogs that were either exposed to, or a puppy of, a rabid dog, dogs that were symptomatic for rabies, and dogs that died or were killed within the 14 days window. If the suspect animal was found sick or dead, the animal head/brain sample was collected by a trained veterinarian and sent to the Regional Animal Disease Diagnostic Laboratory (RADDL) of the Department of Agriculture Bicol in Cabangan, Camalig (municipality in Albay) for laboratory diagnosis by FAT (Fluorescent Antibody Test).

Investigated incidents were classified either as “Healthy”, “Non-traceable”, “Rabies suspect”, “Rabies probable” or “Rabies confirmed” according to the protocol (**Figures 2B**, **S1**). Specifically, dogs that tested rabies positive by FAT were classified as “Rabies confirmed”. Where sample collection was not possible, we classified the investigated dogs as “rabies probable”. Dogs that disappeared before a field investigation could be initiated but showed aggressive behaviour/unprovoked biting were considered “rabies suspect”. Unvaccinated dogs that did not show any clear symptoms at the time of the biting incident and could not be traced after the 14-day observation period were classified as “non-traceable”. All remaining dogs, both vaccinated and non-vaccinated, that showed no behavioural/symptomatic changes after the 14-day quarantine period were considered “Healthy”. Government-led routine field investigations were also conducted if a human rabies death occurred (9).

Clinic registry data, patient interviews and phone follow-up information as well as, field investigations, case classification and laboratory results were recorded using a tailor-made mobile phone-based application (BITERS – Bite Incidence Tool for Enhanced Rabies Surveillance; **Figure S3**). All bite patient cases were initially considered for phone follow-up after 14 days of observation; however, in the instance of the bite incident being entirely provoked by the victim and the biting animal having been vaccinated in the last 12 months (or exclusively kept indoors), the patient/animal owner was advised to observe the animal’s behaviour and report back should any changes occur as opposed to being actively followed up on in 14 days. For all other cases, a strict quarantine was recommended, and for individuals classified as “rabies probable” a relevant LGU officer was contacted to initiate a prompt field investigation. The workflow of the data collection is summarized in **Figure 1**.

Ethical approval was granted locally by the Institutional Review Board at the BRTTH, overseen by the Philippine Health Research Ethics Board (PHREB), and in the UK by the Biomedical and Scientific Research Ethics Committee (BSREC). All interviewed participants provided a written informed consent, and personal data collected from bite patients were anonymized upon case submission to the server maintained by the University of Warwick.

Data Analysis

We used the longitudinal data on patient and dog bite histories to evaluate monthly throughput of patients at the ABTCs, and to categorize the proportion of patients completing PEP.

To investigate whether the reported incidence of bite patient presentations predicts dog rabies cases, we conducted a linear Poisson regression model using the laboratory confirmed rabies

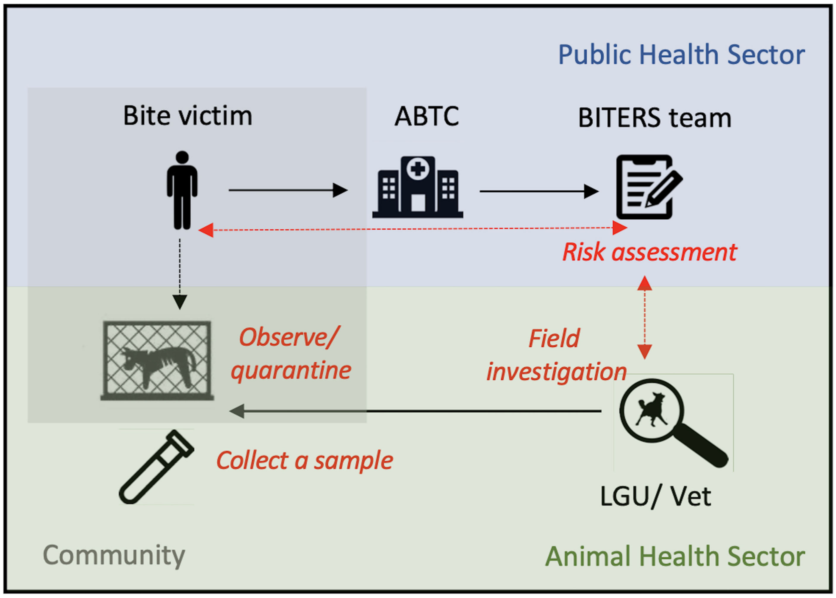

FIGURE 1 | Example of a workflow for intersectoral surveillance approach. Blue shading indicates activities managed by the public health sector, green represents activities falling under the animal health sector and grey shading represents measures that could be undertaken by the communities. Black solid arrows show the workflow, whereas red dashed arrows represent communication between sectors. Bite victims seek care at ABTCs. Upon PEP administration, patient's details are recorded, followed by an interview with a data collector who conducts a risk assessment based on the history of the bite and biting animal. If considered to be high risk, a designated LGU/veterinary officer receives an alert to initiate a field investigation. All patients are advised to quarantine/observe the biting animal, and after a 14-day quarantine period are contacted over the phone by the data collector to provide follow-up information on potential changes in the animal's health condition/behaviour. If relevant, this information is then communicated to the LGU/veterinary personnel to initiate a field investigation to confirm the animal status and where available, collect a brain sample for laboratory testing.

cases as a response variable. To explore potential drivers of reported bite incidence at the barangay level, we tested for a correlation with distance to ABTCs (Euclidean distance to the ABTC from the centroid of patients' home barangay) using a linear regression with Gamma errors and an inverse link function, with PEP shortages using linear regression with Poisson errors and with human population density using linear regression with Gaussian errors.

In order to assess the effectiveness of IBCM for dog rabies detection, we tested for correlation between the number of dogs classified as "probable rabies" and the number of laboratory confirmed rabies cases in each barangay using a generalized linear model with Poisson errors and a log link function. To estimate the odds of finding a laboratory confirmed rabies case (i.e., presence-absence) based on the number of identified "probable rabies" cases, we conducted a logistic regression. To assess the predictive power of the logistic model, we calculated the area under curve (AUC), with values above 0.5 and 0.7 indicating an acceptable and good model fit respectively, although not necessarily the absolute fit. This follows a similar approach to Gorsich et al. (17) and enables us to utilise a well-recognised and easily interpretable assessment metric of the predictive ability of our model.

Lastly, we employed the "probable rabies" case data identified by month and barangay to estimate their predictive power of future rabies occurrence. Specifically, we used logistic regression to model the probability that in a given month a barangay will have at least one laboratory confirmed case based on the number of "probable" cases in the current and previous month (given that the mean generation interval of rabies is ~25 days as in 18), and from across four spatial scales: local barangay (note, this variable was possible only for cases from a previous month in that barangay), local neighbourhood (including neighbouring barangays), municipality and province. Model selection was conducted using backwards elimination based on the Akaike Information Criterion (AIC), with the final model selected when no additional terms could be removed.

We used the population and vaccination data sources to reconstruct spatially refined maps of bite incidence per 1,000 persons/barangay/13 months and rabies presence, and to evaluate the relationship between rabies burden across the province, dog vaccination coverage, and proximity to clinics. To estimate monthly vaccination coverage in each barangay, we projected monthly dog population sizes using the standard logistic growth model, with population growth defined as $r = \frac{\log\left(\frac{N}{N_0}\right)}{T}$ where N and N_0 stand for final and initial population sizes, respectively and denotes the months between two time points. We used the growth rate to project dog population sizes, N_t, at monthly timesteps between March 2018 and March 2019 using dog count data recorded annually by the Albay PVS during mass dog vaccination campaigns. For barangays with missing dog counts we drew the local dog population size from a distribution of barangay-level population sizes within the home municipality. We estimated 183,561 and 210,698 dogs in 2018 and 2019 respectively.

In several barangays, the number of dog vaccines (Rabisin®, Boehringer-Ingelheim) reported as used during the campaigns exceeded the estimated dog population size. This often occurs when there is no visible differentiation between vaccinated and unvaccinated dogs and an individual is likely to receive multiple doses during the same campaign. For barangays that reported >85% vaccination coverage upon campaign completion, we assigned the vaccines proportionally to the overall number of dogs and vaccines reported for the given month and barangay with the probability of getting vaccinated equal to $1 - (\frac{(N_t-1)}{N_t})D_t$ where D_t stands for the number of vaccine doses reported as used in a given month. As such the probability of being revaccinated (i.e., the same dog receives two doses) during the same campaign increases as the number of vaccines used approaches the total dog population.

Vaccinations conducted more than six months after the last campaign were treated as annual dog vaccination campaigns. Vaccination coverage V_t (at the timestep t) was assumed to wane exponentially at rate v, based on the average duration of the vaccine-induced immunity w where $v = \frac{1}{w}$ (w value taken from 19) and the demographic turnover of the dog population bound by the birth rate b and death rate d (parameter values from 20), yielding the following formula for waning coverage: $V_{t+\Delta t} = V_t e^{-(b+d+v)\Delta t}$. For barangays in which repeat vaccination occurred within six months of the previous campaign, we allocated vaccines to previously unvaccinated dogs such that $V_{t+\Delta t} = V_t e^{-(b+d+v)\Delta t} + (1 - V_t e^{-(b+d+v)\Delta t})\frac{D_t}{N_t}$.

RESULTS

Bite Patient Presentations and Relationship to Rabies Cases

Bite incidence in Albay province was very high (685/100,000 persons/13 months) with PEP administered mostly indiscriminately of the WHO (World Health Organization) bite category. A temporary vaccine shortage from April 2018 through to June 2018 led to 911 patients being referred to the private ABC in Daraga where the vaccine was available but not provided free-of-charge. All patients treated at either of the ABTC facilities received at least 1 vaccine dose (8162 patients in total), 82% of patients received 2 doses and 73% patients received 3 doses. Only 17% of patients received the fourth dose, likely due to the cost patients are charged for the last dose. When tabulated by WHO bite category, second and third dose was administered (in this order) to 61% and 57% of patients classified as Category I, 85% and 77% of Category II patients, and 69% and 61% of Category III patients. Additionally, 23% of patients (1887) received RIG.

Ninety percent of patients presenting at ABTCs reported dog bites as their primary injury, followed by 24% reporting scratches and <1% reporting open wound exposures to dog's saliva (several patients reported multiple injuries of different types). Three patients received a full course of PEP after exposure to a "probable" human rabies case; however, no skin rupture occurred. Whilst most patients adhered to the WHO recommended bite management guidelines – washing their wounds thoroughly with water and soap for 15 minutes (indicating widespread awareness on rabies prevention) – a large number also used traditional medicine (i.e., tandok, tambal and herbs).

The total number of patients presenting at the ABTCs varied significantly across months and showed no statistically significant relationship with the number of laboratory-confirmed rabies cases (regression coefficient = 0.0009, p-value = 0.198, residual deviance = 27.25 on 11 degrees of freedom). Instead, the observed pattern likely reflects the vaccine shortage during the first quarter of the year (Poisson regression coefficient = -0.476, p-value < 0.001, residual deviance = 512.42 on 11 degrees of freedom) (**Figure 2A**).

Histories of Bite Incidents as a Predictor of Rabies Risk

The vast majority of bites (76%, n=6906) were reported as provoked by the bite victim, with 4298 animals found unequivocally healthy (i.e., no other classification could be applied) after the 14-day observation period. On the basis of the initial patient triage and phone call follow-up we identified 256 incidents (222 dogs as several patients were bitten by the same animal) classified as "rabies probable" of which 6 patients were from the neighbouring province, Sorsogon, and one patient was from Nueva Ecijia, a landlocked province in central Luzon. For 33 of these "rabies probable" dogs we conducted epidemiological field investigations (as described in the Methods); laboratory diagnostics confirmed rabies in 22 of these animals whilst the other 11 tested negative.

Only a small proportion of the animals that caused the reported incidents were vaccinated (32% within the last 12 months since the incident, and 8% more than 12 months prior to the incident), which was in line with the estimated vaccination coverage achieved during the dog vaccination campaigns (mean=41%, SD=16% for each barangay across the study period). Both the dog vaccination coverage and patient bite incidence in each barangay were negatively correlated with the distance to ABTCs (**Figures 3**, **4A, B** and **Table 1**). As such, barangays located further from the clinics were less likely to have had dog vaccination campaigns during the study period. This pattern also mirrors the distribution of the human population, with densely populated barangays aggregated in and around urban areas where the ABTCs were situated (**Figure 4C**). This result suggests that the dog vaccination campaigns are predominantly targeted at barangays with high population densities although the official strategy recommends prioritizing barangays with high numbers of dog rabies cases. Whether the higher bite incidence occurring in barangays closer to the clinics implies that health seeking behaviour is driven by increased rabies awareness (i.e., greater rabies awareness effort in urban settings) or rather discouraged by the distance required to travel to reach the clinics (or a combination of both) remains unclear.

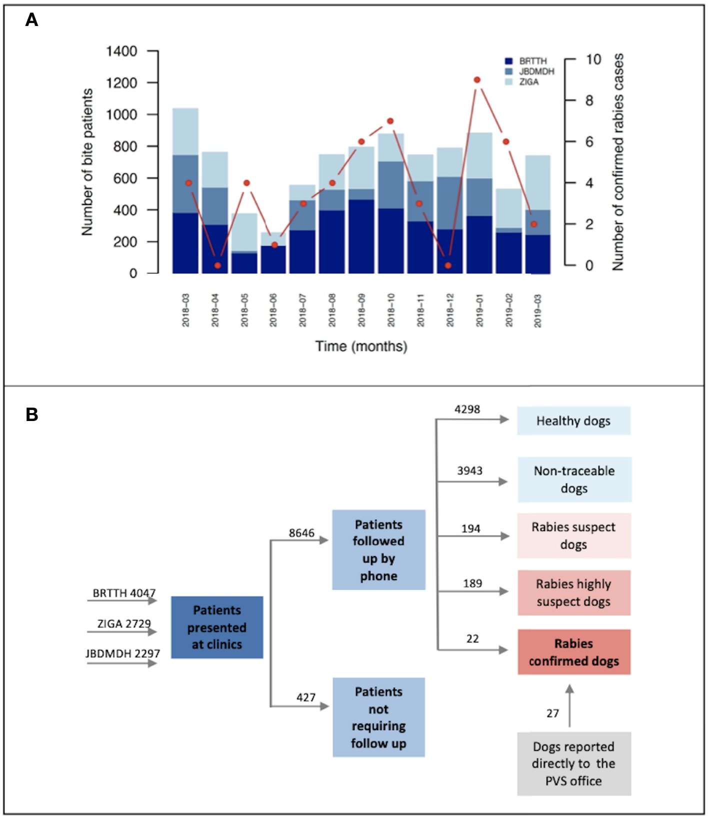

FIGURE 2 | Bite incidence and classification. **(A)** Monthly patient throughput by clinic. Monthly patients presenting at ABTCs in Albay province between March 2018 and March 2019. Red points connected by line segments indicate dog rabies cases confirmed each month with a representative y-axis on the right side of the plot. **(B)** Investigation breakdown from recording of bite victims at the ABTCs to completion of investigations and case classification. Numbers above arrows indicate the sum of cases in each category. BRTTH, Bicol Regional Training and Teaching Hospital in Legaspi City; ZIGA, Ziga Memorial District Hospital in Tabaco City; JBDMDH, Josefina Belmonte Duran Memorial District Hospital in Ligao City.

Interestingly, while the relationship between the distance to clinics and laboratory confirmed cases identified through the study follow a similar pattern as with patient bite cases, the distribution of these dog rabies cases shows a wider spatial range (**Figure 3B**), suggesting wider spread and weaker links between urban centers and rabies transmission in dogs than observed for bite patient incidence.

Impact of IBCM on Detection of Rabies in the Dog Population

In addition to the 22 dog rabies cases that were confirmed following investigations by the BITERS team, a further 27 dog rabies cases were confirmed at RADDL following submission by dog owners, independently of the BITERS team field efforts. Submissions handled by dog owners may have been encouraged by the increased awareness raised through the presence of the field team in the area. All of the laboratory confirmed cases were found nearby the BITERS investigation sites (**Figures 3B, C**). Overall, the case detection increased fourfold over the 13 months of this study, from 12 cases (0.007% of the dog population) confirmed in the previous 13 month period of routine surveillance, to 49 cases during the period with Integrated Bite Case Management (0.02%).

We found that the number of monthly "probable" rabies cases at the barangay level identified by IBCM strongly correlated with both the number of confirmed cases and the probability of case confirmation (**Figure 5A** and **Table 1**), providing convincing evidence that use of IBCM can increase rabies detection compared to routine surveillance in the Philippines. The results of binomial regressions to examine predictors of rabies cases suggest that rabies transmission happens mostly locally (as cases tend to be clustered in space and time) with cases in the focal (i.e., home) barangay from the previous month and current cases from neighbouring barangays most significantly predicting future rabies occurrence (**Figure 5B** and **Table 1**) in comparison to cases elsewhere in the province or the municipality.

DISCUSSION

Despite ongoing rabies prevention programs, the Philippines currently has both high per capita bite patient incidence and

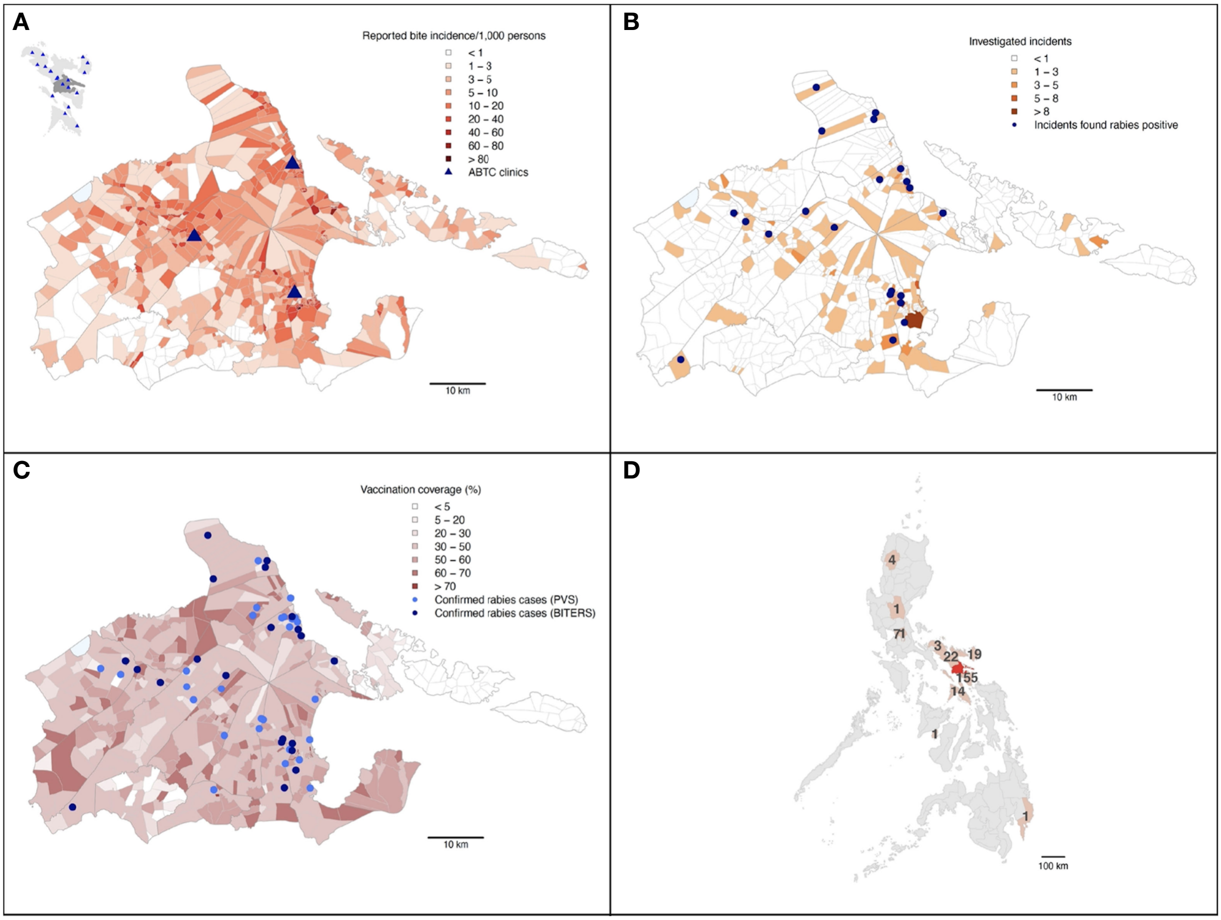

FIGURE 3 | Summary maps of the BITERS surveillance data from Albay province. Albay province shown in **(A)** (with Bicol region as an inset in the top left corner; Albay province indicated by darker grey), **(B, C)**. The Philippine archipelago shown in **(D)**. **(A)** By barangay bite incidence reported at the three ABTCs in Albay province from March 2018 through to March 2019. The inset shows locations of ABTCs (blue triangles) across the Bicol region (lighter grey) with Albay province shaded dark grey. **(B)** Barangays are shaded by the number of bites identified and referred for field investigations through patient triage. Blue points show locations of incidents involving dogs that were found rabies confirmed upon investigation. **(C)** All rabies confirmed dog cases (identified by either the BITERS team or the PVS differentiated as lighter and darker blue points respectively) in relation to average dog vaccination coverage in barangays over the study period. **(D)** The number of bite patients who presented at an ABTC in Albay following being bitten outside of the province. Provinces of the exposures and Albay shown in light and dark red respectively.

human rabies deaths within Southeast Asia (13) and worldwide (1). Here, we found that the patient bite incidence in Albay was high, with PEP administration reflecting vaccine availability and proximity of bite patients to clinics rather than the distribution of dog rabies cases. For example, all patients classified as WHO Category I exposures were administered at least one vaccine dose (with most completing a full course) despite such exposures not requiring vaccination according to WHO guidance (16). Similarly, costly RIG was often provided with substantial delays even though its purpose is to provide protection before the development of vaccine-induced immunity, and it should not be administered beyond day 7 after the initial vaccine dose (21). While consistent with previous reports of generous use of PEP and RIG in the Philippines (6, 22), this is an unnecessary expenditure, especially as evidence suggests rabies vaccines are highly effective even in the absence of RIG (21). Considering that >200,000 USD were spent on RIG over the 13 months of the study, reserving RIG only for Category III patients with multiple exposures, could in theory have saved sufficient funds to procure vaccine for the majority of the dog population in Albay province. This could potentially have greater impacts on public health through the interruption of dog rabies transmission.

ABTCs that provide rabies PEP have been established in every province across the Philippines, but the burden of bite patients falls disproportionately on clinics according to their accessibility and the socioeconomic situation of the region. Often, with free provision of PEP and seemingly unlimited stocks, systematic risk-assessment of patients is lacking both by healthcare providers and patients themselves, leading to substantial precautionary use of PEP (22, 23). In the Philippines, PEP use has been continually rising. This caused PEP shortages across the country in the second quarter of 2018 and was exacerbated by a

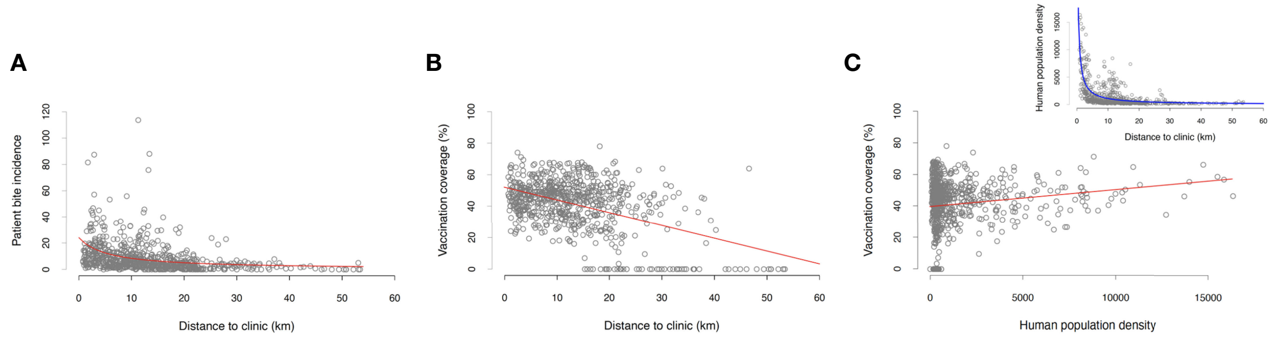

FIGURE 4 | Summary regression analyses using BITERS surveillance data shown as grey points with lines indicating the fitted relationship based on the model output. Relationships between **(A)** barangay-level bite incidence and distance to the ABTC, **(B)** dog vaccination coverage (projected monthly and averaged across the study period) and the distance to the ABTC, and **(C)** dog vaccination coverage (projected monthly and averaged across the time period of the study) and the population density. Inset in **(C)** shows the relationship between barangay population density and the distance to ABTCs; locations of ABTC are located in highly populated urban areas that tend to achieve higher dog vaccination coverage. Statistics of all reported models summarized in **Table 1**.

TABLE 1 | Summary statistics for BITERS regression analyses. All variables are calculated at the barangay level.

Response variable	Explanatory variable	Model regression	p-value	coefficient	residual deviance	DF	AUC	Graphic
Patient bite incidence	Distance to ABTC	Gamma	< 0.005	0.007	1213.3	718	—	**Figure 4A**
Dog vaccination coverage	Distance to ABTC	Linear	< 0.005	-0.008	14.7	718	—	**Figure 4B**
Dog vaccination coverage	Distance to ABTC truncated to 30 km	Linear	< 0.005	-0.006	12.7	674	—	—
Dog vaccination coverage	Human population density	Linear	< 0.005	1.075 e-05	18.6	718	—	**Figure 4C**
Human population density	Distance to ABTC	Gamma	< 0.005	8.912 e-05	549.82	717	—	**Figure 4C** inset
Probability of confirming rabies in the given month and barangay	Monthly rabies probable cases	Logistic	<0.005	0.807	317.84	717	0.63	**Figure 5A**
Laboratory confirmed rabies cases in the given month and barangay	Monthly rabies probable cases	Poisson	<0.005	0.501	252.77	717	—	**Figure 5A** inset
Probability of confirming rabies in the given month and barangay	Rabies probable cases in the local barangay in the past month and current rabies probable cases in neighbouring barangays	Logistic	<0.005 (for both variables)	1.4757, 0.657	591.7	9344	0.6	**Figure 5B**

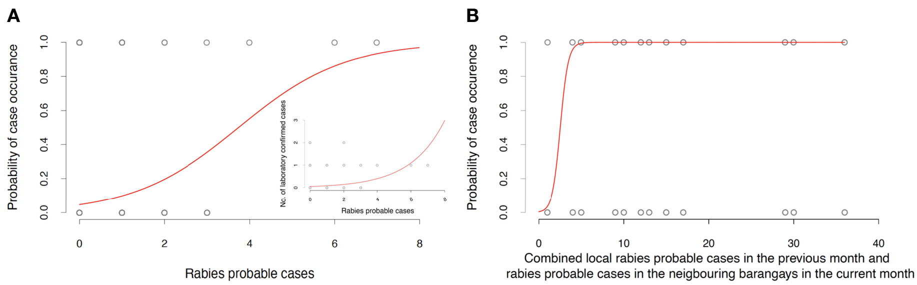

FIGURE 5 | Validation and spatiotemporal analysis of the BITERS surveillance data. **(A)** Relationship between monthly "rabies probable" cases in each barangay and the probability of confirming rabies in a given month and barangay. The inset depicts the positive relationship between monthly "rabies probable" cases in each barangay and laboratory confirmed rabies cases in a given month and barangay. **(B)** Probability of rabies confirmation at barangay level increases with the number of "rabies probable" cases in the local barangay in the past month and "rabies probable" cases occurring currently in neighbouring barangays. Statistics of all reported models summarised in **Table 1**.

wider global vaccine shortage (6, 23). In response to a PEP shortage, many patients were redirected to acquire post-exposure vaccines from private clinics at their own cost, precipitating additional inequity in vaccine access (22). Our results are in line with similar reports from other provinces across the Philippines, highlighting the urgency for discerning high-risk patients for PEP administration and to prevent unnecessary casualties from depleted vaccine stocks (6, 23). Whilst it is an ethical imperative to improve access to PEP for those at risk, indiscriminate PEP administration results in financial strains and vaccine shortages.

Moreover, the risk of rabies exposure persists as long as rabies continues to circulate in domestic dog populations and is highest for marginalized and hard-to-reach communities that tend to have lowest health-seeking behaviour (8, 24). Dog rabies cases are, however, severely underreported as a result of weak surveillance (8), which in turn leads to low prioritization of control programs, especially in settings where rabies competes with other diseases considered to be of greater economic importance. The surveillance protocol piloted in this study facilitated a fourfold increase in the detection of laboratory confirmed dog rabies cases. Our results suggest that such a targeted surveillance approach offers an effective way to locate foci of infection both to obtain better estimates of rabies incidence in dogs and to prevent further spread, through dog quarantine and increased rabies awareness.

The spatial pattern of the detected cases is likely related to the nature of rabies transmission. Rabid dogs typically bite other dogs within 1km of their homestead (18). Our analysis suggests transmission occurs in clusters; a barangay is predicted to experience a case with the highest probability if a "probable" case has been reported in a neighbouring barangay in the same month or locally a month prior. These results make sense given the length of rabies serial interval, but also have helpful management implications. Barangays that are experiencing rabies cases, as well as their neighbours, should be on guard for future transmission, adhering to enhanced surveillance, practicing risk-averse behaviours, quarantining all "rabies suspect" dogs and ensuring communities are aware of the risks. Where vaccination coverage is low and vaccines are available, reactive vaccination may provide some benefits, but will not be sufficient without annual comprehensive mass vaccination campaigns (25). Through the detection of circulating rabies cases, IBCM can also point to areas where routine dog vaccination needs strengthening.

Whilst it is impossible to discern whether the increased case detection resulted purely from improved surveillance or other causes, there is no evidence to suggest that the number of cases during the study period increased due to reduced vaccination coverage in this period. The heterogeneity of coverage may play a role in providing opportunistic pockets of susceptibility, shaping the distribution and size of outbreaks. However, given that the vaccination coverage in Albay province was consistently below the recommended 70% it is more likely that the increase in detected cases was a consequence of the improved surveillance. Additionally, incursions from nearby populations may have contributed to the burden of infection as can be expected in highly connected landscapes such as the Philippines. The number of viral lineages circulating within a region and their origin can be assessed by viral sequence data. Hence, incorporating genomic surveillance into IBCM would generate additional insights regarding the circulation of the virus and could inform the ongoing control program (26).

We conclude that the risk of rabies exposure is disproportionate depending on the geographic location and the socioeconomic background (27, 28), and that the physical access and cost of attending a clinic differs between communities. Integrated One Health approaches to rabies surveillance have potential to substantially increase case detection, and inform more judicious and cost-effective PEP provisioning, while proactively identifying areas and individuals most at risk who would otherwise not receive attention or seek care (29). Similar IBCM/enhanced surveillance studies have been piloted in different settings such as Haiti, Chad, Madagascar, and Tanzania (24, 30–33), generating highly valuable, context-specific information critical to building equitable healthcare systems in resource-limited settings. However, further implementation research is required to operationalize procedures for more judicious PEP administration as healthcare decisions are sensitive and challenging to put into routine practice.

The need to reduce the burden of rabies, and to implement interventions to minimize rabies risk is pressing, with a global strategy in place aiming to achieve zero human deaths due to dog-mediated rabies by 2030 (3). With short-lived or sparse resource allocation for rabies surveillance and control, low-cost approaches tailored to local settings are needed. Here we showed that IBCM offers a cost-effective tool for increased and earlier detection of rabid dogs (by using patient bite-histories to inform field investigations), insights into potential improvements of PEP provisioning that could reduce unnecessary expenditures (by using patient bite-histories and field investigations to inform healthcare decisions) and potential guidelines for strengthened dog vaccination (by using insights generated by analysing the above data to inform canine rabies management). We further advocate for intersectoral collaboration and community participation, including collaborative investments between public health and veterinary services, as the foundation of sustainable and equitable One Health practice.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Institutional Review Board, overseen by the Philippine Health Research Ethics Board (PHREB), and the Biomedical and Scientific Research Ethics Committee (BSREC). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

KR, KH, and MT designed the study. JE, ES, SC, and AA collected the data and conducted field investigations in collaboration with Local Governmental Unit officers. RB and FA oversaw the collection and collating of the data. KR carried out the analysis and drafted the manuscript. KH and MT reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fitd.2022.787524/full#supplementary-material

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Descriptive and Time-Series Analysis of Rabies in Different Animal Species in Mexico

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The spatio-temporal epidemiology of rabies has related the influence of environmental factors and anthropogenic changes on the movements of the hematophagous bat *Desmodus rotundus*. In Mexico, *D. rotundus* is the main transmitter of the rabies virus for different livestock species, modifying annually the fluctuation of the number of cases of rabies and its dissemination in subtropical areas and regions considered free of the disease. The purpose of this study was to perform a descriptive analysis of the distribution of cases of rabies in Mexico, and to perform a time-series analysis to evaluate stationarity and to predict the number of cases for the following year. A total of 3,469 cases were reported in the period of interest, of which the 89.1% occurred in cattle, 4.3% in horses, 1.5% in sheep, 0.6% in goats, 0.01% in pig, 3.1% in vampire bats, 0.3% in cervids, 0.2% in skunks, 0.1% in insectivorous bats, 0.1% in foxes, 0.1% in buffaloes, and 0.02% in coatis; 0.5% were not identified. The most frequent antigenic variants reported were AgV11, AgV5, and AgV3, associated with *D. rotundus.* The distribution of cases in bats correlates with the distribution of cases in domestic and wild animals; however, cases were observed in wild species in non-endemic areas of Mexico, like the State of Chihuahua. The additive model used in the time-series analysis showed a seasonal pattern with a peak of cases at the beginning of each year, from January to March. The model showed a good predicting value; the Pearson correlation coefficient R^2 was 0.705. The highest probability for the occurrence of rabies cases in the different species estimated by Ordinary Kriging was in the coast of the Gulf of Mexico, involving the states of Tamaulipas, Veracruz, Tabasco, Chiapas, and Yucatan. This study confirms that rabies in domestic and wild species is endemic in tropical and subtropical areas—however, cases have been observed in new geographic areas—and provides useful information to support actions to stop the spread of the rabies virus or the reservoir, and for planning vaccination strategies considering time and place.

Keywords: spatio-temporal, analysis, rabies, epidemiology, Mexico

INTRODUCTION

Rabies is a zoonotic disease caused by the rabies virus (RABV) from the genus *Lyssavirus.* This is a neurotrophic virus that affects all kinds of mammal species around the world (1). In humans, rabies is most often associated with dogs; however, the main reservoir for spillover to cattle is the vampire bat *Desmodus rotundus. D. rotundus* is the main transmitter of the RABV in the Americas, from the tropical areas of Mexico to the north of Argentina and Chile (2, 3). In 2019, Mexico was recognized by WHO as the first country free of dog-transmitted rabies as a public health problem (4). However, this virus still circulates in wild animals, such as vampire bats and other wildlife species. *D. rotundus* is the main transmitter of the RABV to cattle causing bovine paralytic rabies (BPR), a disease characterized by limb paralysis, neck flexion, excessive salivation, and death. The spatial–temporal epidemiology of BPR depends of the distribution of the vampire bat. Currently, in Mexico BPR is present in 25 out of 32 States, in the Pacific coast from the south of Sonora to the State of Chiapas, and in the Gulf of Mexico from the south of Tamaulipas to the State of Yucatan (5, 6). Previous studies have reported cases in areas considered free of the disease (7, 8) and have related this to movements of the vampire bat in search of food (8). Even though this paper is about rabies in animals in Mexico in general, emphasis is put on BPR since the cases detected in cattle represent 95% of the total cases observed.

In the last decade, cattle farming in Mexico has changed—cattle are moved to new places in accordance to the human population dynamics, who move from rural to more urbanized areas (8, 9); people need cattle as a source of food. All these changes are an invitation for the vampire bat to also move searching for food (8). *D. rotundus* roost near herds of cattle and feed repeatedly, frequently in the same animal (10). Some animals may have up to four bats feeding at the same time, and 12 bites in a single night have been observed in areas where the vampire population is high (11).

In Mexico, as in some other countries, it is estimated that for every case of rabies reported in cattle, there are 10 that are not (12, 13). The non-notification of suspected cases is currently a major problem for national passive surveillance, and it is estimated that the cattle mortality rate caused by this disease is four times higher than that officially reported (14). Despite reports of low incidence of rabies in *D. rotundus* reported by the National Service of Health, Safety and Agrifood Quality of Mexico (SENASICA, acronym in Spanish) (8), cases of BPR are increasing, causing severe economic losses to the country's livestock sector, especially cattle, which represents 69% of Mexico's livestock production, worth $480 million annually through the export of live cattle (7, 15–18).

Despite the efforts to control rabies transmitted by *D. rotundus* to cattle, such as vaccination of cattle and the control of vampire bats by using chemicals to reduce population size, it is still necessary to understand the complexity of how the disease is disseminated, and spatio-temporal studies are an option to gather useful information, for example, information about factors that influence the presence of cases in areas previously free of rabies. Studies based on multivariate geostatistical methods in Mexico have partially explained the year-to-year occurrence of cases in cattle in endemic areas, and areas where it has been reported for the first time (7, 19). It has been found that environmental variables indirectly affect the ecological behavior of *D. rotundus*; however, these studies have used small databases coming from small geographic areas. It is still unclear whether all cases caused by vampire bats in cattle occur in the place where they are reported, or they were infected in endemic areas and then moved to the reported place before the disease was evident. It is not known either what environmental or anthropological factors are determining the presence of BPR in new or non-tropical areas (7, 8, 19–21).

In general, the use of time-series analysis is for characterizing patterns of behavior occurring in natural environments over time, and for analyzing fluctuations of the variable along the same time period, inferring the impact of an intervention introduced, and forecasting future responses of the variable under study (22, 23). Time series, as epidemiological models, reflect more accurately the dynamics of the epidemiological behavior of a disease despite under-reporting or over-reporting of cases, as occurs in the systematization of data on BPR (24, 25). Therefore, the purpose of this study was to perform a descriptive analysis of the distribution of cases of rabies in Mexico, and perform a time-series analysis to evaluate stationarity, and to predict the number of cases for the following year.

MATERIALS AND METHODS

The Data

Data about cases of rabies in animal species from 2010 to 2019 were obtained from the National Service of Health, Safety and Agrifood Quality of Mexico (SENASICA, acronym in Spanish). The database includes data of all reported cases confirmed by the direct immunofluorescence diagnostic test with monoclonal antibodies against the viral nucleocapsid protein conjugated with fluorescein isothiocyanate, as indicated by Mexican Official Standard Norm (18). Epidemiological information of cases includes state, country, locality, geographic coordinates and meters above sea level (masl), date of report (month and year), information about animal (species, sex, and age), and type of antigenic variant of the rabies virus associated with the hematophagous bats *D. rotundus* (AgV3, AgV5, and AgV11), foxes (AgV7), and skunks (AgV8).

Statistical Analysis

A descriptive analysis was performed for all variables in the data set using frequency tables and graphics for the qualitative variables, and descriptive statistics such as means, SD, and 95% CIs for the quantitative variables. All statistical analyses were performed in SPSS 22.0, released in 2013 (26).

Spatial Analysis

Geographic coordinates for most of the cases were in the data set. Coordinates missing in the data set were obtained using Google Earth. Geographic coordinates were used to elaborate maps and identify areas with probability of cases. Coordinates were first transformed from sexagesimal units to coordinates from the universal transverse Mercator (UTM), and then to decimal units. Mappings of distribution of cases and cases per year and species were performed using ArcMap version 10.1 (27). Prediction of cases was performed with the regionalized variable "number of cases", using ordinary kriging from the geostatistical analyst extension of ArcMap v. 10 (27). The number of cases was aggregated by municipality, for a smoothing process and to reduce the random error. Ordinary kriging is a linear-weighted interpolation method, whose weight not only depends on distance but also on the direction and orientation of the neighboring data to unsampled locations. This method assumes that data collected from a given population are spatially correlated (28, 29).

Time-Series Analysis

An important feature of time-series analysis is that any variable measured over time is potentially influenced by previous observations (autocorrelation). To take advantage of these relationships, time-series models use previous observations as the basis for predicting future behavior. This is the essential difference between time-series analysis and traditional statistical tests for measuring change, such as regression analysis, which rely on variation in independent variables to explain changes in the outcome (30). In this study, for the time-series analysis, the total number of BPR cases was included to describe the distribution by year, and check for seasonality using an additive model. To evaluate the time-series model and to predict the number of cases in the following years, the dataset was divided into two parts, the model was run with data from 2010 to 2017, and tested with data from 2018 to 2019; prediction was made for the year 2018. All statistical analyses were performed in SPSS v.22.

RESULTS

The total number of rabies cases registered during the interest period from 2010 to 2019 was 3,469. Ninety-five percent of the cases occurred in domestic animals (cattle, horse, sheep, and goat); from these, 89.1% occurred in cattle (n = 3,091), the rest occurred in wildlife, 5% (n = 137), including the vampire bats *D. rotundus*, cervids, skunks, insectivorous bats, foxes, buffaloes, and coatis; 17 cases had no record about species affected (**Table 1**). Seventeen States reported at least 50 cases in different animal species. The cases of rabies in cattle are localized mainly in endemic States; however, some others like Sonora, Nuevo Leon, Baja California, Chihuahua, and Durango are not considered tropical or rabies endemic states but have nonetheless reported considerable number of cases (**Table 2**). Seventy-five percent of the cases occurred under 1,000 masl, 21.4% between 1,001 and 2,000 masl, and only 3.3% above 2,000 masl.

Not all cases had data about the antigenic variant of the virus. From the 194 cases that did, 76 had variant AgV11, 87 the AgV5, and 10 the AgV3, all of them associated with vampire bats. Other variants were AgV7 (two cases) associated with foxes and AgV8 (17 cases) associated with skunks. It was observed that in the state of Veracruz, the three antigenic variants associated with *D. rotundus* and the variant associated with skunks were present (**Table 3**). **Table 4** shows the number of cases per month, the highest number of cases were reported in January, February, and March, with 360, 380, and 350 cases, respectively. The lowest frequency of cases occurred in June, July, November, and December. There was an increasing trend of cases from 2010

TABLE 1 | Frequency of the number of cases of rabies in different livestock and wildlife species in Mexico, 2010–2019.

Species	Number of cases	%
Cattle	3,091	89.1
Horse	150	4.3
Vampire bat	109	3.1
Sheep	53	1.5
Goat	20	0.6
Unknown	17	0.5
Cervid	9	0.3
Skunk	7	0.2
Insectivorous bat (3), fox (4), buffalo (2), coati (3), pig (1)	13	0.4
Total	**3,469**	**100**

The bold value 3,469 means the total number of cases and value 100 is the total percentage of the frequency of cases.

TABLE 2 | Total number of cases of rabies by state in the different livestock and wildlife species in Mexico 2010–2019.

State	Number of cases	%
Veracruz	491	14.2
San Luis Potosi	330	9.5
Yucatan	311	9.0
Chiapas	308	8.9
Tabasco	291	8.4
Hidalgo	252	7.3
Puebla	198	5.7
Tamaulipas	193	5.6
Nayarit	187	5.4
Guerrero	159	4.6
Jalisco	107	3.1
Quintana Roo	97	2.8
Campeche	90	2.6
Oaxaca	76	2.2
Michoacan	55	1.6
Colima	53	1.5
Queretaro	51	1.5
Zac (46), State of Mexico (33), Son (32), Mor (28), Sin (22)(24), Gto (17), BCS (10), NL (5), BC (3), Chih (3), Dgo(3), Cd. Mex. (1), Nay (1)	220	6.3
Total	**3,469**	**100**

Number of cases corresponding to Zacatecas (Zac), State of Mexico, Sonora (Son), Morelos (Mor), Sinaloa (Sin), Guanajuato (Gto), Baja California Sur (BCS), Nuevo Leon (NL), Baja California (BC), Chihuahua (Chih), Durango (Dgo), Ciudad de Mexico (Cd. Mex.), and Nayarit (Nay) are shown in parentheses. The bold value 3,469 means the total number of cases and value 100 is the total percentage of the frequency of cases.

TABLE 3 | Number of cases of rabies in different animal species per year, state, municipaliy, and type of antigenic variant in Mexico.

Year	State	Number of cases	Municipality	AgV3	AgV5	AgV7	AgV8	AgV11
2012								
	Veracruz	16	7	–	–	–	–	16
	Guerrero	2	2	–	–	–	2	–
	Puebla	6	4	–	–	–	–	6
2013								
	Veracruz	27	18	–	–	–	–	27
	Guerrero	2	2	1	–	–	1	–
	Baja California	2	1	–	–	–	1	1
	Colima	1	1	–	–	–	–	1
	Oaxaca	2	2	–	–	–	–	2
2014								
	Veracruz	42	30	1	17	–	2	22
	Colima	6	3	–	1	–	5	–
	Hidalgo	1	1	–	–	–	–	1
	Michoacán	2	2	–	–	–	2	–
	Zacatecas	2		–	–	–	2	–
	Guerrero	2	2	1	–	–	1	–
2015								
	Veracruz	65	1	–	65	–	–	–
	Morelos	2	1	–	2	–	–	–
	Michoacán	1	1	–	1	–	–	–
	Guerrero	1	1	–	1	–	–	–
2016								
	Veracruz	1	1	1	–	–	–	–
	Campeche	1	1	1	–	–	–	–
	Puebla	1	1	–	–	1	–	–
	San Luis Potosi	1	1	1	–	–	–	–
	Hidalgo	1	1	–	–	–	1	–
	Sonora	4	1	3	–	1	–	–
2017								
	Sonora	1	1	1	–	–	–	–

Antigenic variants associated to D. rotundus: AgV3, AgV5, AgV11. Antigenic variant associated to foxes: VAgV7. Antigenic variants associated to skunks: AgV8. Of the 194 cases that reported type of antigenic variant, two were atypical.

to 2015, and then a decreasing trend from there on. The lowest number of cases was observed in 2011 (**Figure 1**).

Figure 2 shows the time-series frequency and the predicted number of cases. A slight seasonal effect was observed at the beginning of each year, from January to March. The real and the predicted values of cases in all species for the year 2018 obtained in the time-series analysis used for predicting data for the years 2010 to 2017 are shown in **Table 5**. The model showed good predicting value; the Pearson correlation coefficient R^2 was 0.705.

Figure 3 shows the spatial distribution of cases of rabies in different animal species per year during 2010–2019. There is a clear pattern of dissemination to areas where the disease had not been previously reported, such as the center and north of Mexico. The spatial distribution of cases in all livestock species and vampire bats in the same period is shown in **Figure 4**. It is observed that most cases corresponding to cattle occurred in the coasts the Pacific and the Gulf of Mexico. An area with a high number of cases is observed in the junction of the States of Veracruz, Tamaulipas, Guanajuato, Queretaro, and San Luis Potosi. This area called "The Huasteca" has traditionally been known as an endemic region that needs permanent attention and probably one of the sources of infection for other regions. Surprisingly, cases are observed in the States of Sonora and Chihuahua, which are dry and arid, with an environment hostile for the vampire bat.

Figure 5 shows the risk map obtained by ordinary kriging. This map is based on the regionalized variable of number of cases. The spherical semivariogram model was as follows: $0 * \text{Spherical} * [4.35] + 118.47 * \text{Nugget}$ with a value for the standardized mean of -0.36 and the standard error of 1.15 indicating an acceptable model. Prediction clearly shows high risk in the areas located in the coast of the Gulf of Mexico, involving the States of Tamaulipas, Veracruz, Tabasco, Chiapas, Yucatan, Campeche, and Quintana Roo. In the Pacific coast, the states with higher risk are Oaxaca, Guerrero, and

Nayarit. As expected, the high-risk areas correspond to the typical distribution of the hematophagous bat *D. rotundus*. However, a high-risk tendency was observed in the regions of Huasteca Potosina, Huasteca Hidalguense, the north of the country over the coast line throughout Tamaulipas, and the center of the country in the region known as the Bajio.

TABLE 4 | Frequency of the number of cases of rabies in different livestock and wildlife species by month for the period 2010–2019 in Mexico.

Month	Number of cases	%
January	360	10.4
February	380	11.0
March	350	10.1
April	246	7.1
May	282	8.1
June	236	6.8
July	220	6.3
August	322	9.3
September	282	8.1
October	296	8.5
November	211	6.1
December	221	6.4
Total	3,469	100

DISCUSSION

Our results show that the highest frequency of cases occurred in cattle in both coasts of Mexico, the Pacific and the Gulf of Mexico coast, with an important contribution of the States of San Luis Potosi. These results agree with previous reports (31), who reported the highest number of cases in Veracruz, San Luis Potosi, Yucatan, Tabasco, and Chiapas for data from 2007 to 2015. It agrees also with a recent study in central Mexico, where the State of San Luis Potosi had the highest number of cases in central Mexico (19).

Our study shows that in recent years, cases of rabies have been reported in areas where the disease had not been observed before, such as some regions of the States of Guanajuato and Queretaro, where the climatic conditions are not suitable for the vampire bat *D. rotundus* (19). However, it has been hypothesized that non-environmental, anthropogenic factors have a role in the disease distribution, for example, livestock population density, changes in agricultural activities, new buildings and bridges, and some others (8, 32). Areas with topography with caves also favor the presence of the vampire bat (33). It has been documented that the vampire bat can adapt to different ecosystems as far as it has feeding sources, where livestock might have an important role (7, 34).

The highest frequency of cases was observed in cattle, horses, sheep, and goat, species traditionally affected by *D. rotundus* (35). Livestock management may play a role in this; in some geographic areas of Mexico, it is common to congregate cattle

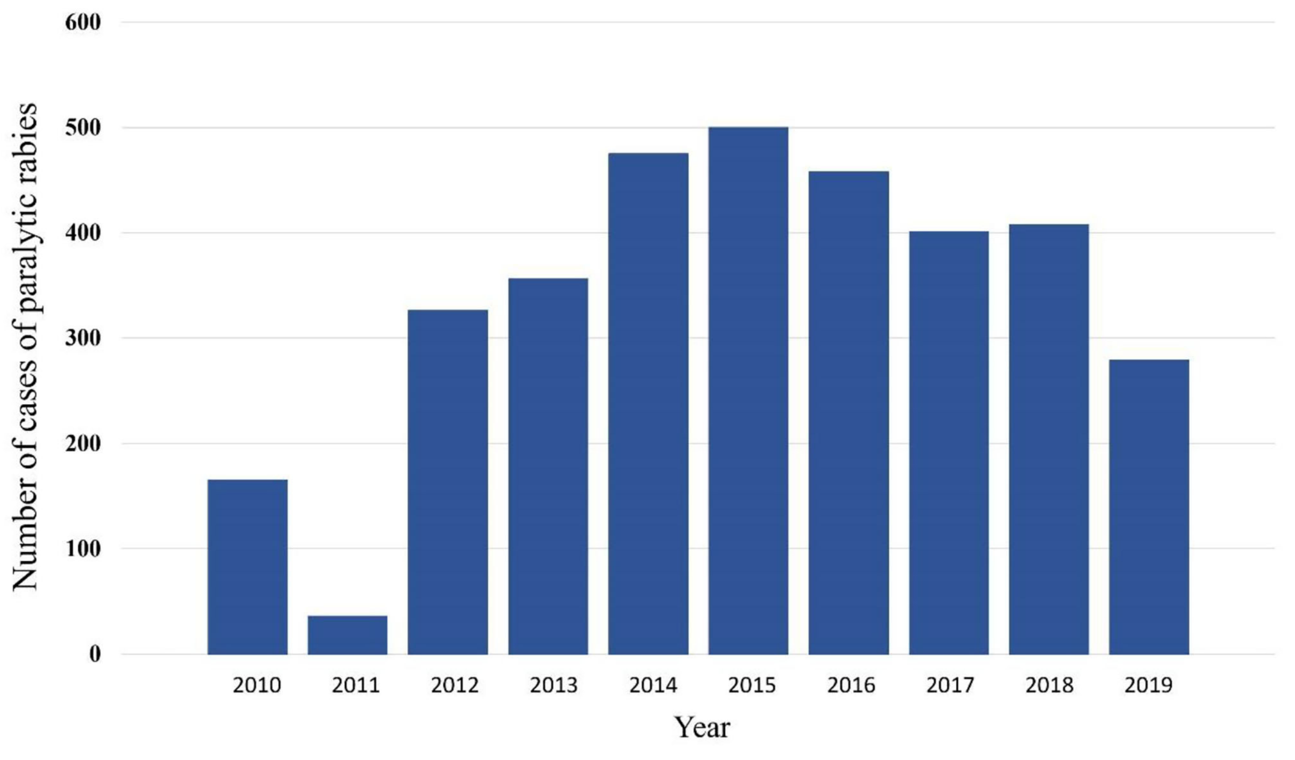

FIGURE 1 | Distribution of cases of rabies in different animal species in Mexico per year, 2010–2019.

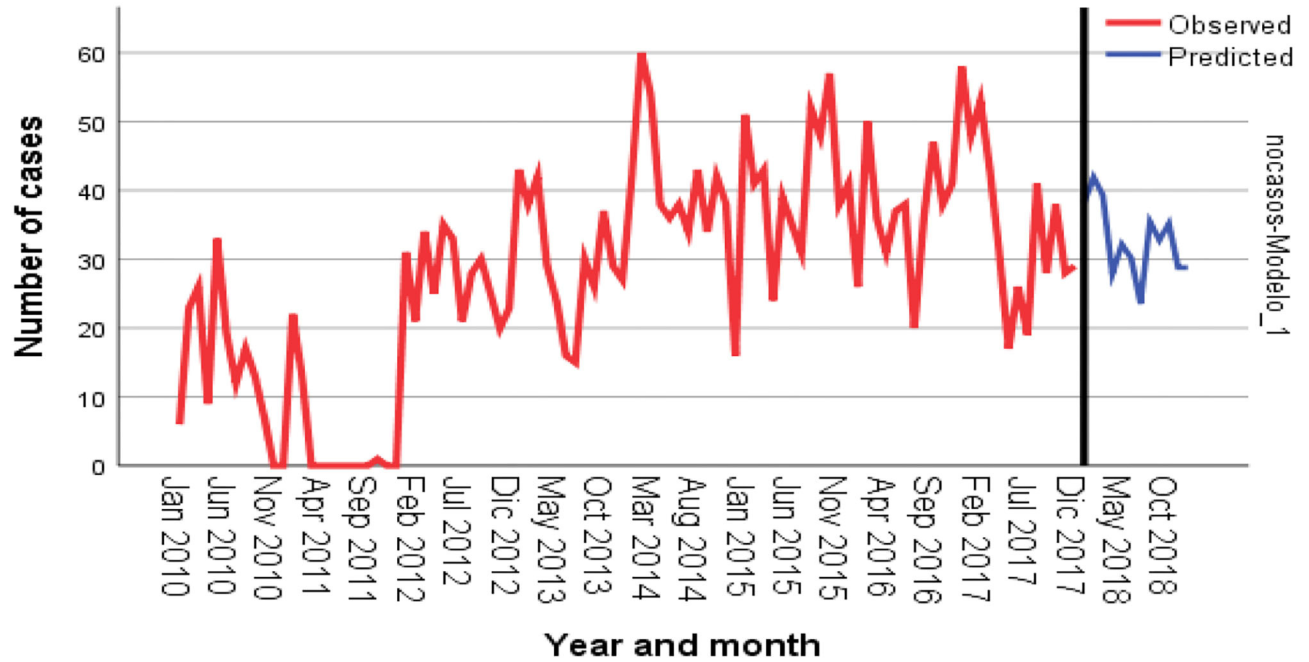

FIGURE 2 | Time-series analysis and prediction of number of cases in different animal species in Mexico. Red line plots the observed values; blue line, the adjusted and the predicted values. Forecasting was performed for the year 2018.

TABLE 5 | Predicted and real values of the number of observed rabies cases in different animal species in Mexico 2010–2019.

Year	Month	Real values	Predicted values	95% CI	
				Low	Up
2018	January	62	38	21	55
2018	February	48	42	23	61
2018	March	44	39	19	60
2018	April	38	28	6	50
2018	May	43	32	8	56
2018	June	22	30	5	56
2018	July	46	24	−3	50
2018	August	49	35	7	63
2018	September	30	33	3	62
2018	October	24	35	5	66
2018	November	0	29	−3	60
2018	December	2	29	−4	62

Trial period: 2010–2017. Prediction period, 2018.

and other domestic animals during the rainy season in corrals close to the family house (21, 36), and this might be an opportunity for *D. rotundus* to attack when roosting near herds of cattle and feed repeatedly and frequently in the same animal (10). Some animals may have four or more bats feeding at the same time and have 12 bites in a single night (11).

Only five types of the antigenic variant of the RABV were observed, which correspond to wild animals, three to *D. rotundus*. The distribution of vampire bat populations matches the distribution of cases in domestic and wildlife animals, where variants AgV11, AgV3, and AgV5 associated with *D. rotundus* have been reported (37). However, the presence of cases in wild species in non-endemic areas of Mexico is remarkable; nevertheless, the variant V8 in skunks in Chihuahua has been previously reported (38).

The highest frequency of cases occurs between February and March each year. This might be due to management practices of cattle during that period. Between November and April, grass is scarce in some tropical regions and farmers gather the animals in corrals to provide silage and hay; this may be used for the vampire to obtain food easily. It might also be explained by the reproductive behavior of bats—the reproductive season of *D. rotundus* occurs during this time, and juvenile vampires require constant feeding to complete their development, meet the energy demands necessary for reproduction, and, in the case of males, to move to other colonies in search of females (39–42). Considering that the gestation period in bats is 205 days, the female bats pregnant in March or April will give birth in September or October, so it is possible that during the months of May, June, July, and August, the cases of rabies decrease a little. This is because near the calving date, the flight hours decrease considerably due to the weight and bulging of the abdominal region, which make the female expend more energy to find food (11, 43).

The annual frequency of observed cases of rabies shows an increasing pattern from 2010 to 2015, and then decreasing from 2016 to 2019. This pattern has been observed in other countries in Latin America (21). The increase in the number of cases in livestock could be more likely related to official policies and

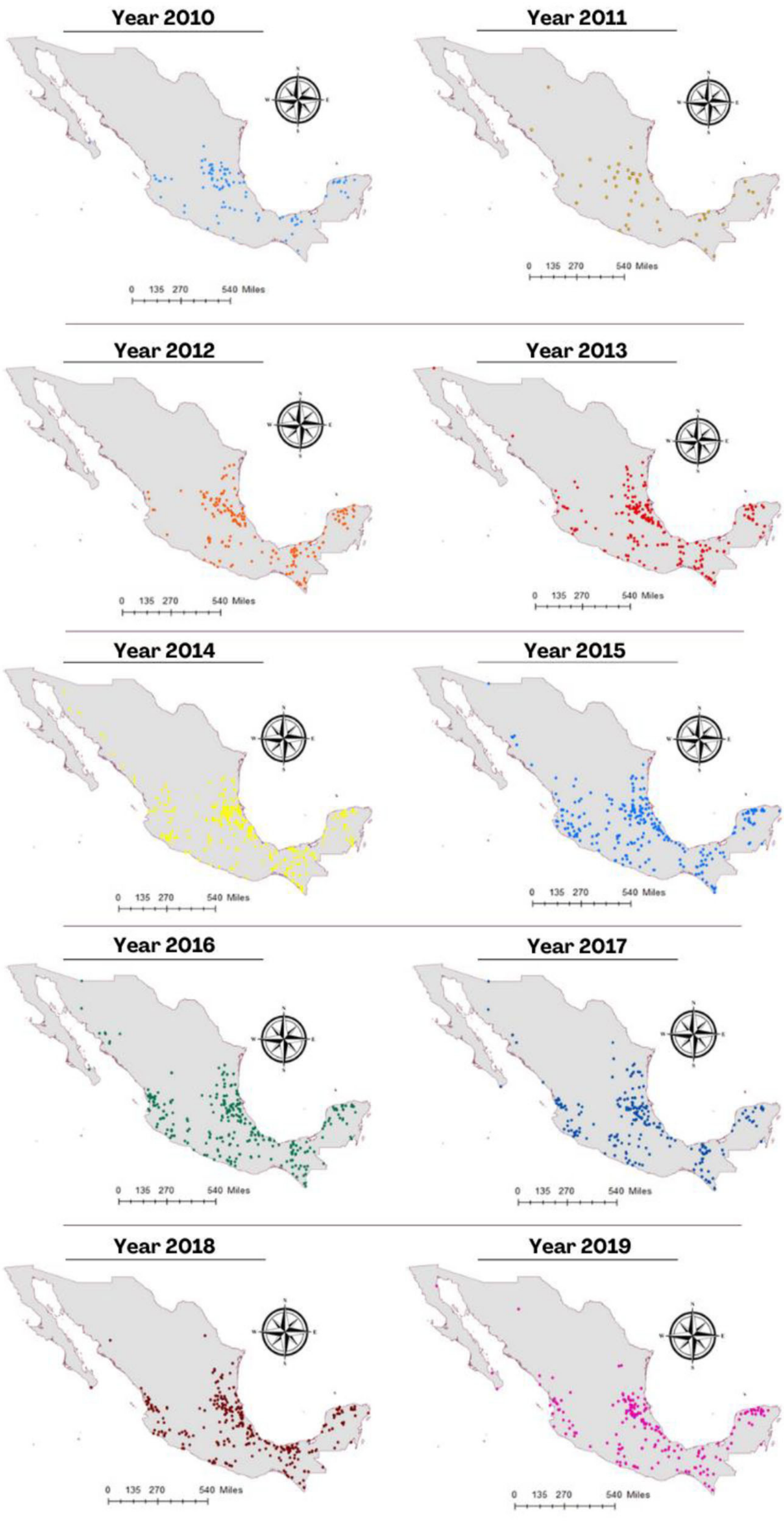

FIGURE 3 | Distribution of cases of rabies in different species animals per year during the period 2010–2019.

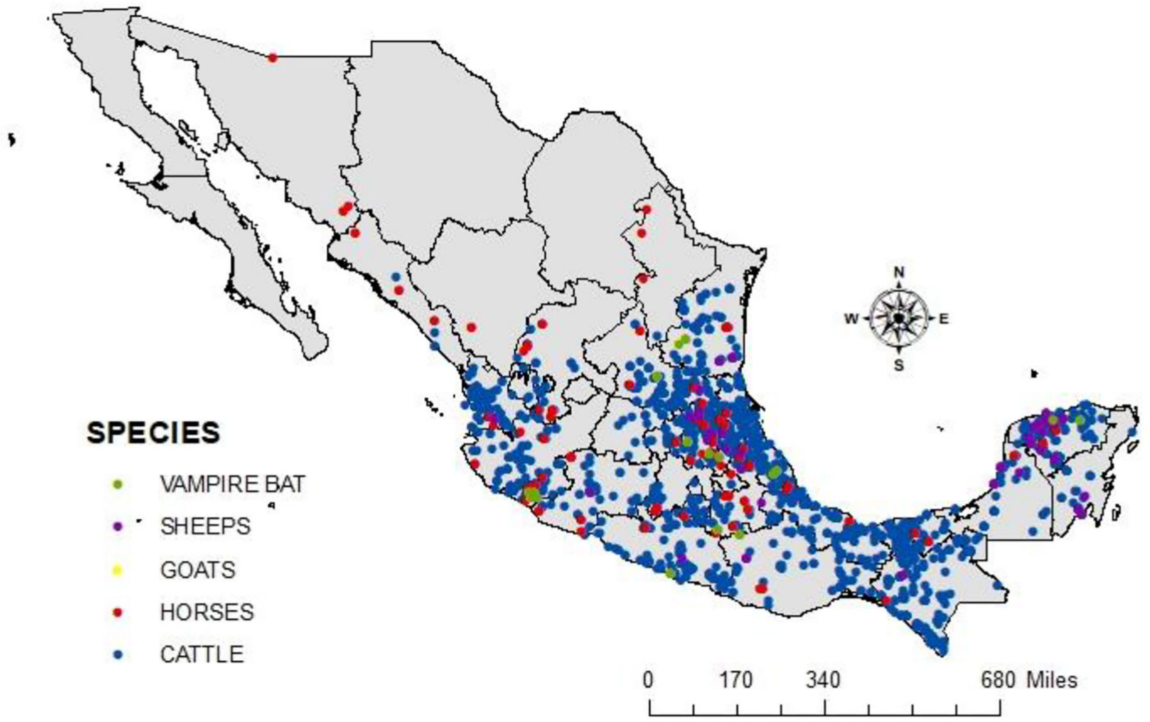

FIGURE 4 | The spatial distribution of cases in livestock species and vampire bats from 2010 to 2019. Rabies cases in cattle are shown in blue, horses in red, vampire bats in green, goats in yellow, and sheep in purple.

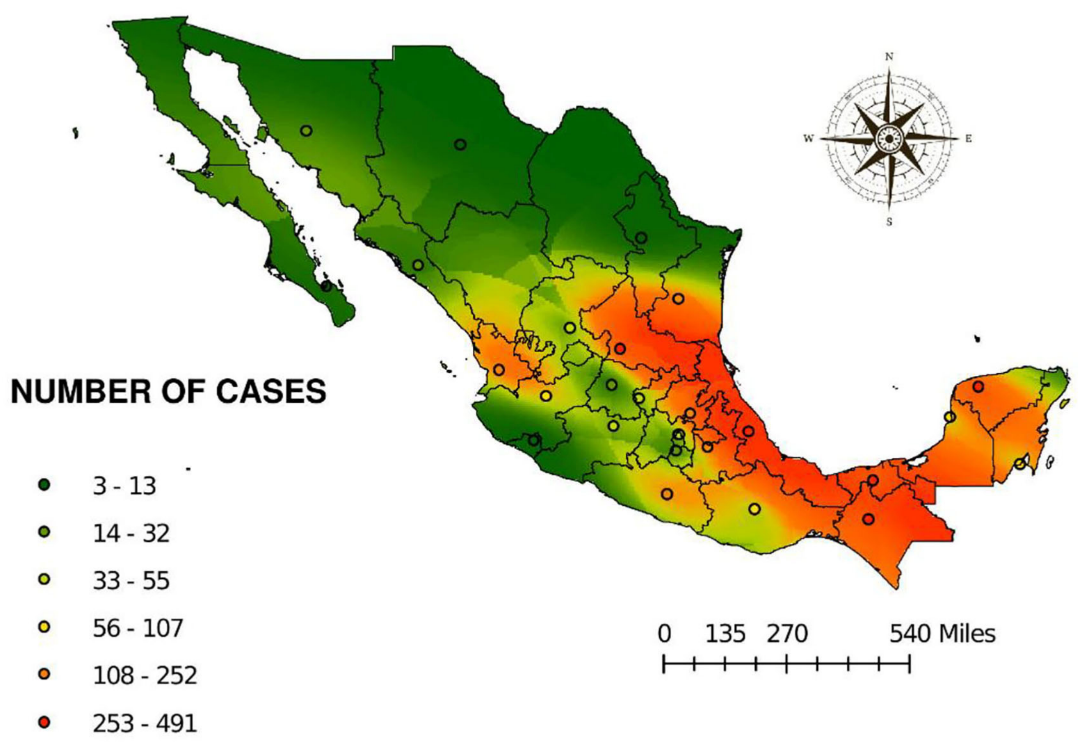

FIGURE 5 | Prediction of risk to cases of rabies in different species from 2010 to 2019 by ordinary kriging.

provision of economic resources to the national rabies control program rather than to a real natural phenomenon.

Underreporting is another issue. The main critical point for surveillance lies in the systematization of case reporting. For example, in the endemic area of southeastern Mexico, 95% of farmers affected with cases of rabies in livestock do not notify to competent authorities about the disease. This may also occur in other geographic regions (21). This is reflected in the fact that the municipalities who send a high number of samples to the laboratory are also the ones with the highest number of detected cases (21, 34, 44).

Results from the time-series analysis reflected a seasonal pattern of cases. This seasonality could be associated with the variability of monthly temperature in some geographic areas. Something similar has been observed in Brazil and Colombia where the higher number of cases in livestock reached the peak in the months of January, April, and October (32, 45). In Mexico, the months of December to March are the coldest months of the year; therefore, it is possible that cattle are calm and close to each other to avoid the cold weather, making easier for the bats to attack. It has been reported that in some regions, the population density of *D. rotundus* in refuges is higher during the spring (46), which could be associated with more cases at this time of the year.

Figure 5 shows the risk for the presence of cases of rabies. It can be observed that there is a tendency of risk toward not endemic or areas free of the disease, suggesting that due to the lack of policies to prevent the spread of the disease, cases could be detected in those areas in the near future, especially if those areas offer microclimates suitable for the vampire bat *D. rotundus* (7, 21, 47).

CONCLUSIONS

This study confirms that rabies in domestic and wildlife species is endemic in tropical and subtropical areas in both the Pacific and the Gulf of Mexico coasts, and that its distribution is clearly associated with the distribution of the reservoir vampire bat *D. rotundus*. However, it has been shown also that cases occur in areas with environmental conditions not appropriate for the reservoir bat, suggesting that the dissemination of the disease depends on factors other than just environmental, and that if actions are not taken to stop the spread, such as well-planned vaccination strategies and bat population control, considering time and place, the problem may grow.

DATA AVAILABILITY STATEMENT

The raw data collection is from the National Epidemiological Surveillance System of Mexico. Requests to access the datasets should be directed to Isabel Bárcenas isabel.barcenas@uaq.mx.

AUTHOR CONTRIBUTIONS

RO-S: investigation, methodology, formal analysis, and writing—original draft. IB-R: conceptualization, statistical model generation, supervision, and writing—review and editing. GC-A: review and editing. JL-C: statistical model generation, review, and editing. R-AE: review and editing. YC-M: review and editing. SG-R: review and editing. BC-G: providing the information on paralytic rabies cases during the 2010–2019 period in Mexico. FM-S: formal analysis and writing—review and editing. All authors contributed to the article and approved the submitted version.

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United Against Rabies Forum: The One Health Concept at Work

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Human deaths from rabies are preventable and can be eliminated by applying a systematic One Health approach. However, this ancient disease still threatens the lives of millions of people in up to 150 countries and kills an estimated 59, 000 people every year. Rabies today is largely a disease of poverty, almost always linked to dog bites, with most deaths occurring in neglected communities in Africa and Asia. The disease places an immense economic burden on its victims, a cost that far outweighs the investment needed to control it. A global framework for rabies elimination in humans is set out in *Zero by 30: The Global Strategic Plan to end human deaths from dog-mediated rabies by 2030*. Despite the existence of proven control strategies and agreement on the path to eliminating human rabies deaths, mortality numbers from rabies remain high, and COVID-19 has set back efforts even further. But COVID-19 has also highlighted the value of a One Health approach to zoonotic disease and pandemic prevention. Rabies control programs offer a practical route to building One Health capacities that can also address other zoonotic threats, including those with pandemic potential. The United Against Rabies Forum aims to accelerate progress on rabies elimination while applying a One Health approach. The Forum promotes cross-sector collaboration among stakeholders and supports countries in their rabies elimination efforts. Increased political engagement and resource mobilization, both internationally and nationally, will be needed to achieve global rabies goals and can also make One Health implementation a reality.

Keywords: rabies, United Against Rabies, rabies elimination, *Zero by 30*, One Health, COVID-19, neglected tropical diseases, zoonosis

INTRODUCTION

Despite being entirely preventable, rabies threatens the lives of millions of people in up to 150 countries and kills an estimated 59, 000 people each year (1). The virus is transmitted through bites and scratches from infected animals, and while a variety of animals can host rabies, more than 95% of human rabies deaths are the result of infection from a rabid dog bite (2). Without prompt access to post-exposure prophylaxis (PEP), infection is fatal (2).

There is considerable global commitment to the elimination of dog-mediated rabies. In 2018, the Food and Agriculture Organization of the United Nations (FAO), World Organization for Animal Health (OIE), World Health Organisation (WHO) (the Tripartite) and the Global Alliance for Rabies Control (GARC) developed *Zero by 30: The Global Strategic Plan to end human deaths from dog-mediated rabies by 2030* (*Zero by 30*) (3). Central to this comprehensive strategy is a One Health approach which recognizes the intimate links between human, animal and environmental health, and promotes intersectoral collaboration to tackle public health challenges (3).

The COVID-19 pandemic has made One Health a policy priority in relation to future pandemic preparedness and the design of disease prevention and control strategies. The challenge is how to turn the One Health concept into practical action, especially in low-income settings. The current crisis has overwhelmed health systems and led to the redeployment of public health resources, resulting in setbacks to the control of endemic and neglected diseases such as rabies (4). However, rabies elimination can be a way to improve human and animal health system collaboration, boost engagement with communities and build workforce capacity, whilst delivering tangible results to the communities most vulnerable to rabies and other neglected diseases.

The United Against Rabies Forum (UAR Forum), an international One Health initiative, sets out to strengthen collaboration and coordination among partners, reduce fragmentation and coalesce cross-sector efforts, as well as supporting countries and regions to progress their rabies elimination efforts. Sustained political commitment and investment into such initiatives will not only end human deaths from rabies but will build One Health capacity to improve the response to other endemic and emerging infectious diseases, including those with pandemic potential.

RABIES AND ONE HEALTH

The global pandemic has forced a major review of global health policy. One Health—based on the intimate links between human, animal and environmental health, has become a policy priority in designing disease prevention and control strategies and ensuring future pandemic preparedness. Rabies control is a model for One Health implementation, as its proven methodologies demonstrate the effectiveness of collaboration at the human-animal interface, including at community and municipal level. However, the pandemic has also set back many countries' efforts toward rabies elimination (4).

A recent survey found that rabies workforce capacity and financial resources were redirected toward COVID-19, movement restrictions limited access to healthcare facilities, and overwhelmed health systems had less capacity to treat cases of rabies exposure (4). Community outreach, including rabies prevention programs in schools, were suspended and the disruption of medical supply chains resulted in restricted PEP access for exposed individuals. The biggest impact on rabies control has been the cancellation or postponement of mass dog vaccination campaigns, a proven cost-effective strategy for saving human lives by stopping the transmission of rabies (3, 4). Rabies mainly affects the poorest communities, and these disruptions exacerbate existing inequalities, increasing the risk of death from rabies and imposing further health and financial burdens on communities already suffering from multiple endemic and neglected diseases (4).

Global policy makers are seeking ways to turn One Health approaches into practical action, including the establishment of the One Health High-Level Expert Panel (OHHLEP) (5). In this context, the elimination of rabies, for which there are effective human and animal vaccines and proven control strategies, can deliver concrete results for affected communities and build the integrated One Health systems that are urgently needed to protect us all (4). Successful rabies control programs require engagement across multiple ministries, public and private sectors, building intersectoral connections and networks which can also help to tackle other public health problems.

For example, Integrated Bite Case Management (IBCM) and contact tracing activities for rabies not only more accurately detect and treat rabies cases but create an active local reporting network that can improve surveillance for other diseases (6, 7). Building a One Health workforce capable of coordinated surveillance and response at community level builds integrated systems that can detect and respond to rabies as well as other zoonotic disease threats.

The burden of rabies is disproportionately borne by underprivileged communities, exacerbated by poor awareness and inequitable healthcare. Often the same health services in these communities are the point of contact for immediate treatment such as PEP, as well as for management of endemic and neglected diseases. Building and strengthening these connections between rabies and other neglected diseases using a One Health approach is likely to better use of existing networks, thus is more efficient, and likely to be more sustained. These are the workforces that have been redeployed during the COVID-19 pandemic (4, 8). The Zoonotic Disease Unit in Kenya is one example of a One Health framework that enhances communication pathways and builds coordinated responses to diseases such as rabies, while also improving systems for outbreak response (9). The One Health capacity-building required for rabies control will ensure that countries have a skilled workforce to respond to both new and existing public health challenges, while alleviating healthcare inequities and helping to break the cycle of neglect (9, 10).

Addressing rabies can also contribute to improved animal welfare, food security, healthier ecosystems and healthier cities. Rabies education and awareness help promote responsible dog ownership, humane dog population management and build more positive community relationships with dogs. There is a significant burden of livestock rabies on many pastoral cattle-keeping households that depend heavily on livestock for their livelihoods, with almost all cattle rabies attributed to rabid dogs in countries such as Bhutan and Ethiopia, and effective rabies control can improve food security by reducing livestock losses (11, 12). Vaccination in domestic canine reservoirs has been shown to effectively control rabies in threatened wildlife species such as African wild dogs and Ethiopian wolves, and a One Health approach which incorporates vaccination programs can contribute to conservation efforts (13). Rapid urbanization and urban slums have corresponded to an increase of waste production and poorly managed garbage disposal which can support free-roaming dog populations and increase the incidence of dog bites and rabies transmission (14, 15). This same issue is associated with other diseases, including dengue virus, leishmaniasis, Chagas disease and toxoplasmosis, and the improvement of waste management as part of an integrated rabies control program will also contribute to the control of other vector-borne and zoonotic diseases (16).

Rabies control strategies build connections across animal and human health systems and require engagement across multiple ministries and government sectors. The capacity-building required for rabies control will not only improve the health and livelihoods of millions of people in underserved and neglected areas, thereby building community resilience to the impacts of significant health events, but will also build health and surveillance systems to detect and prevent other endemic and emerging infectious diseases.

UNITED AGAINST RABIES FORUM: ONE HEALTH IN ACTION

A One Health approach is at the heart of the UAR Forum, which seeks to accelerate the sustained effort needed to deliver on the vision set out in *Zero by 30*. The UAR Forum was announced by the Directors-General of FAO, OIE and WHO (the Tripartite) in September 2020, building on the progress made during the implementation of Phase 1 (start up: 2018–2020) of *Zero by 30* (17).

Phase 2 (scale up: 2021–2025) plans to use the strong foundation established in Phase 1, refined and improved with learning and experience, to expand efforts and truly go global. The UAR Forum facilitates new and more inclusive ways to bring partners together across the world and provides a platform for rabies stakeholders to collectively work toward the goal of eliminating all human deaths from dog-mediated rabies. A key step has been the formation of results-focused working groups to progress priority activities which were defined by stakeholders in the UAR Forum Stakeholder meetings in 2020 and 2021 (18). **Figure 1** shows the United Against Rabies highlights.

Working Group 1, is entitled "Effective use of vaccines, medicines, tools and technologies". Initial work includes identifying minimum data elements to help countries strengthen their rabies surveillance systems and developing an evaluation process to help countries select and adapt existing tools for national control programs. This group will also map global rabies activities to facilitate coordination and assist stakeholders in assessing progress and identifying gaps. Other activities include exploring the benefits of dog identification and vaccination programs, and the review of evidence available for rapid diagnostic testing for rabies.

Working Group 2, entitled "Strategic and Operational Support" is focused on building One Health approaches and capacity, and promoting integrated country and regional strategies to progress rabies elimination. This group has developed a national strategic plan template to help countries develop their own rabies elimination plans and is progressing with the development of a monitoring and evaluation framework and a roadmap that provides countries with guidance and access to technical resources. Other priority activities being addressed by this group include identification of constraints that prevent countries from progressing toward elimination, revision of recommendations on oral rabies vaccines for dogs, and integration of rabies into other disease prevention and control programmes.

Working Group 3 is focused on "Resource Mobilization and Advocacy". Priority activities for this working group include developing strategies and messaging for the engagement of national resource partners, engaging international resource partners to invest in rabies elimination as part of health system

United Against Rabies Forum highlights:

- Inaugural stakeholder event September 2020
- Steering group established
- Three working groups established
- Minimum data elements for surveillance developed
- National strategic plan template developed
- Stakeholder webinar events September – October 2021

FIGURE 1 | United Against Rabies Forum highlights.

improvement, and creating a toolbox of advocacy and investment materials to engage public and private investors, media and civil society.

These working groups actively engage a wide range of stakeholders, representing national and regional bodies, the human and animal health sectors, NGOs, academic and research institutions, social science and private sectors. The UAR Forum encourages country leadership and facilitates access to technical expertise for sustained rabies control measures using a One Health approach (18).

The UAR Forum welcomes members from intergovernmental and non-government organizations, national governmental institutions, private sector entities, philanthropic foundations and academic and research institutions. The UAR Forum is free to join but asks member organizations to demonstrate support for the work, vision and purpose of the Forum, and be willing to actively share knowledge, information and innovations with other members to drive progress toward *Zero by 30* (18).

The UAR Forum website, which is in development, will provide a central platform where members and other stakeholders can access UAR Forum technical resources, while annual stakeholder events build the networks required to collectively overcome the challenges of achieving zero human deaths from dog-mediated rabies.

TRIPARTITE INITIATIVES

To complement this initiative, the Tripartite are working together to promote OIE's dog rabies vaccine bank and the creation of international standards to support rabies elimination efforts (19). The OIE vaccine bank facilitates the procurement of high-quality dog vaccines as a catalyst for mass dog vaccination campaigns. To access this, countries must show strong political will and a robust plan for implementing rabies control measures, and in alignment with the Tripartite's commitment to a collaborative One Health approach, WHO, FAO and other international agencies place procurement orders through the OIE rabies vaccine bank (18).

The Tripartite also encourages countries to apply for OIE endorsement of their national rabies control and elimination programs. This requires countries to adopt an efficient strategy in compliance with OIE International Standards, including intersectoral collaboration (19). Namibia and the Philippines were the first countries to receive OIE endorsement. The endorsements were made during the OIE 88th General Session in 2021 ensuring recognition of government engagement, facilitating access to international expertise and funding, and setting countries on a path toward rabies-free status (20).

OIE endorsement of national control programs may also support improved access to PEP via GAVI, the vaccines alliance. GAVI announced in 2019 that it would extend its portfolio to include human PEP (the vaccines administered if a person is suspected of being bitten by a rabid animal), but noted that this would need to be part of an integrated One Health approach, including the implementation of mass dog vaccination programs (21). However, the COVID-19 pandemic also highlighted challenges of equitable vaccine access, and in order for GAVI's decision to truly translate into improved PEP access for rabies-affected communities, the international community will need to support low-income and middle-income countries in building adequate platforms and infrastructure for vaccine delivery and access (22).

OIE and WHO is facilitating joint International Health Regulations Performance of Veterinary Services National Bridging Workshops (IHR-PVS NBW) with a specific focus on rabies, with the aim of enhancing cross-sector collaboration between human and animal health sectors (19). The OIE PPP Handbook provides guidelines for Public-Private Partnerships, promoting a joint approach to both sectors to work in synergy and share resources to improve outcomes for human and animal health (23). One Health was also identified as a priority area in FAO's new strategic framework launched in 2021, and FAO will further assist countries to strengthen their capacities to achieve the shared goals set out in *Zero by 30* (24).

The Tripartite is committed to using these activities to break down institutional silos and build One Health capacity to address rabies in a coordinated manner at country level. Importantly, the UAR Forum will support countries and other stakeholders by improving awareness of existing platforms and infrastructure, while providing the expertise required to help countries access these resources and progress their elimination efforts.

ONE HEALTH RESOURCE ALLOCATION

We know that control of rabies and the goal of eliminating human deaths from the disease requires sustained investment and political commitment both nationally and at local level. Unfortunately support for rabies elimination is often small-scale and fragmented, and competing priorities mean that stakeholders struggle to allocate sufficient resources to implement sustained rabies control measures (3). This is further compounded by the lack of high quality data on the incidence and socio-economic burden of rabies, without which countries are unable to prioritize rabies and advocate for political engagement and investment (3).

The One Health approach toward the elimination of human deaths from dog-mediated rabies needs to mean more than simply collaborating and sharing expertise across human and veterinary health sectors. There needs to be a systematic approach to One Health financing and integration of other underrepresented and non-traditional stakeholders, for example in tourism, environmental protection and education. The siloed nature of government budgets creates challenges in integrating diverse sectors to serve One Health objectives, however transdisciplinary approaches can reduce duplication, promote the sharing and pooling of resources and maximize the impact of investment. Promoting One Health resource allocation for rabies could create a model of integrated funding arrangements for other zoonoses and One Health needs.

In response to the pandemic, there has been growing commitment at the international level, for example in bringing together the OHHLEP—a valuable step in the right direction. However, One Health needs to be considered in the context of the burden of disease facing different countries and geographical regions. Therefore, national government buy-in is essential for the sustainability of both rabies control and other One Health programs.

Moreover, countries need to be empowered to drive their own progress toward the elimination of human deaths from dog-mediated rabies, prioritizing rabies at the highest political levels and allocating sufficient resources to support implementation of sustainable, One Health-informed, rabies control programs. For endemic countries, control of rabies could be considered a good proxy for the effectiveness of One Health collaboration, and a visible demonstration of commitment both to rabies elimination, and the building of robust national One Health programs. While international donors will inevitably support countries that can demonstrate their dedication to this cause, improving national self-financing, from either public or private sources including corporate foundations or philanthropy, will also work toward reduced international donor reliance.

Investment in rabies control is a money-saver. The cost of implementing effective national strategies to eliminate rabies is significantly lower than the estimated $US8.6 billion that rabies currently costs each year in expensive treatment and lives lost globally—a huge burden for the communities affected (1). The returns on investment in rabies control will be multiplied many times over if they are also made with a view to building One Health capacity to tackle other diseases, improving equity and access to care, and contributing to future pandemic preparedness.

CONCLUSION

Strengthening efforts to eliminate human deaths from dog-mediated rabies offers an opportunity to put One Health into practice and make a significant contribution to health equity, stronger human and animal health systems, and future pandemic preparedness. The United Against Rabies Forum brings together a wide range of technical expertise and commitment to support countries' rabies control efforts and wider One Health implementation. To ensure these One Health approaches are effective and sustainable, the Forum will encourage members to collectively define priority activities and promote a shared responsibility of achieving the vision set out in *Zero by 30.* Focusing on a whole of society approach, the Forum will expand to engage more diverse stakeholders including local authorities and city leaders, social sciences, ecologists, conservationists and the wider NTD community. Political and financial commitment is needed but can deliver multiple returns while also meeting the goals set out in *Zero by 30*.

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

RT wrote the manuscript with input from all authors. All authors have reviewed and approved the manuscript.

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Factors Limiting the Appropriate Use of Rabies Post-exposure Prophylaxis by Health Professionals in Brazil

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Post-exposure prophylaxis (PEP) is necessary to prevent the fatal onset of rabies but requires optimization to avoid overuse in populations at risk of rabies. In Brazil, the incidence of dog bites remains high, with almost half of dog-bite patients not receiving the PEP recommended by the Ministry of Health guidelines between 2008 and 2017. In this study, we aimed to identify the factors that limit the appropriate prescribing of PEP by interviewing health professionals responsible for PEP administration and completion of the 'Information System on Diseases of Compulsory Declaration' (SINAN) form reporting human anti-rabies care for patients seeking health care after a dog bite. We conducted 147 questionnaires (45 questions each) in three Brazilian states (i.e., Rio Grande do Sul, Santa Catarina, Rio Grande do Norte) including questions related to the criteria used by professionals to classify a dog as "suspect" or "rabid", knowledge on PEP prescription guidelines, SINAN and communication with veterinarians. Our analyses showed that most health professionals delivering PEP in these three states struggle to identify a rabies "suspect" dog according to the Ministry of Health guidelines, and to indicate the adequate PEP regimen, with only 11% of professionals prescribing the appropriate PEP under various dog-bite patient scenarios. PEP knowledge score was higher among professionals trained on PEP guidelines and working in facilities with the highest incidence of dog bites. In contrast, PEP knowledge scores did not vary significantly between states, and were not correlated to the professional's level of experience, the number of colleagues available at the health unit or the professional's confidence on prescribing appropriate PEP. Our results suggest that knowledge gaps in PEP administration among health professionals of Brazil can be reduced by implementing training programs to differentiate among rabies risk scenarios, prescribe the corresponding appropriate PEP and improve communication between health and veterinary authorities.

Keywords: dogs, One Health, surveillance, questionnaires, Latin America, bites, PEP

INTRODUCTION

Rabies is a zoonotic virus that causes a progressive neurological infection with a case-fatality rate of almost 100% (1). Although deaths are preventable through the administration of timely post-exposure prophylaxis (PEP), there are no effective therapies once the virus reaches the central nervous system (2). Rabies virus has many domestic and wild reservoirs including dogs, bats, foxes, skunks and primates (3). Domestic dogs are responsible for 99% of human cases worldwide, spreading to people and other animals via mucosal contact during bites or contact between saliva and scratches (1, 4). Successful dog anti-rabies vaccination has reduced canine rabies transmission to only a few endemic areas in Latin America since 2010 (5, 6). Post-exposure prophylaxis is necessary to prevent the fatal onset of rabies in humans and must be provided if there is any suspicion of infection in the biting animal. However, PEP is costly and often has limited availability in low and middle-income countries, requiring efforts to prevent overuse of this scarce resource in at-risk populations (7).

In Latin America, despite the recent substantial progress in reducing canine rabies (6), the use and cost of PEP in patients bitten by dogs remains high. The high cost of PEP challenges the sustainability of rabies elimination programs in countries like Brazil, where more than half a million bitten patients are still treated each year (7, 8). In the last decades, Brazil achieved great progress in reducing human cases of dog-mediated rabies through vaccination campaigns in dogs and provisioning of PEP (5, 8). However, the country still spends dozens of millions of dollars annually in rabies control and prophylaxis [e.g., more than USD 35 million in 2013 (9)], although this cost has not been accurately estimated in the country. Without reducing PEP efficiency, a previous study suggested that PEP costs could be reduced by at least USD 6 million if the Brazilian Ministry of Health guidelines and WHO's 2018 new recommendations were followed (8). The reasons why health professionals still provide inappropriate PEP in around half of bite patients remain unknown (8).

Brazil uses a national standard surveillance protocol to report patients bitten or injured by any mammal including dogs since 1998, known as the 'Human Anti-rabies care' section of the "Information System on Diseases of Compulsory Declaration" (SINAN) form. The SINAN form includes 60 fields with information on the epidemiological background of the animal and the PEP protocol applied. The epidemiological background contains information to guide the correct PEP administration including the type of exposure, characteristics of the injury (e.g., single or multiple wounds, location in the body and severity), the health condition of the dog assessed by the health professional after interviewing the patient (e.g., healthy, rabid, suspect for rabies or dead/disappeared), whether it is possible to observe the dog over a 10-day period, the number of doses of vaccine applied, and if Rabies Immune Globulin (RIG) was administered (8).

The health condition of the dog and characteristics of the wound are essential criteria to guide the indication of PEP (10). Analysis of SINAN forms have previously shown that the incidence of dog bites in Brazil remained relatively constant in recent years, although reports of dogs classified as "suspect" for rabies increased from 17% in 2008 to 25% in 2017 (8). Despite most bites being caused by healthy dogs, almost half of the patients received inappropriate PEP (defined here as not following the PEP recommended by the Ministry of Health and state guidelines), which could be driven by over-assessing a healthy dog as "suspect" for rabies (8, 11). For example, health professionals often report suspect cases of canine rabies in states where dog-mediated rabies is thought to be absent, resulting in more than 2,000 "false-positives" cases reported in SINAN between 2008-2017 compared to the official Regional Information System for the Epidemiological Surveillance of Rabies (SIRVERA) surveillance system, where data is previously curated at the national level before submission (8). Inadequate completion of the SINAN form may either mis-identify cases that pose a real risk (e.g., classifying a dog acquiring rabies from a bat as a "healthy" dog) resulting in not administering PEP when needed, or increase healthcare costs when PEP is unnecessary applied.

Primary healthcare staff that are the first to interact with dog-bite victims play a crucial role in the prevention of rabies (12). Surveys have been widely used in public health to quantify rabies knowledge by health professionals and identify drivers that affect PEP administration (12–14). For example, surveys have shown that only 39% of surveyed physicians in the United States could identify a correct PEP schedule (13) and highlighted poor awareness among nurses on the potential risk of rabies from licks and scratches in India (12). However, to date, no study has evaluated rabies knowledge of health professionals in Brazil and the factors that could hinder rabies prevention measures following dog bites.

In this study, we focused on understanding PEP knowledge and administration by health professionals in three Brazilian states where canine rabies is not currently reported, including the southern states of Rio Grande do Sul (RS) and Santa Catarina (SC), and the northeast state of Rio Grande do Norte (RN). The states of RS and SC have been self-declared as controlled for the canine-rabies variant (AgV2) since 1988 and 1996 respectively (15, 16). Despite these two states not having reported a case of rabies from the canine variant over the last decade, rabies cases in dogs transmitted by bats have been reported (i.e., 2007 in RS and 2016 in SC). RN reported its last case of the canine-rabies variant in dogs in 2016 (17). Additionally, 11 cases in dogs were reported between 2011 and 2016, and 6 cases of the rabies canine variant (AgV2) were detected in the wild crab-eating fox *Cerdocyon thous* between 2015 and 2020 (17, 18). Other wildlife reservoirs of rabies (e.g., bats, the common marmoset *Callithrix jacchus* or foxes) are present in all three states (16–19). We aimed to determine (i) the criteria used by health professionals to identify a dog as rabid or suspicious for rabies, (ii) the factors correlated with prescribing appropriate PEP by health professionals, and (iii) describe how the SINAN form is completed by health professionals.

METHODS

Data Collection

Between March 2019 and April 2020, we conducted questionnaires in Portuguese among health professionals

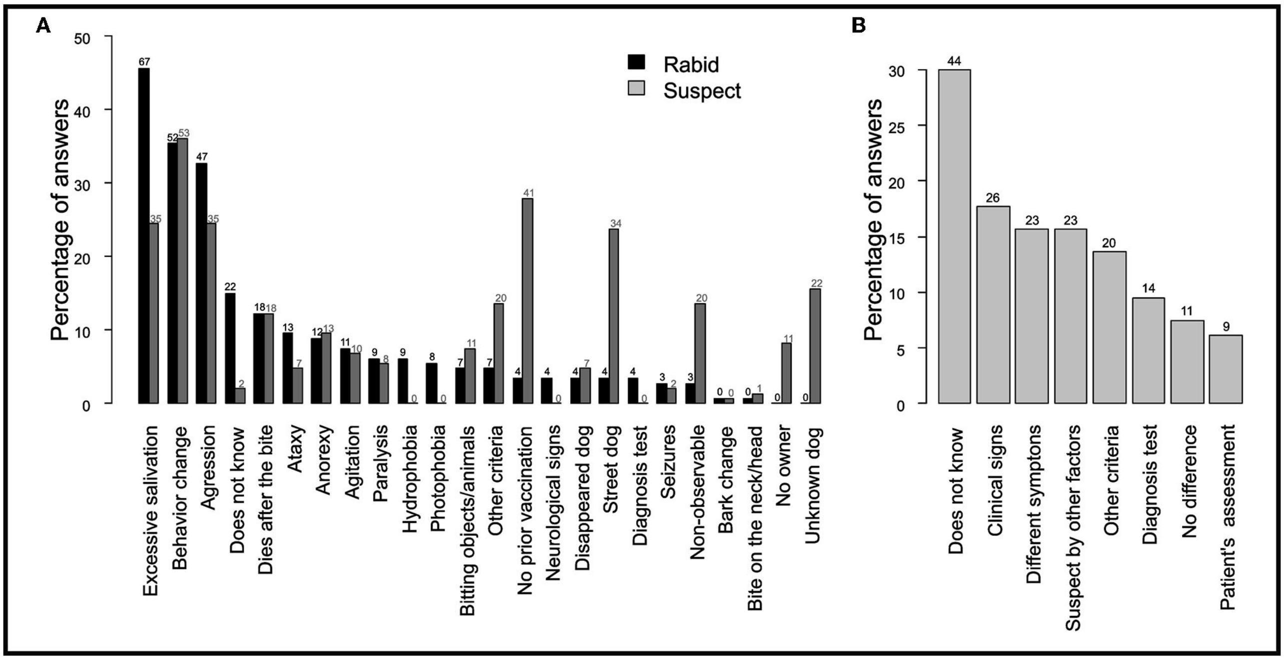

FIGURE 1 | Criteria used by health professionals to classify a dog as "suspect" of rabies or "rabid." Reported criteria used by health professionals to **(A)** define a "rabid" and "suspect" dog, and **(B)** distinguish between a "suspect" and "rabid" dog. The y-axis represents the percentage of professionals that included that criterion (out of 147 respondents). Respondents could mention more than one criteria.

responsible for filling the SINAN form and prescribing PEP including doctors, nurses and technicians in public health centers of the states of RS, SC and RN. In each state, we first obtained a list of all public health centers in each municipality administering PEP and completing the SINAN form from the state's health secretary. Each health center was then ranked according to the total number of patients/notifications received in 2017, and we then selected up to 10 municipalities with the most notifications (i.e., "high notification center group") and up to 25 municipalities with the fewest (i.e., "low notification center group" with 1–4 notifications/year). We aimed to undertake ∼25 questionnaires per group per state, which was assessed by the state's health secretaries as an attainable objective during a 1 month-collection period in each state. In municipalities including several health centers, we invited health centers with the highest notification rates to participate, not exciding 5 health centers per municipality. All professionals willing to participate in the study were interviewed at each health center. We interviewed all available professionals until the 1-month period was completed. Data were entered using the free open source KoBoToolbox software (https://www.kobotoolbox.org/) on a mobile phone. All participants were given a document describing the study objectives and a data confidentiality statement before confirming their voluntary enrolment in the study. Professionals were given the team's contact information for further inquiries.

Our questionnaire included 45 questions divided into 4 sections. Section 1 included 13 questions on the criteria used to assess the dog's health condition (6 questions), the 10-day observation period (4 questions), bite severity (1 question) and the professional's trust in correctly assessing the dog's health condition (2 questions). Section 2 included 12 questions on PEP administration, with four questions related to specific simulated scenarios of dog bites where the professional was asked to choose the appropriate PEP according to the Ministry of Health guidelines (20). Section 3 included 13 questions on how and when the SINAN form was completed (9 questions), the utility/feedback of the SINAN results (2 questions), and 2 questions related to PEP training. Section 4 included 7 questions regarding the current dog rabies and PEP situation in the area. Most questions were multiple-choice answers and if the answer given by the participant was not included in the available choices, it was recorded in a "other response" category along with the specific answer. All questions are detailed in **Supplementary Table S1**. The questionnaire was first validated on five health professionals from RS contacted by the state's health secretary to test its clarity and comprehension, and revised where necessary. To maintain the professional's anonymity, we did not record individual variables during questionnaires (e.g., age, gender or specific profession).

Data Analyses

We built a PEP Knowledge Score (KS) using four questions aiming to assess whether the health professional could identify the appropriate PEP according to the Ministry of Health and state guidelines under four different bite patient scenarios, which

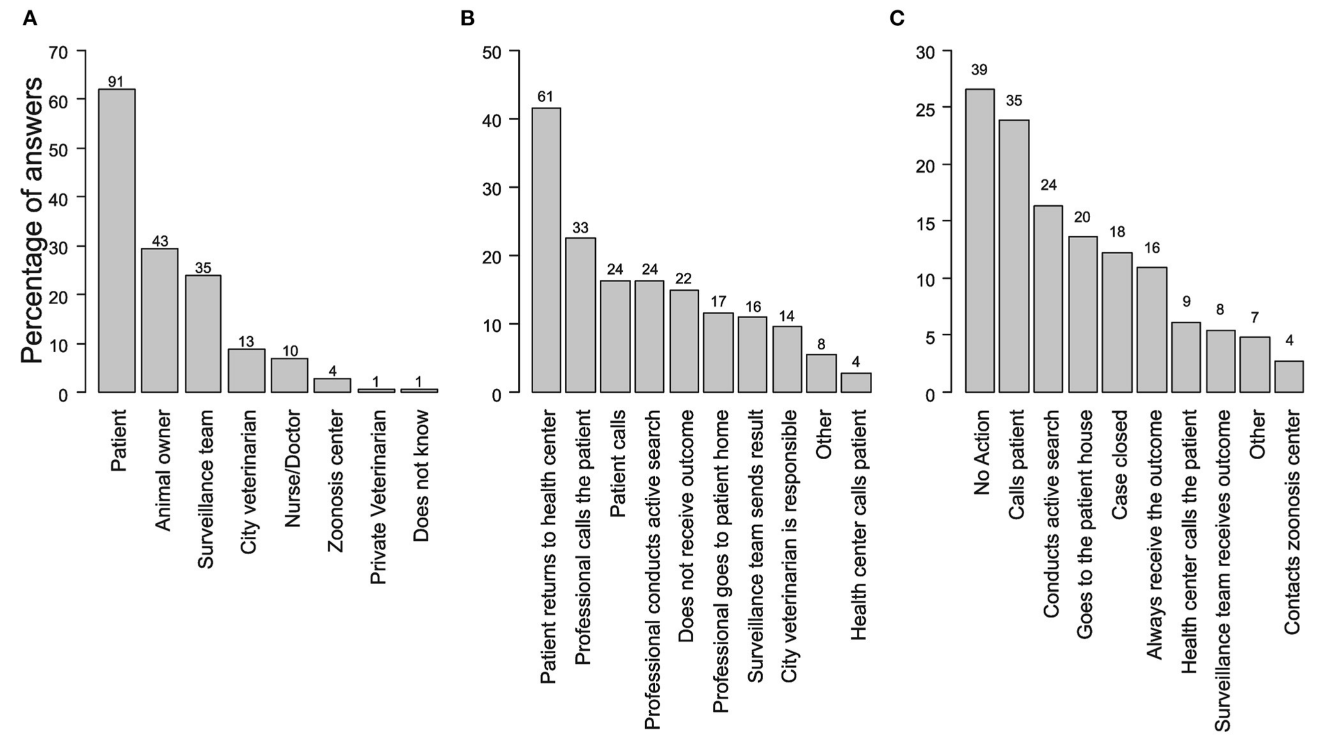

FIGURE 2 | Actions of health professionals related to a dog's 10-day period observation required to assess its rabies status. **(A)** Person in charge of the observation period. Respondents could mention more than one criterion. **(B)** Reported actions allowing the health professional to receive the outcome of the 10-day observation period **(C)** Action taken by the health professional in the case that the observation outcome is not received.

were discussed with professionals from the health secretary of RS. The PEP KS ranged from 0 (four incorrect answers) to 4 (four correct answers). The four questions related to hypothetical scenarios are given in **Supplementary Table S1** (questions 15–18). We then tested the correlation between the PEP KS and several variables obtained during the questionnaires including the state ID, whether the professional was in a high or low notification health center, the number of years working in that center, if he/she had received PEP training, the number of colleagues at the center that can complete the SINAN form or administer PEP, and the professional's level of confidence in PEP decisions.

We performed a multivariable analysis to test the influence of the above variables on the PEP KS using a generalized linear model (GLM) with Poisson errors, given the count nature of our response variable (i.e., KS from 0 to 4). All statistical analyses were conducted in R 3.4.3 (21). The GLM model was build using the *glm* function in R. Variable significance was tested using the Wald's test.

Ethical Approval

The study was approved by the Ethics Committee in Research of the Faculty of Medicine of Botucatu at the São Paulo State University (UNESP), representing the National Platform Brazil of the National Committee of Health of Brazil (project number: 3.029.348).

RESULTS

A total of 147 questionnaires were conducted, including 80 questionnaires conducted in high notification centers from 17 cities and 67 questionnaires in low notification centers from 46 cities. 62 questionnaires were performed in RS, 51 in SC and 34 in RN. Questionnaires were conducted through an individual interview performed in person at a health facility ($N = 69$) or by phone ($N = 78$).

Evaluation of the Dog's Health Condition and 10-Day Observation Period

Professionals used 24 different criteria to classify a dog as either "rabid" or "suspect" in the SINAN form. The percentage of each answer per criterion are provided in **Figure 1A**. Common distinctive signs of a rabid dog such as excessive salivation (46%, $N = 67$ out of 147 respondents), behavioral change (35%, $N = 52$) and aggression (33%, $N = 48$) were among the most used criteria to define a dog as "rabid" by health professionals. In contrast, 15% ($N = 22$) did not know a specific criterion to classify a dog as rabid. Similar criteria were used to assess a dog as "suspect," except for four criteria that were more used to classify a dog as "suspect" including "no prior vaccination" (28%, $N = 41$), being a "street dog" (24%, $N = 35$), "a non-observable dog" (14%, $N = 20$) or an "unknown" dog (16%, $N = 23$). When asked how often they could reliably assess a dog's health condition, 49% (N

TABLE 1 | Factors influencing the professional's PEP Knowledge Score (KS).

Variables	Estimate	Standard Error	*P*-value
Training on PEP administration (yes)	0.30	0.13	0.02*
Rio Grande do Sul	−0.22	0.16	0.16
Santa Catarina	−0.20	0.17	0.23
Cities with low notification level	−0.31	0.13	0.01*
Number of professionals filling the SINAN form	−0.00	0.01	0.99
Number of years completing the SINAN form	−0.01	0.01	0.22
Professional's confidence on applying PEP (none)	−0.22	0.15	0.14

Results from a generalized linear model (with Poisson distribution) testing the correlation between professional's PEP KS (0–4) and several variables. The state ID variable used the state of Rio Grande do Norte as the baseline. Professional's confidence on applying PEP was included as a binomial variable divided between "none" or "low/medium/high," which was selected over a variable with 4 categories (i.e., none, low, medium and high) based on the model's higher AIC when including the latest.
**Significant effect.*

= 172) affirmed to often know how to assess it, 26% ($N = 38$) only occasionally and 7% ($N = 10$) never. Eight different answers were provided when professionals were asked how to differentiate between a "rabid" and a "suspect of rabies" dog. The percentage of answers per response are provided in **Figure 1B**. While 30% ($N = 44$) of professionals indicated that they did not know how to differentiate a "suspect" from a "rabid" dog, only 18% ($N = 26$) used 'specific clinical signs', 10% ($N = 14$) differentiated both categories based on laboratory testing, and 7% ($N = 11$) declared that there was no difference between the categories.

The majority (62%, $N = 91$) of professionals reported that the patient is responsible for the 10-day period of observation of the dog (**Figure 2A**). The observation outcome was primarily communicated (41%, $N = 61$) when a patient returned to the health unit, while 15% ($N = 22$) of health professionals did not receive feedback on the observation (**Figure 2B**). Not acting (27%, $N = 39$) and calling the patient (24%, $N = 35$) were the most common actions if a professional did not receive the observation outcome (**Figure 2C**).

Indication of PEP

Health professional's Knowledge Score (KS) on the correct application of PEP to four different scenarios ranged from 0 to 4, with 11% obtaining a KS = 0, 23% a KS = 1, 31% a KS = 2, 24% a KS = 3 and 11% a KS = 4. No overdispersion was found on the GLM model studying the influence of several variables on KS (Dispersion test, *AER* library in R, $p > 0.05$). We present the full model including all variables (AIC = 463). The reduced model including only significant variables ($p < 0.05$) provided similar estimates and AIC (459) than the full model. The KS was lower in low notification cities (GLM, estimate = −0.31, $p = 0.01$), and increased among health professionals who received specific PEP training (GLM, estimate = 0.30, $p = 0.02$) (**Table 1**). In contrast, the confidence level of the professional on prescribing PEP [tested as a binominal variable (none/confidence) or a multimodal variable (none, low, medium or high confidence)], the number of years completing the SINAN form, the number of colleagues completing the form/administering PEP at the center and the center's state did not significantly correlate with the KS (GLM, $p > 0.05$) (**Table 1**).

When asked to identify seven different potential wounds as "light" or "severe" according to the Ministry of Health guidelines, only 3% ($N = 5$) of professionals classified all seven wounds correctly, on which "punctiform injury without bleeding" and "multiple light wounds on the back" presented more than 60% of errors. A patient not returning to the center was the main obstacle to successfully administering the complete PEP regimen for 39% ($N = 57$) of professionals, followed by vaccine shortages (18%, $N = 27$) and a reduced number of centers administering PEP for the entire population (14%, $N = 20$).

The majority (58%, $N = 86$) of health professionals felt 'medium confidence' on indicating the appropriate PEP, followed by 'low confidence' (24%, $N = 35$) and 'high confidence' (16%, $N = 23$), while only 2% ($N = 3$) stated that they never felt confident. Most professionals (82%, $N = 120$) responded that they "often" or "always" knew the correct PEP to apply, 65% ($N = 96$) stated that they would call a colleague if they had a doubt, while 22% ($N = 53$) would search the available guidelines. Only 54% ($N = 79$) of professionals received training in PEP administration.

Completion of the SINAN Form

The majority (81%, $N = 119$) of professionals reported to understand all the form fields and 71% ($N = 105$) declared to fill the entire SINAN form. Most professionals (44%, $N = 64$) took 11 to 20 min to fill out the form, followed by 0 to 10 min (38%, $N = 56$), and 21 to 60 min (18%, $N = 27$). The majority (82%, $N = 120$) did not regularly ask for help to a colleague when assessing a patient and completing the SINAN form. When asked about the multiple methods to fill up the form that they would use, the majority (62%, $N = 91$) preferred to complete the form on a computer, followed by on paper (46%, $N = 67$) or via a mobile app (22%, $N = 32$). Even though 75% ($N = 110$) of professionals did not receive feedback on the forms they sent, the majority (88%, $N = 129$) believed that the form was used to optimize PEP administration.

One Health Interactions With Veterinarians and Knowledge of the Rabies Local Situation

The majority (66%, $N = 97$) of health professionals reported not requesting help from a veterinarian, 33% ($N = 421$) requested help to assess the 10-day period of observation, and only 8% ($N = 11$) to determine the dog's health condition. When asked how to improve interactions with veterinarians, 45% ($N = 66$) mentioned having a veterinarian in the state or city's epidemiological surveillance team could help, 10% ($N = 14$) mentioned training together with veterinarians, and 7% ($N =$

10) mentioned having more meetings with the epidemiological surveillance team. Regarding tracking secondary bites, most professionals (77%, $N = 113$) did not seek other people bitten by the same animal, 38% ($N = 56$) reported they would call the health secretary if they suspected a rabid dog, and 29% ($N = 42$) said they would not warn any authority and would just complete the form and provide PEP. A large proportion (41%, $N = 60$) of professionals did not know if dog/cat rabies cases had been reported in their state during the last 2 years, whereas 29% ($N = 43$) did not know if dog/cat rabies cases had been reported in their municipality during the last 5 years.

DISCUSSION

In Brazil, only half of dog-bite patients received appropriate PEP following a dog bite during the last decade (8). Our analyses showed that most health professionals delivering PEP in the states of Rio Grande do Norte, Rio Grande do Sul and Santa Catarina struggled to differentiate a "rabid" from a "suspect" dog according to the Ministry of Health guidelines and to indicate the appropriate PEP regimen, with only 11% indicating the appropriate PEP when given four different bite patient scenarios. Professionals' PEP Knowledge Score (KS) was higher in cities with higher incidence of dog bites and among professionals who were specifically trained on PEP guidelines. In contrast, PEP KS did not vary significantly between states, with the professional's experience level, the number of their colleagues at the health center or with their level of confidence on delivering appropriate PEP. Therefore, the inability to identify a rabid dog and poor PEP knowledge resulting from insufficient training of health professionals in areas of low or self-declared controlled dog-mediated rabies could be a main factor explaining the high levels of PEP misuse in Brazil.

The percentage of reported suspect dogs among dog-bite patients attending public health centers increased from 17% in 2008 to 25% in 2017 in Brazil, despite an average of just 1 to 3 laboratory-confirmed dog-mediated human rabies cases each year since 2006 (5, 8). Optimizing PEP use thus requires understanding what generates this relatively high percentage of dogs assessed as suspect for rabies. To assess the health condition of a dog and administer the appropriate PEP, the evaluation should include the observation of clinical signs of rabies (e.g., excessive salivation), the condition of the attack (provoked or not) and whether the dog is observable (20). Our results show that health professionals often rank a dog as "suspect" not only based on classic signs of rabies, but also on whether it is a "street dog," a dog "without prior vaccination" or a dog "that cannot be observed during the 10-day period". In particular, health professionals often reported to classify a "non-observable" dog as "suspect" instead of "healthy" and "non-observable," which could be contributing to the observed increase in "suspect" dogs reported in Brazil (8), despite not necessarily changing the appropriate PEP to be administrated. Additionally, dog's vaccination status is not a criterion to report or used to select PEP according to the Ministry of Health guidelines. The role of street dogs in PEP use requires future research in Brazil, which has an estimated population of 25 million abandoned dogs and cats (22). In the United States, 40% of PEP use was considered inappropriate and mainly related to exposures of domestic animals not available for observations (23, 24). Implementing national strategies to reduce dog bites from street dogs could therefore be a key component in reducing unnecessary use of PEP.

Our surveys highlight the difficulty for health professionals to distinguish between a "rabid" and a "suspect" dog. In fact, most professionals used the same criteria to classify both types of dogs, 28% reported not knowing how to differentiate them, and 7% declared that they were indistinguishable. Only the observation period followed by laboratory testing upon death can definitively discriminate between both cases, allowing PEP to be discontinued if the dog is not confirmed rabid. However, only 10% of health professionals stated that laboratory testing could differentiate these dogs, highlighting the need for better training. In fact, despite a relatively high degree of confidence in assessing the dog's health condition, this misclassification could lead to excessive PEP use (e.g., RIG) in patients bitten by dogs wrongly classified as rabid. Most professionals stated that the bitten patient or the dog owner are responsible for the 10-day observation period and should report the outcome. However, patients might not be able to observe the dog or will not return to the health center (25), with 39% of professionals expressing that the patient not returning to the health center is the main obstacle to successful completion of PEP. Although dog-observation by health and veterinary services is likely to not be feasible in all cases given the number of bites registered in Brazil (more than 500.000 bite-injury patients/year), other strategies such as phone communication could be encouraged to reliably complete the dog's observation period.

Since rabies is a zoonotic disease, overcoming rabies in humans requires the use of a One Health approach including the collaboration of human and veterinary medicine (26). Our questionnaires showed that most health professionals do not consult veterinarians to assist in assessing a dog's health condition. This could be due to their confidence in reliably classifying a dog or trusting the patient's recommendation. However, almost half of dog-bite patients in Brazil do not receive the appropriate PEP including over-use (8), similar to the United States where physicians apply PEP even in low-risk exposures if the 10-day observation period cannot be assured (23). Thus, improving PEP administration by a more appropriate dog evaluation could require increasing health professional awareness of the need of a more informed opinion including veterinary help. Improving communication could benefit from including a veterinarian in the city or state's surveillance team, as expressed by health professionals, which could also contribute to the largely overlooked tracking of secondary bites of potentially rabid dogs. Specific interactions during complex cases could be promoted using mobile apps connecting professionals from these two sectors as developed for endemic rabies in other regions and for leishmaniasis in Brazil (27–29).

The high rate of PEP administrated not following the Brazilian Ministry of Health guidelines can be partly due to poor knowledge of health professionals on PEP (8). Our study supports this hypothesis, since poor knowledge on PEP administration was significantly correlated with a lack of training on PEP guidelines and low incidence of bites, similar to poor PEP administration in areas of low rabies incidence or low awareness in the United States (23). In our study, PEP KS did not increase with the number of other professionals present to support the PEP decision, which could be partially explained by a lack of communication among professionals, with 82% reporting not asking for help when completing the form. Moreover, PEP knowledge was not higher in more experienced professionals, in contrast to more experienced workers having more awareness of practices to control rabies in India (12). Most health professionals (97%) in our study were unable to correctly classify wounds as "severe" or "light" according to Ministry of Health guidelines, which also strongly influences PEP outcome in Brazil and other countries (27, 30). Therefore, our study suggests that more frequent training should help increase PEP knowledge and reduce its misuse. Future studies could also test other individual factors that can influence PEP knowledge not included in this study such as the professional's socio-economic background, risk perception, specific profession, age and gender.

Besides providing the appropriate PEP to people at risk, adequate surveillance is also key in reducing rabies burden (31). Reducing data entry errors or inconsistency and ensuring form completion are key to using SINAN in local and national surveillance for rabies (8). However, many SINAN records include major errors (e.g., rabies false positives) or missing data (8, 30). Errors in the SINAN data could arise from the relatively limited amount of time (<20 min for most professionals) to complete the form, the large number of fields (e.g., 60), errors when digiting paper forms, and a lack of feedback about errors (e.g., false positives) detected. In fact, 75% of participants did not receive feedback on the forms. Lack of feedback was reflected in less than half of professionals knowing if dog or cat rabies cases were reported in their state or municipality in recent years. Thus, we suggest that improving form completion and accuracy could be achieved by online training that highlights the utility and use of these data. For example, a scenario-based online module was successfully applied to increase knowledge of rabies and PEP in the USA (32), while a user-friendly mobile App guiding rabies prophylaxis among health-care professional was successfully implemented in India (33). Likewise, a mobile-phone-based health tool (mHealth) facilitated large-scale data collection on rabies in Tanzania, triggering automated text messages (SMS) to alert patients of vaccination schedules (34).

Overall, this study identified several knowledge gaps of health professionals assessing bite patients in Brazil, from evaluating the dog's health condition to selecting the appropriate PEP regimen. This lack of knowledge could contribute to the observed misuse of PEP over the past decade (8). Our results highlight that in Brazil, a decrease in dog and human rabies does not necessarily generate a proportional reduction in PEP demand as previously suggested in Latin America (35), unless appropriate training on dog's health condition and reduction of bites caused by "non-observable dogs" is achieved. Improving communication between public health professionals and veterinarians using One Health approaches could improve PEP administration and can potentially be achieved by applying an approach of Integrated Bite Case Management, which has previously shown potential to reduce PEP use by 40–60% through more accurate identification of dogs posing a risk for rabies (7, 36).

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Platform Brazil of the National Committee of Health of Brazil (project number: 3.029.348). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JAB, KH, and JM conceived and designed the study. RMDS collected and analyzed the data. JAB analyzed the data. RMDS, JAB, and KH drafted the article and edited the article. AASC, CSH, and FSM supported data collection. All authors read, commented, and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.846994/full#supplementary-material

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Immune Response After Rabies Vaccination in Owned Free-Roaming Domestic Dogs in Flores Island, Indonesia

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Vaccination is the main tool to prevent the circulation of rabies in dog populations. The development of an immune response after vaccination differs between individual dogs and depends on many factors such as dog characteristics, management, or genetics. Here, we first investigated the level of, and associated factors for, the presence of binding antibodies in 130 healthy dogs from Flores Island, Indonesia. Secondly, we identified factors associated with the development of binding antibodies within 30 days after vaccination among a subsample of dogs that had a binding antibody titre <0.5 EU/ml at the day of vaccination (D0, $N = 91$). Blood samples were collected from the individual dogs immediately before vaccination at D0 and 30 days after vaccination (D30). The rabies antibody titres were determined using ELISAs. Information on potential risk factors such as the dog's age and sex, history of vaccination, type and frequency of feeding, and BCS (body condition score) were gathered during interviews at D0. Regression analyses were performed to identify the risk factors associated with the presence of binding antibody titre ≥0.5 EU/ml at D0 for the 130 dogs and the development of binding antibody titre ≥0.5EU/ml at D30 for the 91 dogs. The results showed that the proportion of dogs with antibody titre ≥0.5 EU/ml was 30% (39/130) at D0. The only factors found to be significantly influencing the presence of binding antibodies titres ≥0.5 EU/ml was previous vaccination within 1 year before D0 [46.8 vs. 14.7%, Odds ratio (OR) = 3.6, 95%CI 1.5–9.3; *p*-value = 0.006], although the same trend was found for dogs of higher age and better BCS. Eighty-six percent (79/91) of dogs whose rabies binding antibody level was <0.5 EU/ml at D0 had developed an adequate immune response (≥0.5 EU/ml) at D30. Almost a significantly higher proportion developed an adequate immune response in dogs of good BCS compared to those of poor BCS (95.3% vs. 79.2%, OR = 4.7, 95%CI 1.1–32.5; *p*-value = 0.057. Twelve (13.2%) dogs retain binding antibody level <0.5 EU/ml at D30, indicating poor immune response after vaccination. A majority of them did not receive vaccine before D0 according to the owner and had poor BCS (83.3%; 10/12). Our findings show the high effectiveness of rabies vaccine in under field conditions to develop measurable immunity and the importance of a good BCS, often achievable by good dog keeping conditions, for developing efficient immunity after parenteral vaccination in dogs.

Keywords: rabies antibody, free roaming dogs, vaccine, Flores Island (Indonesia), body condition score (BCS), history of vaccination, serology

INTRODUCTION

Rabies remains an important global health concern, with an estimated global annual human death of 25 to 159 thousand cases (1). Most of these cases occur in African and Asian developing countries, with 60% of the global disability adjusted live years lost due to rabies occurring in Asia (1). Human rabies cases in developing countries are mostly transmitted by domestic dogs. Although they are mainly kept free-roaming, dogs in developing countries have a function, as for example in Indonesia where they are often kept as guardians for properties and to chase away wild animals, particularly monkeys and wild pigs (2, 3). Vaccination of dogs has been repeatedly shown to be the most effective strategy to control rabies in a sustainable way. However, diverse challenges, from political to organisational, have been identified to reach a high enough vaccination coverage in dog populations (4). As a consequence, the annual vaccination coverage is most often lower than the vaccination coverage of 70% recommended by WHO for preventing the circulation of rabies virus among dog population, as for example 53% that was reached in Flores Island, Indonesia over the period 2000–2011 (5).

In addition to a high coverage of mass dog vaccination, providing high-quality dog vaccine is key for the success of canine rabies elimination programs (6). High quality and effective rabies vaccine exist on the market, with laboratory trials documenting 93% seroconversion in vaccinated dogs after a single dose (7). However, development of immunity under field conditions might be hampered by several factors. Identifying them, helps to inform effective rabies vaccination campaigns.

Several studies have been conducted in European countries to identify risk factors of development of antibodies in dogs after vaccination (8–13). Age and breed are amongst the most commonly identified factors correlated with the lack of detectable immunity production after vaccination in dog population (9–12). For example, Keneddy et al. studied the antibody response of 10,483 dogs tested at the Veterinary laboratories Agency, Weybridge UK and found that adult dogs aged 1–7 years were more likely to have an antibody titre >0.5 International Units per ml serum (IU/ml) than young dogs (<1 year) (9). Berndtsson et al. investigated factors associated with the development of antibody titre of 6,789 vaccinated dogs in Sweden and found that larger breeds were at higher risk of having antibody titre <0.5 IU/ml (12). However, most of the above studies were conducted in developed countries for the purpose of international pet travel. Field studies to identify risk factors of development of antibodies in free-roaming domestic dogs in developing countries are limited (14, 15). In a randomised controlled study conducted in Tanzanian dogs, Lugelo et al. found that there was a positive correlation between body condition score (BCS) and seroconversion in which those dogs with good BCS were more likely to seroconvert than dogs with poor BCS (14). In contrast, Morters et al. investigated risk factors of antibody response of the free-roaming domestic dogs after vaccination in South Africa and found that the vast majority of the dogs seroconverted 30 days after vaccination, regardless of the health status of vaccinated dogs (15).

The majority of free-roaming dogs in Indonesia, as in most of developing countries, are aged <1 year and have a poor BCS as a consequence of insufficient nutrition uptake or parasitic diseases (16). The influence of BCS on the losing antibody titre has been reported in the previous studies (17). In a prospective study, Wera et al. found that the dogs with low BCS tend to more rapidly lose detectable antibody titres after vaccination than dogs with high BCS (17). However, to which extent the dogs develop binding antibodies after vaccination has not yet been analysed in these populations. In this study, we first investigated the level of and associated factors for the presence of binding antibodies in 130 healthy owned free roaming dogs from Flores Island, Indonesia. Some of them have previously been vaccinated, and the vaccination status was based on owners' reports. This study approach was previously used in the literature (10, 12). However, the low level of antibodies in the investigated dogs does not fully represent the development of immunity, because dogs may have already lost detectable binding antibodies after vaccination, although cellular immunity may still be present (18, 19). Therefore, we secondly investigated binding antibody titres and identified factors associated with the development of antibodies within 30 days after vaccination among a subsample of dogs that had a binding antibody titre <0.5 Equivalent Unit per ml serum (EU/ml) at the day of vaccination.

MATERIALS AND METHODS

Study Site and Data Collection

Study site and data collection has been described in a study previously conducted by the authors (17). In brief, blood sampling and questionnaire surveys were conducted in dog-owning households in two rural and one urban area in Sikka Regency, Flores Island, Indonesia between July and September 2018. As the dog sampling was part of a larger project on the investigation of dogs' roaming behaviour (3, 16), research cohort included every available healthy dog aged 3 months and more in the study area of 1 km^2 and excluded sick dogs. In addition, pregnant dogs were excluded from the study to avoid miscarriage due to stress. Dog blood samples were collected from 256 dogs on the day of vaccination (D0) and each dog was vaccinated subcutaneously with 0.5 ml of OIE pre-qualified vaccine (Rabisin vaccine, Boehringer Ingelheim) immediately after the blood sampling. Blood was further sampled from the exact same dogs 30 days after vaccination (D30), in case they were available. Information on dog characteristics, history of vaccination (previous vaccination performed and date of vaccination), kind of daily food (leftovers, rice, corn, and fish), frequency of feeding, and BCS was collected during interviews. The history of vaccination in the present study was mainly based on the dog owners' declaration. The number of dogs feeding with corn and fish is too low (nine dogs) to be analysed, therefore, rice, corn, and fish were collapsed into "others." The investigators were equipped with the pictures of BCS categorisation according to The American Animal Hospital Association (20) at D0. The BCS was assigned by the investigators (based on the pictures) using a 5-point system and categorised as "too thin" (BCS 1), "thin" (BCS 2), "ideal" (BCS 3), "overweight" (BCS 4), and "obese" (BCS 5). For purpose of data analysis, categories were collapsed as "good" (BCS 3, 4, 5) and "poor" (BCS 1 and 2) BCS. The interviews were conducted in Bahasa by the research team.

Of 256 blood samples collected at D0, 126 (49.2%) blood samples showed lysis due inappropriate handling after blood collection and had to be excluded from laboratory analysis. Consequently, 130 samples were eligible to be tested for the presence of rabies antibodies. For the first analysis, the 130 dogs with a serology result at D0 were investigated. For the second analysis, as the aim of the study was to identify the development of binding antibody titres within 30 days after vaccination and risk factors associated with binding antibody development, the analysis was conducted on the 91 dogs that manifested an antibody titre <0.5 EU/ml at D0.

At the same day of sampling, full blood was centrifuged and the serum was extracted, which was then dispensed into 3 ml labelled Eppendorf tubes. The tubes were stored at +4°C until shipment to the veterinary laboratory (Disease Investigation Centre, Denpasar) in Bali. The presence of rabies antibodies of the serum samples was tested by ELISA as described elsewhere (15). Samples were categorised as negative, i.e., having inadequate level of binding antibodies, if the titre was detected to be <0.5 EU/ml, and positive otherwise.

Statistical Analysis

Categorical variables were presented as numbers with their corresponding percentages. Univariable and multivariable logistic regression analyses were conducted to investigate the effect of the independent variables (**Table 1**) on the presence (D0 model) and development (D30 model) of binding antibodies after vaccination (outcome variable). All independent variables that had *p*-values lower than or equal to 0.25 in the univariable analyses were subsequently included in the initial models for the multivariable analyses (21). The final multivariable logistic models were derived by comparing the Akaike Information Criterion (AIC) of the models build with all possible combinations of the variables selected for the multivariable analysis. The model with the lowest AIC was considered as the final model. The fit of the final models to the data was determined using the Hosmer-Lemeshow goodness-of-fit test (21). The final models were considered a good fit for the data if the *p*-value of the Hosmer-Lemeshow test was greater than 0.05. All statistical analyses were performed with SPSS version 19 and R version 4.1.0 (function *glm* of the *stats* package for the regression analysis and function *hoslem.test* of the package *Resource Selection* for the Hosmer-Lemeshow test). We assumed a level of significance at 0.05.

RESULTS

Study Population

A total of 130 free-roaming owned dogs from 98 households in two rural (Pogon and Hepang) and one urban (Habi) area were eligible to be tested for antibody against rabies at D0 (**Table 1**). The majority of dogs were younger than 12 months (56.9%), had a poor BCS of <3 (50.8%), and had either no previous vaccination or was vaccinated more than 12 months before D0 (52.3%). These characteristics were comparable with the characteristics of the 91 dogs sampled and included in the analysis at D30 (**Table 1**).

Antibody Titres and Factors Influencing the Presence of Adequate Binding Antibodies Levels at D0

At D0, 39 of the 130 dogs (30.0%) had antibody titres ≥0.5 EU/ml. A higher proportion of female dogs had antibody titre ≥0.5 EU/ml compared to male dogs (**Table 2**). Similarly, the proportion of rural dogs with antibody titre ≥0.5 EU/ml was higher than urban dogs, but the differences were not significant in the univariable analysis ($p > 0.05$) (**Table 2**). Furthermore, of the 62 dogs that had a history of vaccination within 12 months before D0, 29 (46.8%) had antibody titres ≥0.5 EU/ml against rabies. Only 10 (14.7%) of the 68 dogs with either no previous vaccination or were vaccinated more than 12 months before D0, had antibody titres ≥0.5 EU/ml, which was significantly less than dogs vaccinated within the last 12 months before D0 (**Table 2**). Dogs older than or equal to 12 months were significantly more likely to have antibody titres more than ≥0.5 EU/ml (18.9 vs. 44.6%, $p = 0.002$) than those age <12 months. In the multivariable analyses, the history of vaccination was the only factor significantly associated with the proportion of binding antibody titres ≥0.5 EU/ml at D0 (**Table 3**). Being of age more than 12 months and having a good BCS also increased the odds of antibody titres being >0.5 EU/ml, however not on a significant level according to our defined significance level.

Antibody Titres and Factors Influencing the Presence of Adequate Binding Antibodies Levels at D30

At D30, 79 (86.8%) of the 91 dogs with detectable levels of rabies antibody titres <0.5 EU/ml at D0 developed antibody titres ≥0.5 EU/ml. No significant differences were found for factors age, sex, breed, geographical area of dogs (urban vs. rural), kind of daily food, and frequency of feeding (**Table 4**). Univariable analyses showed that almost significantly (OR = 4.4, 95%CI: 0.9–21.4; $P = 0.066$) more dogs (37/39, 94.9%) with previous vaccination within 12 months before D0 developed antibody titres ≥0.5 EU/ml at D30, compared to 42 (80.8%) of the 52 dogs without

TABLE 1 | Demographic characteristics of dogs surveyed in Flores Island, Indonesia on the days of vaccination, D0 (n = 130) and at 30 days after vaccination, D30 (n = 91; only dogs with antibody titres <0.5 EU/ml at D0 were considered for the analysis).

Variable	D0[a] (*N* = 130 dogs)		D30 (*N* = 91)	
	Frequency (*n*)	Percentage (%)	Frequency (*n*)	Percentage (%)
Sex				
Female	88	67.7	59	64.8
Male	42	32.3	32	35.2
Age				
<12 months	74	56.9	60	65.9
≥12 months	56	43.1	31	34.1
Breed				
Local breed	126	96.9	88	96.7
Other	4	3.1	3	3.3
Geographical area				
Urban	69	53.1	50	54.9
Rural	61	46.9	41	45.1
History of rabies vaccination				
Vaccinated <12 months before D0[a]	62	47.7	39	40.7
Never vaccinated or vaccinated >12 months before D0[a]	68	52.3	52	59.3
Origin of dogs				
Born at home	54	41.5	38	40.7
Given or bought	76	58.5	53	59.3
Kind of daily food				
Leftovers	121	93.1	83	90.1
Other[b]	9	6.9	8	9.9
Frequency of feeding				
<3 times per day	76	58.5	52	57.1
≥3 times per day	54	41.5	39	42.9
Body condition score /BCS[c]				
Poor	66	50.8	48	52.7
Good	64	49.2	43	47.3

[a] *D0 is the day of vaccination within this study.*
[b] *Other daily food like rice, corn, fish.*
[c] *BCS ranged from 1 to 5 and was categorised as poor for scores lower than 3 and good for scores of 3 or higher.*

history vaccination within 12 months before D0 (**Table 4**). Forty-one (95.3%) of 43 dogs with good BCS had antibody titres ≥0.5 EU/ml at D30, which was significantly more than the 79.2% among dogs with poor BCS. Of the 12 (13.2%) dogs that had an inadequate immune response at D30 (i.e., binding antibody level <0.5 EU/ml), 10 dogs (83%; 10/12) did not receive vaccines within 12 months before D0 and had poor BCS, while the other two dogs had vaccination within 12 months before D0, but had a poor BCS.

Multivariable analyses showed that BCS was almost significantly associated with the development of binding antibody levels of >0.5 EU/ml at D30 with almost a significantly higher proportion of adequate immune response found in dogs with good BCS compared to those poor BCS (95.3 vs. 79.2%, p-value = 0.057) (**Table 5**). Dogs being vaccinated <12 months before D0 also tended to have a higher odd of developing an antibody titre of >0.5 EU/ml, however the effect was observed to be lower than for the BCS (**Table 5**). In both multivariable regression models (**Tables 3**, **5**), the Hosmer-Lemeshow goodness-of-fit test p-values were non-significant ($p = 0.982$, $p = 0.999$ for model D0 and D30, respectively), indicating the model fitted the data well.

DISCUSSION

The success of a parenteral mass vaccination campaign in the field depends on many factors. These include dog owners' behaviour, infrastructure, the sustainability of vaccination campaign programs, and immunity coverage after vaccination. A good balance of these factors could contribute to effective prevention of rabies both in dogs and humans. For example, 2 years mass dog vaccination campaigns in Bali using long-acting vaccines with a coverage of >70% in 2010 and 2011 have led to the reduction of dog and human rabies cases by ∼90% (5). Similarly, dog and human rabies incidence in Latin America and Caribbean has successfully decreased by 90% due to sustainable

TABLE 2 | Frequency (*n*) and percentage (*n/N*) of dogs having a level of ≥0.5 EU/ml of binding antibodies at D0, stratified by different demographic characteristics of the dogs.

Variables	Sample size (*N*)	Binding antibody titre ≥0.5 EU/ml (*n*)	Percentage (*n/N* in %)	OR	95% CI of OR	*P*-value*
Sex						0.287
Male	42	10	23.8	1.00		
Female	88	29	33.0	1.57	0.68–3.64	
Age						**0.002**
<12 months	74	14	18.9	1.00		
≥12 months	56	25	44.6	3.46	1.58–7.58	
Breed						1.000
Local breed	126	38	30.2	1.30	0.13–12.86	
Other	4	1	25.0	1.00		
Geographical area						0.541
Urban	69	19	27.5	1.00		
Rural	61	20	32.8	1.28	0.61–2.72	
History of rabies vaccination						**<0.001**
Vaccinated <12 months before D0[a]	62	29	46.8	5.10	2.21–11.76	
Never vaccinated or vaccinated >12 months before D0[a]	68	10	14.7	1.00		
Origin of dogs						0.938
Born at home	54	16	29.6	1.00		
Given or bought	76	23	30.3	1.03	0.48–2.21	
Kind of daily food						0.277
Leftovers	121	38	30.0	3.66	0.44–30.33	
Other[b]	9	1	11.1	1.00		
Frequency of food						0.641
<3 times per day	76	24	31.6	1.20	0.56–2.58	
≥3 times per day	54	15	27.8	1.00		
Body condition score[c]						**0.026**
Poor	66	14	21.2	1.00		
Good	64	25	39.1	2.38	1.10–5.17	

The influence of demographic parameters was explored by univariable logistic regression analyses.
OR, Odds ratio; CI, Confidence interval.
[a]*D0 is the day of vaccination in this study.*
[b]*Other daily food include rice, corn, or fish.*
[c]*BCS ranged from 1 to 5 and was categorised as poor for scores lower than 3 and good for scores of 3 or higher.*
**p-value shown in bold represents p ≤ 0.25; these variables were used in the subsequent multivariable logistic regression analysis.*

TABLE 3 | Determinants associated with developing of adequate level of binding antibodies at D0 in dogs on Flores Island, Indonesia, using multivariable logistic regression analysis.

Variable	*n/N*	percentage	OR (95% CI)	*p*-value
History of rabies vaccination before D0*				0.006
≥12 months	10/68	14.7	1.00	
<12 months	29/62	46.8	3.63 (1.4–9.30)	
Age				0.141
<12 months	14/74	18.9	1.00	
≥12 months	25/56	44.6	1.94 (0.80–4.70)	
Body condition score				0.127
Poor	14/66	21.2	1.00	
Good	25/64	39.1	1.91 (0.84–4.44)	

OR, Odds ratio; CI, Confidence interval.
**D0 is the day of vaccination within this study.*

TABLE 4 | Frequency (*n*) and percentage (*n*/*N*) of dogs developing a level ≥0.5 EU/ml of binding antibodies within 30 days after vaccination, stratified by different demographic characteristics of the dogs.

Variable	Sample (*N*)	Binding antibody titre ≥0.5 EU/ml (*n*)	Percentage (*n*/*N* in %)	OR	95% CI of OR	*P*-value*
Sex						0.747
Male	32	27	84.4	1.00		
Female	59	52	88.1	0.73	0.21–2.51	
Age						0.745
<12 months	60	51	85.0	1.00		
≥12 months	31	28	90.3	1.65	0.41–6.58	
Breed						1.000
Local breed	88	76	86.4	NA		
Other	3	3	100			
Geographical area						0.800
Urban	50	43	86.0	1.00		
Rural	41	36	87.8	1.17	0.34–4.01	
History of rabies vaccination						**0.066**
Vaccinated <12 months before D0[a]	39	37	94.9	4.41	0.91–21.41	
Never vaccinated or vaccinated >12 months before D0[a]	52	42	80.8	1.00		
Origin of dogs						0.525
Born in house	38	34	89.5	1.00		
Given or bought	53	45	84.9	0.66	0.18–2.38	
Kind of daily food						0.301
Leftovers	83	73	88.0	2.43	0.43–13.75	
Other[b]	8	6	75.0	1.00		
Frequency of food						0.929
<3 times per day	52	45	86.5	0.94	0.28–3.24	
≥3 times per day	39	34	87.2	1.00		
Body condition score[c]						**0.023**
Poor	48	38	79.2	1.00		
Good	43	41	95.3	5.39	1.11–26.22	

The influence of demographic parameters was explored by univariable logistic regression analyses.
[a] D0 is the day of vaccination within this study.
[b] Other daily food like rice, corn, fish.
[c] BCS ranged from 1 to 5 and was categorised as poor for scores lower than 3 and good for scores of 3 or higher.
**p-value shown in bold represents p≤0.25; these variables were used in the subsequent multivariable logistic regression analysis.*

TABLE 5 | Determinants associated with developing of adequate level of binding antibodies 30 days after rabies vaccination in dogs on Flores Island, Indonesia, using multivariable logistic regression analysis.

Variable	*n*/*N*	percentage	OR (95% CI)	*p*-value
Body condition score (BCS)*				0.057
Poor	38/48	79.2	1.00	
Good	41/43	95.3	4.72 (1.12–32.45)	
History of rabies vaccination before D0*				0.107
≥12 months	42/52	80.8	1.00	
<12 months	37/39	94.9	3.75 (0.08–25.93)	

OR, Odds ratio; CI, Confidence interval.
**BCS ranged from 1 to 5 and was categorised as poor for scores lower than 3 and good for scores of 3 or higher.*

efforts in mass dog vaccination campaigns using a high quality of rabies vaccines (22). A high quality of rabies vaccines is expected to guarantee protection in dogs from rabies, resulting in a high immunity coverage. Vaccination coverage of 70% after sustained annual vaccination is necessary to interrupt the rabies virus circulation among dogs and is thus more cost-effective than PEP alone for preventing human rabies (23, 24). In Flores Island, mass dog vaccinations are carried out annually, targeting all dogs older than 3 months, achieving vaccination coverage around 53% on average (5). This low vaccination coverage was unable to eliminate rabies in Flores Island (25, 26). Furthermore, we earlier reported that the loss of detectable immunity is substantial within 1 year after vaccination in dogs on Flores Island, indicating that vaccination campaign on annual basis may be insufficient (17). Currently, the development of binding antibodies after vaccination has not been investigated yet in these populations, which was the objective of this study.

The present study highlighted the proportion of dogs with antibody titre ≥0.5 EU/ml increased from 30% (39/130) at D0 before vaccination to 86.8% (79/91) at D30 (30 days after vaccination). Increasing the proportion of dogs with antibody titre ≥0.5 EU/ml 1 month after vaccination is well-documented in the literature and findings lay in the range of what was found in the present study (7, 14, 27). Lugelo et al. reported that the proportion of dogs having a titre higher than 0.5 IU/ml increased from 4% (D0) to 85% (D30) (14). Similarly, Minke et al. studied the antibody titre in a group of 30 laboratory dogs vaccinated with Rabisin rabies vaccine and found that the proportion of dogs with a titre ≥0.5 IU/ml increased sharply from 0% at D0 to 93% at D30 post-vaccination (7). Furthermore, Kallel et al. studied the immune response of 1,000 vaccinated domestic dogs under field condition in Tunisia and found that the proportion of dogs with a protective antibody titres at D0 and D30 was 30 and 91%, respectively (27). These results indicate that the vaccine used in the present study was as effective as in comparable studies in producing protective antibody against rabies in the field.

Although development of immunity has already been studied in other contexts, it is of interest to investigate the effectiveness of internationally recognised vaccines in field condition, particularly in areas with limited animal health infrastructure and services, like Flores Island. Moreover, the socio-economic status of dog owners differs between countries, leading to varying availability of resources for ideal dog keeping practises. The results of the present study showed that around 13.2% (12/91) of vaccinated dogs have not produced a sufficient level to be protected against rabies despite the use of an international commercialised rabies vaccine well-known for its efficacy in inducing a strong humoral response (7). Failure to produce a strong humoral response 30 days after vaccination (D30) was also previously found in laboratory experiment (7) and under field conditions (14, 28). Although virus challenge studies would be needed to definitively prove the lack of protection against rabies, for the development of immunity, measuring antibody titres are a useful tool (18, 19). In an experimental study, Auber et al. provided evidence that animals with neutralising antibody titres at the time of challenge towards rabies virus were better protected than those without neutralising antibodies (18). Therefore, these 135% of dogs ($n = 12$) are very likely to be not protected at D30, because of their lack of reaction towards the vaccine.

In our study, failure to produce a strong humoral response was linked to low BCS. The multivariable logistic regression analysis results indicated that the main determinant of antibody development at D30 was the BCS. The results are consistent with a field study in Tanzania, in which 412 free-roaming domestic dogs following single dose of rabies vaccination, demonstrated the significant association between BCS and seroconversion (14). Similarly, Wera et al. have reported significantly higher proportion of antibody titres >0.5 EU/ml in dogs with good BCS vs. poor BCS at 90, 180, and 270 days after rabies vaccination (17). These findings suggest that the present study provides consistent evidence for BCS influencing the development and presence of binding antibody titres following rabies vaccination.

This observed association between presence of antibody titres and BCS may be attributed to the health status of dogs at the time of vaccination because other dog characteristics such as age, sex and breed, the location they live (urban vs. rural), and management practises (feeding, origin and role of the dog) did not show any significant relation to the presence of antibody titres. Dogs with poor BCS are malnourished and most frequently found with high burden of endo and ecto-parasites (own unpublished data from West Timor, Indonesia). This is most likely due to the fact that dogs in developing countries are free roaming day and night and therefore encounter more dogs and potentially contaminated environment (2, 15, 16). They have, thus, a high potential to be infected by parasites. This further depresses the immune system and consequently reduce antibody production (29). Booster vaccination focusing on dogs with low BCS and educational campaigns to improve knowledge on dog management practise, promote responsible dog ownership including good feeding and health practises, is expected to improve BCS of the dogs, which would enhance producing immunity after vaccination. We thus argue that investing into improved dog keeping practises leading to an increase of dog health and BCS, could highly improve the efficiency of rabies vaccination campaigns in the future.

In our study, we exclude obviously sick dogs because another part of the project is aimed at investigating normal dog roaming behaviour (3, 16, 30). Investigating the effect of the dog's health status in addition to the effect of BCS on the development of immunity after vaccination in the field should be of interest for future studies.

The present study found that 46.8% of dogs that had a history of vaccination within 12 months before D0 had evidence of anti-rabies antibodies at D0. Having a low level of antibodies (<0.5 EU/ml) does not always indicate poor reaction towards vaccination, because it may be that the dogs have developed immunity after the vaccination in the past, but had a decay in the detectable antibodies until blood sampling. Low coverage of antibody titres >0.5 EU/ml after the mass vaccination against rabies in dog populations was also reported by other authors (27, 31, 32). Tepsumethanon et al. described that 42% of vaccinated dogs in Thailand failed to present rabies antibody titres >0.5 IU/ml 360 days after vaccination (31). Similarly, Cliquet et al. reported low immunity coverage (36%) 1 year after vaccination in a dog population in Tunisia (32). These findings suggest that a large number of dogs are potentially susceptible to rabies 1 year after the vaccination campaign, although cellular immunity that is not measured by the antibodies may still be present (18, 19). It is noteworthy that in our study, the majority of dogs with antibody titres <0.5 EU/ml at D0 are dogs having no history of vaccination before D0 (63.7%; 58/91), which was found to be a significant factor for the D0 titre. Therefore, booster vaccination (for example, 6 months after first vaccination) targeting on dogs without previous vaccination is crucial to increase the immune response between yearly campaign and eventually eliminate rabies both in dogs and humans.

The history of vaccination in the present study was mainly based on the dog owners' declaration. This approach is also reported by other authors (33–35) and may lead to an over- or underestimation of the proportion of vaccinated dogs, as dog owners could misclassify the dog's vaccination status. Dog vaccination campaigns in Flores Island are carried out on a yearly basis by public veterinary offices without providing vaccination certificate for dog owners. Despite this limitation, the results of this study illustrate the link of history of vaccination and the presence of a strong immune response in owned free-roaming domestic dogs in Flores Island. Finally, our study revealed that 14.7% ($n = 10$) of dogs with either no previous vaccination or more than 12 months before D0, had antibody titres ≥0.5 EU/ml at D0. According to the statement of the owners, most of these dogs were previously vaccinated more than 12 months before D0. However, for four (3% of the entire study population of 130 dogs) dogs, owners reported that the dogs were unvaccinated during their life. Although it may be that the statement of the owners was wrong (for example due to lack of memory or change of the dog's owner over time), natural immunity due to exposure to rabies virus not leading to fatal disease can be the reason for this observation (36, 37).

CONCLUSION

Our findings showed a high immune response in owned free-roaming domestic dogs vaccinated under field conditions in Indonesia. History of vaccination and good BCS were significantly associated with the presence of rabies antibody at D0 and the development of antibodies at D30. Given the significant association with BCS, an educational campaign focusing on dog management practise is expected to improve the health status and BCS of the dogs, which would enhance producing detectable immunity after vaccination.

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/s.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Ethics Commission of the Veterinary Medicine Faculty, Nusa Cendana University (Protocol KEH/FKH/NPEH/2019/009). Written informed consent for participation was not obtained from the owners because dog owners in Flores were not familiar with written informed consent.

AUTHOR CONTRIBUTIONS

EW, CW, SD, PMB, and MMS conceived the study. EW, CW, PMB, and MMS collected the field data. EW, CW, SD, and PMB conducted statistical analyses. EW wrote the original draft of the manuscript. CW, SD, PMB, and MMS contributed to reviewing the manuscript. All authors read and approved the version of the manuscript to be published.

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Where Rabies Is Not a Disease. Bridging Healthworlds to Improve Mutual Understanding and Prevention of Rabies

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Deeply embedded in local social, cultural, and religious settings, traditional healing is part of dog bite and rabies management in many rabies endemic countries. Faith healing, which usually encompasses a more holistic approach to health including physical, mental and social dimensions, is rare in the context of rabies. In Gujarat, Western India, the Hindu goddess Hadkai Mata is worshiped by low-caste communities as the Mother of Rabies in the event of a dog bite to a person or their livestock. This belief might influence people's attitudes and behaviors toward rabies prevention but has never been investigated. Through 31 in-depth interviews with healers and staff of Hadkai Mata temples, this paper explores the system of knowledge around dog and human rabies that is built and shared in these places of worship and healing. Qualitative and quantitative data were analyzed looking for convergences and divergences with the recently launched National Action Plan for dog-mediated Rabies Elimination. Results suggest that while the etiology of human rabies as a social illness is usually explained as the goddess's wish to correct misbehaving people and restore positive interpersonal relations, there is some appreciation for the biological processes of infection that lead to rabies as a physical disease. Hadkai Mata is believed to cure rabies if her patients undergo the necessary process of moral growth. Although conventional post-exposure prophylaxis is not opposed *per se*, it is often delayed by patients who seek traditional treatment first. Some reluctance was expressed toward mass dog vaccination because it is seen as an interference in how the goddess controls dogs, by enraging them—hence infecting them with rabies—and sending them to bite wrongdoers. Addressing these cultural perceptions is likely to be critical in achieving effective control of dog rabies in this region. The study highlights the value of multidisciplinary approaches in the control and elimination of rabies, as well as other zoonoses. This includes the importance of understanding different culturally- and religiously- mediated ways in which humans relate to animals; and looking for points of convergence and mutual understanding, upon which context-tailored, linguistically-accurate, locally acceptable, feasible and effective strategies can be designed.

Keywords: dog bites, dog-mediated rabies, human rabies, faith healing, healthworlds, mass dog vaccination, One Health, post-exposure prophylaxis

INTRODUCTION

Rabies constitutes an important public health concern and causes an estimated 59,000 human deaths, with the highest burden falling on the rural poor of Asia and Africa (1). This viral infection is usually transmitted through animal bites, mostly from domestic dogs, and is fatal once clinical signs appear (2). Based on the One Health concept that recognizes the interdependence between human and animal health, the global strategic plan to end human deaths from dog-mediated rabies by 2030 ("Zero by 30") consists of vaccinating dogs to stop transmission at its source, providing post-exposure prophylaxis (PEP, which uses rabies vaccine and, in case of severe exposure, rabies immunoglobulin) to exposed individuals, and increasing community awareness about these two measures and dog bite prevention generally (3). In most endemic countries, each of these components faces challenges. The design of mass dog vaccination campaigns is often poorly informed by an understanding of people's attitudes toward dogs and perceptions around dog vaccination (4); PEP delivery struggles to reach those that need it most (5); and awareness about how to manage dog bites is often low not only amongst the public (6) but also healthcare providers (7). The common denominator of these issues is frequently a disconnect between research and policy (8), hence the design of strategies that are not context-specific (9).

India shares one-third of the global human rabies burden (10) and typifies these problems. Despite the high burden of disease, rabies was made notifiable only in September 2021 (11) and therefore its on-the-ground reality is still poorly understood. Inspired by "Zero by 30," India recently launched its National Action Plan for dog-mediated Rabies Elimination (NAPRE). Enthusiastic about this plan, a Union Minister claimed that *"The mere mention of hadakwa* ["rabies" in the language of his native State, Gujarat] *induces terror in rural areas. They* [villagers] *will actively help the government in this noble endeavor"* (12).

Based on worldviews, beliefs, and symbols, faith healing consists of religious and spiritual practices, performed by the patient or an intermediary, to alleviate suffering or restore health. Health is usually understood not only as the absence of disease, but more specifically as the co-presence of physical, mental, and social wellbeing, and can be achieved in several ways (for example, through divine energy, the power of healer's hands, a process of spiritual growth). In relation to rabies and dog bites, faith healing is usually considered part of traditional medicine, together with the more common, both in India and abroad, application of substances on the wound (13, 14). India also has the unique distinction of having, besides biomedicine, six other official systems of medicine (i.e., Ayurveda, Siddha, Unani, Yoga, Naturopathy, and Homeopathy), the first three of which are used in the field of rabies (15). For example, a previous study found evidence for herbal therapy and "magico-religious [practices]" being sought by rabies bite victims in 60% of fatal cases in India (10).

By influencing individual and community dog keeping practices and concepts of animal welfare, religion also impacts mass dog vaccination, but this aspect of rabies control is rarely studied (16), even by anthropologists (17). For example, people in India feed free-roaming dogs more because of the belief in karma (i.e., good deeds in the current life lead to a happier rebirth) than a genuine concern for dog welfare (4).

Here we explore the role played by the Hindu goddess Hadkai Mata (literally, the Mother of Rabies) in the understanding of and approach to dog and human rabies in Gujarat, the only Indian State where the goddess is venerated. To our knowledge, Hadkai Mata is, among the world's current religions, one of the very few rabies-related deities who inspire the performance of faith-healing practices. In the past, the figure of Lyssa in Greek mythology and the Christian saints Quiteria and Denis were associated with rabies, but not with a specific treatment. Saint Hubert was a notable exception. Until the early twentieth century, people in Europe hung metal nails known as the keys of Saint Hubert on the walls of their houses to protect themselves from rabies. In case of a bite, the key was heated and used to cauterize the wound, and a thread—supposedly from the saint's stole—was inserted in a little incision on the patient's forehead for 9 days, during which time dietary restrictions were followed (18).

Theoretically, this paper reflects on two very different approaches to the understanding of people's behaviors; the extended parallel processing model and the concept of "healthworlds." Within health behavior change theories, the extended parallel processing model states that, for people to take protective action against a health threat and eventually improve community participation in disease control programs, health communication campaigns should focus on two factors. They are people's fear—or "terror," to echo the abovementioned Union Minister's words—and perceived self-efficacy (i.e., a person's confidence in their capacity to put a proposed solution into practice) (19). Despite the lively debate on whether interventions based on behavioral theory really work in the real world (20), with rabies, fear appears to be a potent driver of community engagement and compliance with human and dog vaccination, for example in both Tanzania (21) and Peru (22). In this paper, we challenge this assumption in two ways. First, by highlighting the importance of understanding rabies dynamics in local, context-specific variations. Second, by acknowledging that behaviors are not the mere result of individual choices and cognitive determinants (23), but they reflect people's healthworld. Crucial to understanding "the empirical complexity of health beliefs (importantly, including religion) and behaviors" (24), healthworlds include large-scale social and economic relationships. They are concerned with disease (i.e., the pathological deviation from a biological norm) and illness (i.e., the patient's experience of ill health). They also adopt expanded definitions of medical pluralism [i.e., "shopping and switching" between multiple modalities of care available, in a complementary or alternative way—(25)] that include faith-based options. According to this concept, which resonates with systems-based approaches to health like EcoHealth and One Health (26), "individuals' healthworlds are shaped by, and simultaneously affect, the socially shared healthworld constituted by the collective search for health and wellbeing" (24). What inspired the development of this concept, and its use in this paper, is the ultimate, practical purpose, *via* communicative action (27), of having different people seek to reach an understanding about something so that they can coordinate their plans of action by way of mutual agreement.

No religious or devotional literature exists on Hadkai Mata, because this deity exists only in the oral tradition. Scholarly research is extremely limited (28, 29) and the relation between Hadkai Mata and rabies management has never been investigated. In this paper, based on discussions with traditional healthcare providers at Hadkai Mata temples, we analyze some aspects of the cult of this goddess to understand whether and how it influences the way this deity's followers manage dog bites and rabies. In particular, we ask the following questions: (1) what knowledge on rabies is collectively built and/or circulated in Hadkai Mata temples?; (2) what do this deity's believers do when they are bitten by a dog, and why?; and (3) what is the opinion of the traditional healthcare providers about dog vaccination (which has never been done at scale in rural India before), and why? The ultimate purpose of this work is to identify convergences and divergences (30) between this local perspective on rabies and the recently launched NAPRE. This understanding may enable us to propose tailored, bottom-up recommendations that address the wellrecognized limitations of top-down public health interventions that can be disconnected from local realities. Anthropology and implementation research literature already abound with examples of wasted resources and ineffective interventions as a result of mistrust from communities, and aggravated social fractures over power imbalances and communicative injustice (31–34). Given the size, geographic variety, federal political system, cultural diversity, and socio-economic complexity of India, the risk of failure of a one-size-fits-all strategy is evidently high.

MATERIALS AND METHODS

Study Area

Of the 36 States and Union Territories of India, Gujarat ranks fifth by area (196,024 sq km) and ninth by population [60,439,692 people, in 2011—(35)]. Agriculture and industry lead its economy, with the pharmaceutical sector playing a key role in the country's drug manufacturing. Gujarat ranks 10th by per capita gross state domestic product [3,200 USD, in 2019—(36)] and its 18,225 villages benefit from a good infrastructure, with eight villages out of 10 reachable through all-weather roads and nearly all of them connected to the electrical grid (37).

Nevertheless, 17% of Gujaratis live below the poverty line [20 USD per month, in 2012—(38)] and in 2019, the state ranked 21st by human development index (39), mainly because of caste- and religion-based social inequality (40) and insufficient spending on social security, rural development, and education (41). Most Gujaratis are Hindus (89%) and live in rural areas (57%). The population is classified as 15% Scheduled Tribes (geographically, and often economically, isolated communities), 7% Scheduled Castes (groups that face caste-based ostracization), 51% Other Backward Castes (historically oppressed communities, who continue to face some social and educational isolation), and 27% Other Communities (upper-caste Hindus and most non-Hindus) (35).

The percentage of the State budget spent on healthcare (5%) is far below the 8% recommended by the National Health Policy, and the privatization of the health sector is on the rise, with public health infrastructure being primarily affected (42). With regards to PEP, unlike in most Indian States, Gujarat's government hospitals usually have good stocks of rabies vaccines (43), which should be provided free of cost at such facilities, as per a nationwide policy. Similarly, hospitals in the state provide human rabies immunoglobulin (HRIG) for free (44). In most of the country, HRIG, which is generally preferred by doctors to its cheaper, equine alternative, is usually available only in private clinics and for a fee that only high-income patients can afford.

Study Sites

Merging information from Google Maps, YouTube videos of religious festivals, and village-by-village exploratory trips undertaken by DN (anthropologist) in September 2019, 139 places of worship were located. Before the COVID-19 pandemic interrupted fieldwork, DN visited 65 of these, selected by convenience and purposive sampling, starting from those in Anand and Ahmedabad districts and the most famous ones in the research area. After excluding little roadside shrines and unattended temples, the remaining 31 places of worship were included in the study (**Figure 1**) located in six districts of central Gujarat [total population in 2011 = 16,208,494—(35)], mostly in rural settlements.

Data Collection

In the 31 study sites, DN and her research assistant, RR (a half-Hindu, half-Christian woman born, raised, and college-educated in social work in the city of Anand), met for the first time and interviewed the bhuva (healer) or, when this person was not reachable, the sevak (temple caretaker). The purpose of this research, including the production of scholarly articles, was explained. Participation was entirely voluntary and verbal consent was obtained. On 25 occasions, either one or both among the bhuva and the sevak as well as some co-villagers participated in the discussion. The interviews, lasting 20 to 70 mins, were conducted in Gujarati, in or outside the temple (or, rarely, at the bhuva's home) and were audio-recorded with informed consent. A pre-defined semi-structured interview sheet consisting of 36 open-ended questions guided the conversation, but participants were encouraged to raise unaddressed issues. The first two

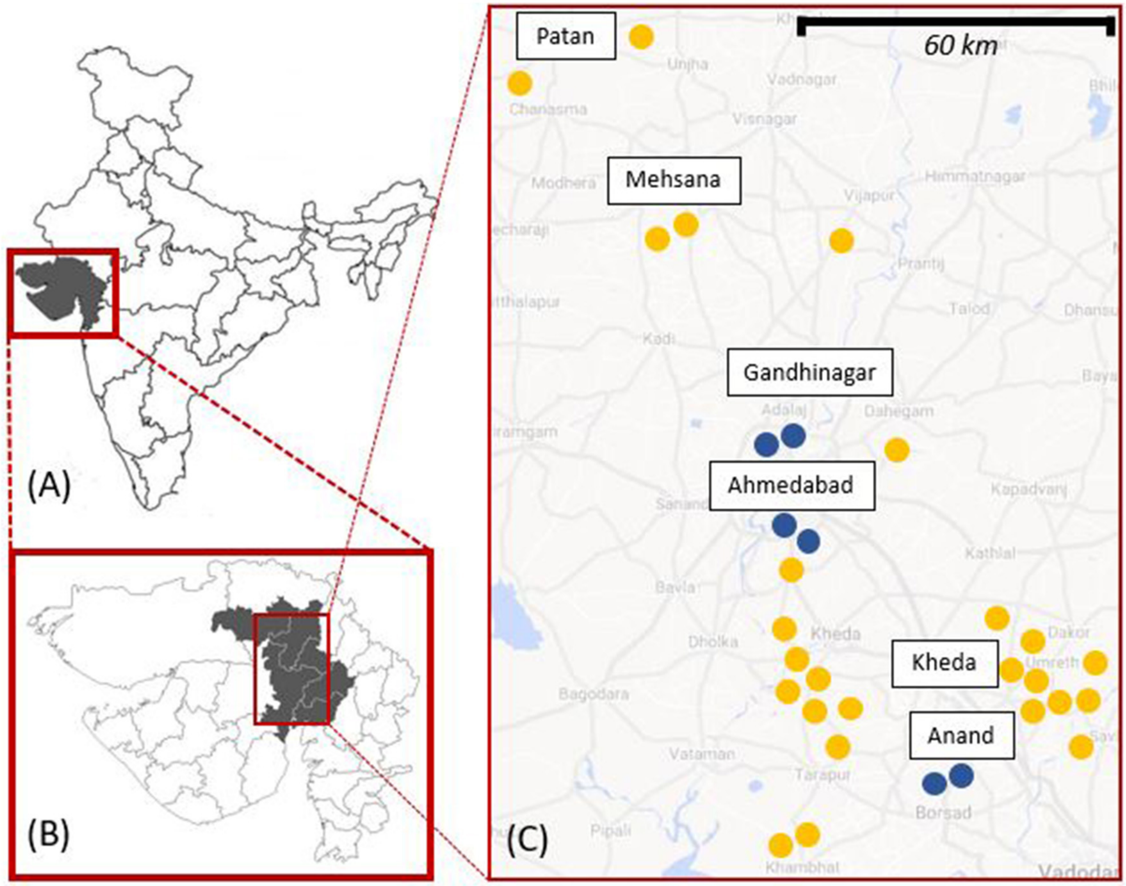

FIGURE 1 | Map showing: **(A)** Gujarat; **(B)** the six districts of Patan, Mehsana, Gandhinagar, Ahmedabad, Kheda, and Anand; and **(C)** the approximate location of the 31 study sites (25 rural, in yellow, and 6 urban, in blue).

interviews served as a pilot, but they were exploitable for analysis. All the participants we approached agreed to be interviewed.

Pictures and videos of the temples, Hadkai Mata's sacred images, and ritual paraphernalia were taken, where allowed by temple staff. The research team were often asked to treat the image of the goddess with respect, for example by refraining from printing her pictures to avoid them being accidentally dropped or stepped over. Observation was performed during four healing sessions and the ceremony in which a new bhuva was elected. During and after each temple visit, DN took notes of her own observations, feelings, comments, and thoughts, as well as those of the research assistant and three drivers. The presence of the drivers, all men, proved crucial for facilitating the researchers' approach to those temples where (local) women are not allowed and starting the conversation in vernacular Gujarati.

Data Analysis

The interviews were verbally translated into English and transcribed by the research assistant, and checked for accuracy by another Gujarati native speaker. Intelligent transcription was chosen to retain substance and accuracy, while ignoring fillers and expressions of agreement (the latter being very frequent, as the answers of the bhuva were rarely contested by the other respondents). Interview transcriptions were then inductively open coded by DN in Nvivo 12, where a double and iterative approach was taken. First, in each transcription, the answer to each of the key questions was identified and coded and the process was repeated for all interviews. Then, all the transcriptions were considered in their entirety and transversal themes were created. Photos, pictures, and field notes were used to enrich the data and referred to for triangulation. All data were pseudo-anonymized for analysis following transcription.

Quantitative data were analyzed in Excel 2019. In the case of multiple respondents and disagreement among them, the opinion of the highest-in-charge person was considered.

Direct quotes, chosen for their representativeness and revealing quality, appear in italics. Data are presented in order of frequency.

RESULTS

Characteristics of the Study Participants

The age of the study participants ranged from 25 to 85. All the bhuva inherited their role, which is male-only, from their fathers or older brothers. One election ceremony, observed by DN, where a new bhuva was chosen based on his ability to fall into trance, is a rare event.

All the interviewed bhuva claimed that they provide their service for free, as they usually have another job from which they generate their income, and which allows them to rush to the temple as needed. Elderly bhuva maintained that they are financially supported by their family. While personal donations are officially not accepted, offerings to the temple, both in the form of money (customarily, 5 to 20 rupees, 0.06 to 0.30 US dollars) or food for the deity, are. In the case of healing, donations can be more substantial. The biggest Hadkai Mata temples included in this study are managed by a Trust, which has access to the offerings and decides how to use them. In the smaller temples, this issue was not addressed during the study.

Most of the Hadkai Mata's bhuva and sevak who participated in this study belong to communities historically considered out- (39%) or low- (52%) caste (Scheduled Castes and Other Backward Castes). Almost the totality of the surveyed temples are located in the area of villages designated to these people. Only in a small proportion of the surveyed temples, the bhuva or sevak belonged to a mid (3%) or high (6%) caste. According to the interviewees, high-caste people are rarely involved in Hadkai Mata devotion. The research assistant, drivers, and other Hindu people met during fieldwork confirmed this.

Reason for Dog Bites and Rabies Infection

When asked the reason for dog bites, 84% of the informants provided an Hadkai Mata-related answer, while the remainder mentioned ethological or biological reasons (for example, part of dog behavior, pups' protection, reaction to danger, and rabies-induced aggression). According to an example often cited by those in the first group, there must be a reason why seven people walk together and a dog comes and bites only the one in the middle. This reason was explained as "*sin*," "*karma*," "*being at fault*," or "*having done something wrong*," in general or against Hadkai Mata. General mistakes commonly include lying, dishonoring promises, stealing, being stingy, overindulging in alcohol, and disrespecting relatives. The misbehaviors against the goddess mentioned by participants included committing perjury by her name, insulting her or her image, or "*taking her lightly and believing that she has no strength*." The latter misconduct is considered particularly dangerous, because "*like parents who let go the mistakes of their children once, twice, and then slap them if they repeat it, she* [...] *doesn't let go of many mistakes*," "*she doesn't tolerate anything wrong*," and "*she doesn't forget and doesn't forgive*." The most distinctive feature of Hadkai Mata is her strict and resolute nature, to the extent that she is commonly referred to as "*judge*," "*magistrate*," "*Supreme Court*," and "*Nyaya ni Devi*" (literally, the Goddess of Justice). She is famous for being the most severe among the deities that "*when other goddesses have complaints against their* [unruly] *followers, they register a case with her* [...] *as we do at the police station*," and they ask her to "*teach them a lesson*."

Like most Hindu deities, Hadkai Mata has a companion animal and animal that she rides, which, in this case, is a dog. In the Hadkai Mata-related explanation of rabies, dog bites are the means through which the deity punishes misbehaving people, by infecting them with rabies. She usually gives people a first warning, by sending a "*good* [not rabid] *dog*" to bark at the wrongdoer or nibble on their clothes. If they misunderstand or belittle this signal, she "*sits on a dog*" that, because of the presence of the angry goddess on them, immediately becomes rabid and bites them upon her command. As soon as the bite occurs, "*Mataji's rabies starts*" in the victim. According to one respondent, rabies could start in a person even if the biting dog is not rabid, or without any bite at all. "*If she wants to make a case, her power is enough*."

Once "*Mataji's rabies starts*," there are two possible scenarios. People who do not take the goddess seriously or do not care about correcting their mistakes will invariably die. An informant reported of a man that belittled the bite he had received from a pup reckoning that the dog was just playing and not biting upon Hadkai Mata's command. In cases of exceptional devotion, the bitten person may even accept death as the only fair consequence of their misbehavior. This was reported to have been the choice of one bhuva who had operated in one of the surveyed temples until a few years ago. The mistake he ascribed his rabies infection to was kicking a dog, which had eaten his food, while he was on duty at the temple. According to his successor, he refused both the goddess' and doctors' help, asked to be locked up in the temple, developed rabies, and died.

The second scenario applies to those who acknowledge their misdeed and want to fix it. If they follow the necessary healing process, described below, "*Hadkai Mata turns a bad* [rabid] *bite into a normal bite*" and "*takes rabies back*."

Healing Process

The healing process as reported by the study participants consists of three consecutive, equally necessary steps.

Step 1

Step 1 usually involves the visit to an Hadkai Mata temple, where at least one of the activities illustrated in **Figure 2** is performed. The most important ritual, mentioned in 95% of the interviews, is *badha* (literally, bond, restriction), or tying a thread around the wrist or neck of the patient, while the latter makes their promise to the goddess. The informants speculated that in most cases, the promise involves a future offer to Hadkai Mata in exchange for her doing the requested "*work*" for the follower. Provided that "*the promise is made with your full heart*," then "*everything* [the person's chances of healing] *is in the thread*," which purpose is to "*build faith*" and maintain it during Step 2. The wound is treated very rarely, because "*Mataji is the medicine and she is the doctor*," so "*people apply faith and go*."

Investigation

In one-third (n=11) of the temples, the bhuva runs a test to understand the presented bite case and how to handle it. Ten bhuva use wheat grains and one uses water, which is splashed on the patient's face to check their reaction. A bunch of wheat grains is thrown on a flat surface several times and, depending on the sequence of even and odd results, the answer to the bhuva's query is understood. There are usually three kinds of queries.

FIGURE 2 | Number of interviewees who mentioned the actions to take (by the bhuva, B, or the patient, P) during the first step of the healing process.

The first one concerns the status of the dog and the dangerousness of its bite: "*good dogs*" have a "*sweet bite*" that is usually not a concern, while "*bad dogs*" have a "*bitter bite*" for which the healing process must begin immediately in the hope that "*Hadkai Mata will make it sweet*." "Good dogs" are usually pet dogs or dogs whose bite was provoked, for example when a person inadvertently steps on their tail. "Bad dogs" are those who show the signs of rabies (described by the interviewees as hypersalivation, aggressiveness, attempts at chewing objects, restlessness, and tail bent between the legs) or unknown dogs that "*act upon Mataji's order*," so they appear only to bite the designated person and then run away.

The second query regards the mistake that led to the dog bite. Most bhuva leave the patients to determine their misbehavior on their own, but in a few temples, a more public acknowledgment is considered crucial for the recovery process to help the victim be fully aware of their fault.

The third query is about the chances of success of the healing treatment. When the victim shows evident signs of rabies, the bhuva will tell, by reading wheat grains as described above, whether the case can still be solved by Hadkai Mata or if "*it is spoilt*" (or ruined, doomed to fail).

Start of the Healing Process

In most cases, the thread is tied by the healer, but in some circumstances, this can also be done at home by the bite victim themselves, if a relative of them goes to the temple and collects the thread. These circumstances include (1) people who live too far from the temple; (2) people who are too worried to wait until going to the temple; (3) women who are menstruating (who would in any case be treated outside the temple, as it happens to any women in those temples where only men and girls are allowed); (4) people who belong to a caste lower than the one that runs the temple (where caste segregation rules are applied); and (5) people whose faith is particularly strong. When the healing process begins at the temple, Tuesdays or Sundays are the best days for two reasons. First, these are the most auspicious days to receive help from Hadkai Mata. Second, while the biggest temples are usually open 7 days a week, minor ones are attended by the bhuva only on these 2 days.

All the informants agreed on the importance of starting the healing process as soon as possible and within 3.5 days in all cases. "*Even if they* [bite victims] *come half an hour* [...] *before this time, then she can save them. After that, Mataji says that 'My time is over, now I cannot do anything. Nor can any doctor do anything'*."

According to most study participants, 3.5 days is also the time it takes for rabies clinical signs to show. A few mentioned a period of 1, 3.5, or 6 months for rabies signs to become evident, but a bhuva observed that "*she will wait for 2 years and she will show the symptoms. Like if I owe you some money, I will forget, but will you forget?*" Similarly, Hadkai Mata never forgets.

If a person reaches the temple later than 3.5 days since the bite, the healing process can still begin, if Hadkai Mata expresses her approval through the wheat grains, but the bhuva "*cannot give any guarantee*" of success. High diabetes was often taken as an example to explain the difficulty of avoiding death when "*rabies is full*," and 42% of the informants claimed that there is no chance of survival if the person starts the healing process after the 3.5-day limit. Death is expected to occur within—again—3.5 days since the onset of symptoms, or, if rabid patients are splashed with water or exposed to bright light, right away.

Seven informants reported cases of people that developed rabies symptoms earlier than expected, were rushed to an Hadkai Mata temple within 3.5 days, and were cured as soon as the bhuva asked the goddess for an emergency healing and performed their rituals (i.e., having the patient drink oil or waving peacock feathers around them). Critical cases are often sent to the temple in Kotha that, unlike minor temples that "*are like simple lawyers, where people can only make their promises*," "*is like a court*," where final decisions are made. "*People with full rabies*" were described as being tied up because of their agitation, salivating abnormally, scared to drink, bathe, and look at a light, fire, or red objects, restless when air is blown on their face, and unable to stand still.

Role of the Bhuva

This section, and any other mention of the role of the bhuva, presents the position of the healers from their own perspective. Their patients may provide a very different portrayal of the bhuva.

Bites from dogs—and dogs only—to people or their livestock are the main reason why people go to Hadkai Mata temples and benefit, if they wish to, from the services of the bhuva. A wide range of other deities are locally available for other needs. However, as with other powerful goddesses, people occasionally address Hadkai Mata also for generic reasons, which do not require the bhuva's assistance, such as having a (male) child, finding a good job, or solving visa-related issues. On Sundays, some major Hadkai Mata temples, such as the one in Patan, often become the informal venue for the members of a specific community, the Devipujaks, who consider Hadkai Mata their tutelary goddess. This is an occasion to spend the day together, celebrate the deity, and discuss, among themselves and with the bhuva, mundane issues such as disputes over money borrowing or extramarital affairs.

In almost all the interviews, the informants specified that the main role of the bhuva is being available as middle-persons that can easily communicate with Hadkai Mata. All but one of the interviewed bhuva strategically claimed that they never present themselves as those who have the power to cure, because that can be done only by the goddess. While this denial of personal responsibility emerged clearly in the interviews, what bhuva say they are happy to do is telling Hadkai Mata something like "*'Cure everyone, they are all your children. If they have done some sin, slap them and give them some small diseases, but don't give them rabies'*."

Similarly, they always stressed that the matter is resolved between the goddess and the patient, not the bhuva. Bite victims are encouraged to discuss their situation directly with Hadkai Mata, as a sevak explained: "*I tell them 'You tell Mataji yourself, this is not my work'. This is the era of science* [compared to the previous generation of bhuva, today's bhuva increasingly deal with patients who have received a formal education]*; we should not fall into this* [today's bhuva should not put themselves in a position that makes them vulnerable to criticism by educated people]." Promises too must be addressed to the goddess, not the bhuva. "*We are only taught that by worshiping her, rabies can be cured. We don't know what the secret behind it is. We have faith in the goddess. We follow moral values, rituals, and tradition*," a bhuva clarified.

Step 2

From the moment the promise to the goddess is made, patients must abide by precise rules that, according to most informants, are often already known by the patients themselves (**Figure 3**).

Almost all the study participants agreed that these rules apply for 5 weeks. One respondent claimed that 8 days are enough, while two said it depends on the personal agreement between the patient and Hadkai Mata (for example, when the person has to attend a wedding during the 5 weeks).

Diverging opinions were given with regards to what happens if these rules are broken. Going to funerals or to houses where a child was born, drinking alcohol, or eating meat is particularly risky and an immediate, probably lethal, "*rabies attack*" is likely to happen. According to one bhuva, this fatal event happens even if the person is undergoing PEP but infringes a rule. In particularly stubborn cases, the goddess may have a rabid dog bite not the offender themselves, but a dear family member or their livestock. Even worse, "*Mataji will have such a full power that rabies can be spread from person to person via touching*." In case of minor offenses, patients "*have to face the anguish of Mataji*" in any case but, upon the payment of a fine to Hadkai Mata (for example, a coconut or a sum of 0.35 or 0.70 USD), no negative consequence will occur. To minimize risk, an informant pleads with people to temporarily free themselves from the promise by untying the thread during the break they need to take. Another said that sometimes bite victims, especially men who travel a lot for their work and cannot always be sure about the food they are offered, have their wives keep the promise on their behalf and follow the rules that this implies.

Step 3

At the end of the 5 weeks, bite victims must go back to the temple to make an offering to Hadkai Mata. Sweets are the most common thanksgiving presents, together with dog-shaped toys or metal statuettes. According to four informants, patients should also do things like throwing away the clothes they wore the day they were bitten, lighting a lamp, drinking oil, or undergoing a water test. Clean water is poured into a vessel and shown to the bite victim: if they see "*insect-like things*," it means that "*they are still at fault as per Mataji*," so "*she is still in them*." In this case, the person has to consult any healer in their village to understand the precise mistake the deity is punishing them for and adjust the healing process accordingly.

If a person forgets to go back to the temple, ignoring the dogs that they see in their dreams, where they are sent to by Hadkai Mata as a reminder, they will get punished by being re-infected with rabies, through a second dog bite or simply upon the will of the goddess. No matter what the patient does, this time they will invariably die because the goddess feels she has been cheated.

Final Outcome

If people act appropriately during the entire healing process, this typically ends successfully because Hadkai Mata "*always*

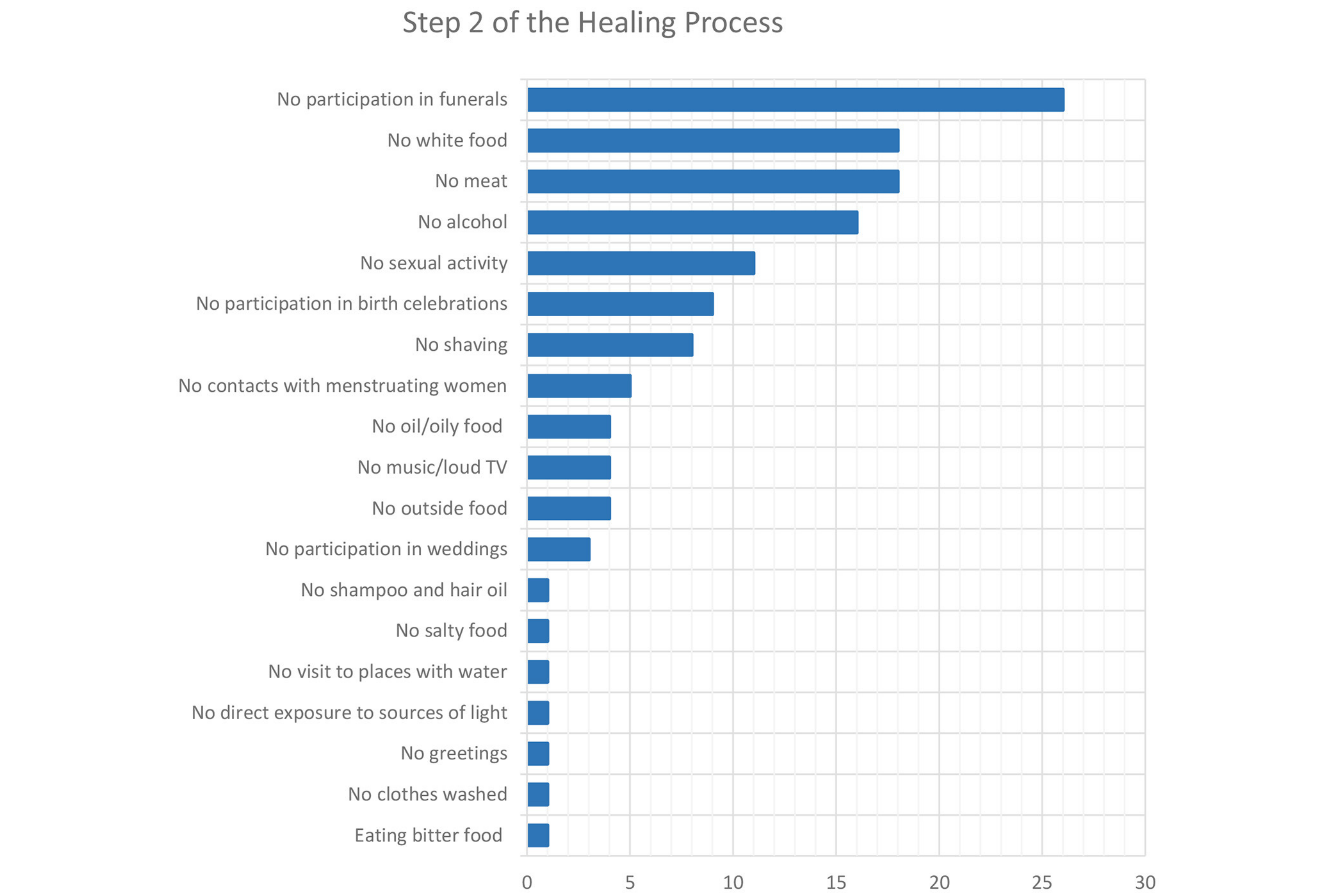

FIGURE 3 | Number of interviewees who mentioned the rules to follow during the second step of the healing process.

deals with people in accordance to their behavior." Patients are considered cured or out of danger when they do not develop any rabies symptoms within 3.5 days since the bite, when their symptoms disappear, or, according to one informant, when they excrete in their feces white tiny *jiv* (literally, germs). In rare cases, the bhuva may suspect that "*the case is still pending*" and recommend that the rules for Step 2 be extended for some months. This precaution is taken especially when Hadkai Mata is punishing the wrongdoer on behalf of another, unsatisfied goddess, who may work on a different schedule.

All the informants stated that Hadkai Mata is always able to "take rabies back" from a person, if she chooses to do so. Nevertheless, fatalities occasionally happen, when (1) people do not have complete faith in her, so "*their mind is not satisfied*"; (2) "*they think that this is all superstition*"; (3) "*they have already sinned a lot*"; and (4) they are too late in seeking her help.

Opinion About Post-exposure Prophylaxis

The question as to whether PEP should or should not be administered divided respondents. Eight were against this option and tell their patients that there is no need for vaccination, because Hadkai Mata has enough power to cure rabies. According to one study participant, if people go to the hospital, rabies symptoms immediately start because "*the doctor and Mataji are enemies.*" Another said that hospitals and doctors cannot be mentioned in the goddess' presence. "*For fever and other natural diseases, we have to go to the doctor,*" a sevak explained, "*but that is a different thing.*" When the person starts PEP, "*she won't take any responsibility*" and she can deny help. Several informants justified their opinion by observing that doctors also go to the temple in case of a dog bite, and that hospitals often refer their rabies cases to them. They also stressed that, as PEP is free in government hospitals, people must prefer temples over hospitals not because of money issues, but because of their faith.

Eight of the respondents reported believing that PEP can be taken, but only in two situations. The first is that the person goes to the temple first and asks, *via* wheat reading by the bhuva, and obtains permission from Hadkai Mata. The general opinion is that "*if they first climb the steps of the hospital, then nothing will work here* [at the temple]," both because Hadkai Mata would be annoyed and because people would have wasted precious time. Should patients take PEP despite the goddess' negative answer, "*their case will be spoilt for sure.*" That said, the underlying idea that "*Why do we need vaccination when we have Mataji sitting* *here for us?*" is found in this group too, and is legitimized by the fact that "*here it never happened that someone came and didn't get cured*." The second circumstance in which PEP can be taken is when symptoms are so severe that Hadkai Mata could struggle to cure the patient.

Seven study participants had a neutral opinion and prefer not to discuss PEP with their patients. Some said that they cannot explicitly recommend PEP, but they are fine if their patients take it—especially the doubtful ones and those who belong to communities that are not particularly devout to Hadkai Mata. This also reflects the fact that they do not want to be blamed in the future for advising them against vaccination. That said, most of these informants personally believe that Hadkai Mata's help is sufficient.

Eight informants considered PEP necessary and claimed that they usually recommend taking it, specifically within 24 h. Even if "*Mataji says that if you have any doubt, then you take vaccination*," according to these informants "*95% of people don't need vaccination because they have faith in Mataji*."

Three informants spontaneously mentioned the number of injections that, to their knowledge, are administered for PEP; 5, 14, and 20 injections.

Management of Rabid Dogs

"Bad," rabid dogs are always described as free-roaming and mostly unowned. Unlike people and livestock, Hadkai Mata cannot cure them because "*no one would go that far for a street dog*," by trying to catch them and committing to a 5-week obligation on their behalf. Hence, rabid dogs invariably die—again—after 3.5 days from the moment they were ridden by the goddess and became rabid, or as soon as somebody throws water on them. Pet dogs could potentially be cured, but it is very unlikely for them to become rabid, because Hadkai Mata only selects dogs that can move freely and walk long distances—"*even 100 km*"—to bite the targeted person. Only two respondents said that rabid dogs can be cured, either by giving them buttermilk to drink or when they spontaneously perform pradakshina (i.e., the ritual circumambulation of an Hadkai Mata temple).

Diverging approaches to rabid dogs were found. Half of the respondents said that dogs are killed, both to keep people safe and to avoid, especially during the wedding and festival seasons, that bite victims are bound to Step 2-rules and cannot enjoy social events. The common belief that Hadkai Mata would not cure her dogs anyway, because of the lack of a human intermediary, was often provided as a justification for dog killing. In two temples, food is offered to Hadkai Mata as a self-imposed community fine when a dog is killed.

The other half of the interviewees claimed that rabid dogs cannot be killed, because the goddess is sitting on them and she cannot either be hurt or disrespected by killing her companion animals. A bhuva tells his co-villagers "*to shut their doors and* [. . .] *wait for the dog to pray at the temple* [when they finish the job of biting people assigned to them by Hadkai Mata]" and leave the village for good. In one village, food is offered to the animal as a ritual before their departure.

Opinion About Mass Dog Vaccination

All the 11 informants with whom mass dog vaccination was discussed hypothetically said they dislike this measure. The objection is that dog bites are useful to and wished by Hadkai Mata and the other goddesses that request her assistance to discipline their followers. Mass dog vaccination is considered a risky interference in Hadkai Mata's work, from which "*it is always good to stay away*," because of the power of the goddess. One bhuva made his position clear by saying that "*rabies is not a disease, it's the wish of Mataji*," so he would not support anything that goes against her will.

DISCUSSION

For many diseases, endemic countries have developed specific policies, often supported by the WHO Traditional Medicine Strategy (45), to strengthen the role traditional medicine plays in keeping populations healthy. Because of the clinical incurability of rabies, no such policy exists for this disease. Yet, work from Vietnam shows that traditional healers can be successfully engaged in rabies control programs to better connect with local communities and increase the chances that bite victims, especially in rural areas, receive precise information about PEP from a person they trust (46). Even though faith healers can be harder to involve than other traditional healers (47), encouraging examples can be found in the field of mental illness where healers and psychiatrists work as a team. For example, in central Gujarat, the Department of Health and Family Welfare has established the collaborative project "Dava & Dua" (literally, medicines and prayers) at a famous Muslim shrine (48).

In the context of devotion to Hadkai Mata, many key points of divergence—but also some of convergence—with the technical solutions of the NAPRE can be observed and, hopefully, discussed and worked upon. The participants in this study, including those who do not believe in vaccination, never expressed feelings of hostility or resentment toward biomedicine or doctors (for example, rumors about PEP and stories of disservices). However, this could be due to the lack of confidence in expressing this view to an unfamiliar research team. Nevertheless, they were proud of having—according to them—doctors among their patients, and cases referred from hospitals. Incidentally, the first time DN heard about Hadkai Mata was in a newspaper article that mentioned the presence of the goddess' portrait in a rabies clinic in Ahmedabad. Additionally, at least in minor temples, bhuva are not materially compensated for their service (even though they benefit from high social recognition) and this presumably reduces the risk of non-cooperation because these people's source of income lies elsewhere. That said, the fact that one quarter of the interviewed bhuva are opposed to PEP, and only another quarter explicitly recommend it, is worrisome and calls for dialogue and engagement with local faith healers. The success of this dialogue depends on the external interlocutors keeping in mind that Hadkai Mata controls not only rabies but, through it, social ill-being. Looking back at the extended parallel processing model presented at the beginning of this paper (according to which fear and self-efficacy are the main drivers for effective health-seeking behaviors), in this context, the factor of fear is anything but straightforward. Anti-social behaviors are feared as much as, if not more than, rabid bites, so these two issues need to be discussed and addressed as a unit.

According to the complex explanatory model of rabies that we started to explore in this study, this disease is considered supernatural and triggered by a person's misbehavior (usually in the domain of social relations) that forces Hadkai Mata to take the action that is expected from her. This is particularly evident in the belief (or at least in the profitable recommendation, from the perspective of bhuva) that if a person forgets to go back to the temple at the end of their healing process to duly thank Hadkai Mata, the will of the enraged goddess is able—alone, with no dog bite—to fatally re-infect the unruly patient. This etiological explanation is unusual, as causes of disease are usually sought, and found, *outside* the victim and their responsibility [for example, evil eye, witchcraft, immigrants, poor—(49)]. Nevertheless, those informants who provided ethological and biological explanations for dog bites and the frequent mention of germs in the wound suggest the co-presence, in this healthworld, of a vague, distant, underlying biological source of disease (i.e., the rabies virus), and a much more concrete, close, immediate cause of illness (i.e., Hadkai Mata's punishment). This double interpretation has been observed in other contexts of medical pluralism (50). Rabies as an illness, perceived as a priority for the psychological and social repercussions of misconduct, is treated at the temple. There, the patient and the healer share the same conceptualization of ill health and the same socio-cultural background and related challenges (for example, caste-based ostracization), and almost wordlessly work toward the same goal of moral refurbishment. Rabies as a disease can, meanwhile, be managed—just in case—via PEP, as most bhuva and sevak do not see vaccination and Hadkai Mata's healing powers as incompatible.

In our study, the idea that "*Hadkai Mata is for 3.5 days*" is central. The 3.5-day limit for seeking treatment leads to two observations. First, the belief that healthcare should be sought first and foremost at the temple, preferably on Tuesdays or Sundays, dangerously delays PEP (for those who choose it). In India and abroad, traditional medicine has already been found to delay vaccination (51). This is an important concern, because wound washing is rarely performed (52, 53) and timely access to PEP is already delayed for a wide array of reasons, such as, according to a study done in Baroda, Gujarat, work pressure (especially for daily laborers that do not have a fixed salary), forgetfulness, low bite severity, travel, and financial constraints (54). The fact that treatment at temples takes only 15 mins, there is minimal to no waiting time, bhuva are always available on Sundays and usually easy to source during the rest of the week, and family pressure is high makes faith healing more easily and quickly accessible than hospitals. At least in (urban) areas where anti-rabies clinics are open 24 × 7, information about the need for immediate PEP and the opening hours of clinics should be widely circulated. Importantly, other factors that undermine the access to healthcare by socially marginalized communities, such as caste-based discrimination by healthcare providers, need to be structurally addressed (55).

The second observation is that, despite the popularity of treatment at temples, the urgent need for PEP in case of a dog bite was also recognized by some respondents. This provides a solid starting point for consolidating and expanding access to vaccination. Unlike in other parts of India (56), most hospital-based studies from Gujarat found that the majority of patients sought medical care within 24 h, or 48 at the latest, of the bite (57), probably because of sufficient road connections and widespread health infrastructure. In India, government hospitals are preferred to private ones by dog bite victims (52) and in our study area, local Primary Health Centres (58) are preferred. In these facilities, it is therefore essential to ensure support for effective delivery of PEP, including the continuous availability of vaccine and RIG stock, staff expertise (for example, especially on intra-dermal vaccination and RIG administration), wound washing facilities, convenient opening hours, and, more importantly, a cost-free service. When fees are charged (43), economically marginalized communities are disproportionately affected, and this may increase the preference for traditional and faith healing. At most of the visited Hadkai Mata temples, donations are usually discretionary. Furthermore, no more than two temple visits are necessary, while at least three hospital visits are necessary for PEP, increasing travel-related costs.

Given that the onset of clinical signs and symptoms is not a central crux in the Hadkai Mata-related exploratory model of illness, awareness messages should stress the fact that PEP can *prevent* this from happening. Particular attention has to be paid to the translation, across conceptual and linguistic healthworlds, of "preventable," "treatable," and "curable." Messages should also be clear about the necessary number and prescribed site of PEP injections. Even though the use of Nerve Tissue Vaccines was discontinued in 2004, its painful and numerous injections in the abdomen still persist in the memory of adults and the elderly in many parts of India (59), and probably informed the belief that dog bite victims become pregnant with the puppies of the biting dog (60). The prospect of 10 to 14 injections during as many hospital visits (58) is evidently discouraging for impoverished bite victims, if only for its devastating impact on income and travel-related expenditure. In a survey in rural Anand district, hence in the same area of our study, one-third of the respondents believed that rabies is curable, and mentioned tetanus injections as a first-aid measure. In the city of Ahmedabad, the biggest city in Gujarat, 61% of interviewees are not aware of the fatality of rabies and 55% would not get PEP if they do not have any symptoms (61). Increasing awareness about the correct, short, and pain-free modern PEP calendar may increase compliance after the initial PEP doses, which is a problem both in India (57) and abroad (62). This information should be shared in a detailed yet easily understandable format that takes into account the different levels of literacy and biomedical knowledge in the population (for example, by clearly distinguishing life-saving PEP from tetanus vaccination, which can also be essential). A similar "awareness update" on wound washing and PEP technicalities should target also healthcare providers, whose practical, first-aid knowledge is not always adequate in endemic countries (63). As one sevak observed, "*this is the era of science*" so, as is already the case abroad (64) and in some Indian States (65), simple but practical information should be spread to all school children, as a long-term investment in their health and that of their communities.

In our study, the main point of divergence is dog vaccination. Resistance can be due to the local interpretation of rabies transmission, in which dog-to-dog transmission receives little attention, but also to the inexperience of rural residents in this matter. Dog rabies vaccination campaigns are rare in rural India, and among dog-related issues, rabies is a low-priority concern for Indians (66). A study in Anand, in our fieldwork area, found that only 6% of respondents believe that dog vaccination controls human rabies, while 76% did not consider rabies vaccination important in pet dogs (58). In rural India, veterinary consultation for dogs is low (10), arguably due to both the negligible economic value of dogs compared to livestock and also the generally low position of (free-roaming) dogs in Hinduism (67) and Islam (68). Structural drivers such as poverty and limited interest in dog health from the government veterinary sector contribute to poor dog welfare standards. We, therefore, make three recommendations. First, even though some respondents in our study mentioned dog-to-dog transmission of rabies, human and dog rabies are deeply interlinked in the Hadkai Mata-related healthworld. Hence, an internally congruent and synchronized One Health strategy is fundamental. For example, it could be emphasized that dog vaccination, while preventing a dog from becoming infected by rabies, does not alter dog behavior [as feared in South Africa—(69)], or automatically reduce dog bites (which may already be commonly experienced from healthy dogs). In the beginning, insisting on dog bite prevention could be counterproductive, because of the value assigned to biting dogs as a means to social health. Second, given that in rural India pet dogs are usually free-roaming, the message should be conveyed—in a culturally-sensitive way—that these dogs can also be rabid. Third, instead of focusing on individual dog ownership practices, it seems more sensitive and effective to work on community-based dog keeping practices and values (as demonstrated, for example, by the strong social cohesion and shared responsibility that emerge when villagers agree with not killing rabid dogs and waiting for them to leave). Considerable work will be needed to develop strategies for controlling rabies through dog vaccination [including oral vaccination approaches—(70)] that can be implemented effectively and acceptably in these communities.

Strengths and Limitations

This anthropological paper is the first on Hadkai Mata, probably the only goddess, in any current world religion, worshiped specifically in case of rabies exposure. We provided rich and from-the-ground-up data on the Hadkai Mata-related healthworld with regards to both human and dog rabies. Having all the interviews carried out by the same research team allowed for consistency in data collection. For many of the discussed topics, saturation was reached and courtesy bias seemed minimal. Nevertheless, because of the pandemic, we could not discuss our results with the study participants and collect more interviews. More importantly, we could not perform the planned contact tracing interviews starting from Hadkai Mata temples, to retrospectively chart the patients' journeys through faith-based and, probably, biomedical healthcare; document their actual actions and underlying reasons (research question 2); collect their views on dog keeping and dog vaccination; and—above all—understand the structural challenges they face as socially and economically marginalized people in accessing livelihood assets and, when exposed to rabies, healthcare (71). This contact tracing study would have provided us with quantitative information about the extent to which the bhuva's recommendations are actually followed by dog bite victims. From a qualitative point of view, as most of the dog bite victims that seek Hadkai Mata's protection are likely to belong to the same social group of most bhuva—low-income, low-caste, poorly educated—we do not expect major discrepancies in how Hadkai Mata and her management of rabies are imagined. Nevertheless, the perspective of temple goers is necessary to complete the description of the bhuva and their role in attaining physical and social health. Coding was performed only by one researcher (with a background in South Asian studies), but problematic passages were discussed with the research assistant. As this study refers to a particular social and religious context, it may have low external generalizability. However, traditional and faith healing for rabies likely exists in all endemic countries. The approach we used to explore this issue can be applied to all healthworlds, of rabies and any other disease.

CONCLUSIONS

This study approached the system of knowledge of rabies collectively built by faith healers around the goddess Hadkai Mata in rural central Gujarat through the concept of "healthworld," an important analytical tool for the field of health in its social context and a perspective toward decolonizing research and public health agendas (72). In the social and religious setting under study, dog bites are both feared and valued, demonstrating once more the multidimensionality of the human-dog relationship (59), and the possibility that, from the point of view of One Health, animals could be "more-than-animals" (for example, emissaries of deities). From a practical point of view, the sustainability of rabies control programs in contexts of medical pluralism can be enhanced with: a better understanding of interventions from the perspective of the multiple actors who are involved on the ground, rather than narrowly emphasizing the efficiency of technical solutions; consideration of the social dynamics, norms, values, needs, and inequities; work with—not only for—communities; and starting from points of convergence and enabling factors in order to transform alternative conceptions of healing from obstacles "into opportunities for understanding the worlds of health in which people live, move and have their being" (24).

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical approval was obtained from the University of Washington (STUDY00006267)—with reliance approved by the University of Glasgow—the Ashoka Trust for Research in Ecology and the Environment (IRB/CBC/0005/ATV/07/2019), and the Indian Health Ministry's Screening Committee (2019/7418). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SC, KH, DN, and TL: conceptualization and funding acquisition. DN: data curation, visualization, writing (original draft), and methodology. DN and RR: investigation. SC, KH, TL, DN, and ATV: project administration. SC, KH, and TL: supervision. SC, KH, TL, RR, and ATV: writing (review & editing). All authors read and approved the final manuscript.

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Implementing a One Health Approach to Rabies Surveillance: Lessons From Integrated Bite Case Management

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As part of the 'Zero by 30' strategy to end human deaths from dog-mediated rabies by 2030, international organizations recommend a One Health framework that includes Integrated Bite Case Management (IBCM). However, little is understood about the implementation of IBCM in practice. This study aims to understand how IBCM is conceptualized, exploring how IBCM has been operationalized in different contexts, as well as barriers and facilitators to implementation. Semi-structured interviews were conducted with seventeen practitioners and researchers with international, national, and local expertise across Africa, Asia, and the Americas. Thematic analysis was undertaken using both inductive and deductive approaches. Four main themes were identified: 1) stakeholders' and practitioners' conceptualization of IBCM and its role in rabies elimination; 2) variation in how IBCM operates across different contexts; 3) barriers and facilitators of IBCM implementation in relation to risk assessment, PEP provisioning, animal investigation, One Health collaboration, and data reporting; and 4) the impact of the COVID-19 pandemic on IBCM programs. This study highlights the diversity within experts' conceptualization of IBCM, and its operationalization. The range of perspectives revealed that there are different ways of organizing IBCM within health systems and it is not a one-size-fits-all approach. The issue of sustainability remains the greatest challenge to implementation. Contextual features of each location influenced the delivery and the potential impact of IBCM. Programs spanned from highly endemic settings with limited access to PEP charged to the patient, to low endemicity settings with a large patient load associated with free PEP policies and sensitization. In practice, IBCM was tailored to meet the demands of the local context and level of rabies control. Thus, experts' experiences did not necessarily translate across contexts, affecting perceptions about the function, motivation for, and implementation of IBCM. To design and implement future and current programs, guidance should be provided for health workers receiving patients on assessing the history and signs of rabies in the biting animal. The study findings provide insights in relation to implementation of IBCM and how it can support programs aiming to reach the Zero by 30 goal.

Keywords: dog-mediated rabies, rabies elimination, rapid diagnostic tests, mobile health, post-exposure prophylaxis, zoonosis, implementation research

INTRODUCTION

Effective rabies vaccines for humans and animals have been available for over a century, providing means to eliminate this fatal and incurable zoonotic disease (1). Through mass dog vaccinations starting in the 1920s, rabies has been eliminated from domestic dog populations in high-income countries across western Europe, North America, and parts of Asia, such as Japan and Taiwan (2, 3). Additionally, low- and middle-income countries (LMICs) in Latin America have successfully reduced dog-mediated rabies cases by over 98% through large-scale coordinated dog vaccination programs (4, 5). Despite this progress, an estimated 59,000 people still die from rabies every year, with the vast majority in resource-poor countries in Africa and Asia (2, 6).

There are several challenges for LMICs in reducing the burden of rabies within their populations. First, to control and eliminate rabies a high vaccination coverage, typically around 70% of the susceptible dog population, must be sustained for 3-7 years *via* recurrent annual campaigns (2). Yet many LMICs have not initiated routine dog vaccination (7). Furthermore, countries face the threat of reintroductions if endemic rabies circulates at their borders (8). Second, post-exposure prophylaxis (PEP)—which is needed immediately after a bite from a rabid dog to prevent the fatal onset of rabies—is often expensive for both bite victims and governments. As a result, PEP availability is frequently limited, especially in rural areas (9, 10). PEP costs can even drain the finite budget available for rabies, without impacting the incidence of rabies in dog populations that are the source of exposures (11, 12). Third, surveillance in LMICs is typically weak and does not capture accurate data on either human or animal rabies cases (13). This significant under-reporting leads to lack of awareness and understanding of the burden of rabies, which further results in limited community/stakeholder engagement and inadequate funding. Thus, the absence of robust surveillance gives rise to a cycle of underestimating the disease burden and consequently neglecting control measures, such as dog vaccination and PEP provisioning (12, 14).

To overcome these challenges, the Tripartite [World Health Organization (WHO), World Organisation for Animal Health (OIE), Food and Agriculture Organization (FAO)] along with the Global Alliance for Rabies Control (GARC) developed the 'Zero by 30' global strategic plan to end human deaths from dog-mediated rabies by 2030. Within this strategy, international organizations jointly recommend a One Health framework, recognizing the interconnections between the health of humans, animals, and their shared environment (11). To control and eliminate rabies, the strategy advocates Integrated Bite Case Management (IBCM) (15). WHO describes IBCM as an advanced surveillance method involving "investigations of suspected rabid animals and sharing information with both animal and human health investigators for appropriate risk assessments" (2). Through multisectoral collaboration and communication, this One Health approach aims to enhance surveillance by increasing detection of animal cases and human exposures, as well as to improve PEP allocation and compliance (4, 13, 16).

While the objectives, aims, and benefits of IBCM are becoming better known by international organizations and experts in the field, this approach is relatively new and has only been implemented within the last decade. Official guidelines for IBCM and risk assessments in relation to biting animals were first mentioned in WHO guidance in 2018 (2). Peer-reviewed empirical evidence about the impact or implementation of IBCM remains limited. A PubMed search using keywords "novel surveillance" OR "integrated bite case management" AND "rabies" identified only eleven studies from four countries: Chad (10, 17), Haiti (4, 14, 16, 18–20), the Philippines (12, 21), and Tanzania (13). Although several IBCM programs have been implemented within the last decade, the approaches undertaken and the lessons learned from these programs have not yet been synthesized. This study aims to understand how IBCM is conceptualized and practiced by stakeholders involved in rabies prevention and control programs and barriers and facilitators to its implementation.

MATERIALS AND METHODS

This qualitative study was conducted among experts with experience designing, implementing, and/or managing IBCM programs in different epidemiological and geographical contexts (**Table 1**). Purposive sampling of known professional networks

Abbreviations: FAO, Food and Agriculture Organization; GARC, Global Alliance for Rabies Control; IBCM, Integrated Bite Case Management; LMIC, low- and middle-income countries; OIE, World Organisation for Animal Health; PEP, post-exposure prophylaxis; RDT, rapid diagnostic test; WHO, World Health Organization.

TABLE 1 | Economic and epidemiological context of countries included in this study.

Country	Continent	Population (2020)	GDP (2020, US $/Bn)	HDI (2019)	Rabies elimination stage	Level of dog vaccination	Owned, free roaming dogs	Deaths per year	Policy on PEP (cost per course)	Bite incidence (per 100k)	Estimated HDR ***	Source
Chad	Africa	16,425,860	10.1	0.40	Endemic	Not routine	>90% >90%	550	Patient pays* (US$ 80-100)	480-570	1: 7.8 (1: 5.2-40)	(10, 22, 23, 24)
Kenya	Africa	53,771,300	98.8	0.60	Endemic	Not routine	>90% >90%	2,200	Patient pays* (US$ 85)	290	1: 4-8	(22, 25)
Madagascar	Africa	27,691,020	13.7	0.53	Endemic	Not routine	>90% >90%	1,000	Free	190	1: 8-25	(26, 27)
Malawi	Africa	19,129,955	12.0	0.48	Endemic	Not routine	>90% >90%	900	Free	230	1: 23 (1: 14-31.8)	(22, 28)
Tanzania	Africa	59,734,210	62.4	0.53	Endemic	Not routine	>90% >90%	650	Patient pays (>US$ 80)	12-120	1: 20.7 (1: 7-181.3)	(9, 22, 29)
Brazil	Americas	212,559,410	1,445.0	0.77	Elimination*	Routine*	>90% <50%	<10	Free	230-280	1: 4.2-7	(30, 31)
Guatemala	Americas	16,858,330	77.6	0.66	Endemic	Routine	>80% >70%	0-8	Free	150-280	1: 6.4 (1: 1-10)	(30, 33, 34)
Haiti	Americas	11,402,530	13.4	0.51	Endemic	Routine	>90% >50%	350	Free	200	1: 5.2	(22, 35)
Peru	Americas	32,971,850	202.0	0.78	Emerging*	Routine	>80% >40%	0-10	Free	200-600	1: 3.8	(30, 36, 37)
India	Asia	1,380,004,390	2,623.0	0.65	Endemic *Goa State - elimination	Highly variable	>60% >40%	>15,000 *Goa State: 0 since 2018	Free	1,300	1: 11-36	(22, 38, 39)
Indonesia	Asia	273,523,620	1,058.0	0.72	Endemic*	Not routine	>90% >70%	3,300	Free	200	1: 8.3-360	(22, 41, 42)
Philippines	Asia	109,581,090	361.5	0.72	Endemic	Routine but variable	>80% >50%	200-300	Free	1,100	1: 4-10	(43, 44, 45)
Vietnam	Asia	97,338,580	271.2	0.70	Endemic	Routine but variable	>80% >50%	500	Patient pays (US$ 150)	400	1: 10-38	(22, 46, 47, 48)

*GDP, Gross Domestic Product, HDI, Human Development Index, PEP, post-exposure prophylaxis, HDR, Human to Dog Ratio. Population and GDP data from the World Bank OECD National Accounts data files, 2020 (https://data.worldbank.org). HDI data from the United Nations Development Programme 2020 Human Development Index Ranking (https://hdr.undp.org). Rabies elimination stage, level of rabies control, and policy on PEP were all reported from interviews. Deaths per year, annual bite patient incidence, and estimated human:dog ratios were from the literature. *Brazil is close to elimination, but rabies has continuously circulated in the state of Maranhão (47). *Peru is close to elimination, but rabies has continuously circulated in the border state of Puno and re-emerged in the city of Arequipa in 2015 (36). Canine rabies is endemic in India, but the State of Goa is now close to elimination and has not had a human rabies death since 2018 (47). Indonesia has endemic dog rabies in 26 provinces, while 8 provinces are rabies-free (39). *Dog vaccination is routine in 24/27 Brazilian states, but has been discontinued in 3 southern states: Parana´, Santa Catarina and Rio Grande do Sul. There is considerable variability in the degree of routine dog vaccination reported in Asian countries. *Patients pay for PEP in Chad, but PEP was provided for free during the IBCM project (2016-2018). *Patients pay for PEP in most of Kenya, but PEP is free in a few counties (e.g., Makueni). Bite patient incidence (presentations to health facilities) is reported rather than cross-sectional surveys which typically are much higher. ***Variability in HDRs relates to culture (with major differences between religions).*

was used to identify and recruit experts. All participants were contacted by email and provided with a participant information sheet. Participants provided consent prior to interviews, which were all conducted in English.

Seventeen participants were interviewed: five international-level, six national-level, and six local-level experts. The majority of participants had a degree in veterinary medicine (twelve); a doctoral research degree (nine); or both (five); and one had a MSc in medical statistics. All participants had some educational/experiential background in epidemiology. Their work experience ranged from academic, government, non-profit, and international organization, with most having experience with more than one. Fourteen IBCM programs were included in the study representing thirteen countries in the Americas, Africa, and Asia (**Table 2**). All IBCM programs were/are being implemented in countries with endemic dog rabies, with the exception of Rio Grande Do Sul, a state in Brazil which has not reported a dog or human case since the 1980s.

Semi-structured one-to-one interviews (40-65 minutes) were conducted between January 2020 and August 2021. One interview was conducted face-to-face before COVID-19 restrictions and the other sixteen were conducted over the videoconferencing platform, Zoom (49). Interview topic guides were generated for each level of expertise: international, national, and local. The questions were designed to be open-ended and encourage experts to share their experiences and elaborate on the issues they felt to be important.

Interviews were audio-recorded, transcribed verbatim, pseudonymized, then uploaded into NVivo 12 Pro software (50).

Data analysis was conducted by the first author and supervised by the last author, an experienced qualitative researcher. The data was analyzed using a six-step thematic analysis (51). All transcripts were read for familiarization to develop initial codes. An inductive approach was used to develop descriptive codes identified from similar patterns, topics, and elements of the intervention, which were then collated into themes, categories, and subcategories. Transcripts were also coded deductively using assumptions underlying a logic model of IBCM, depicting the relationship between program activities and the intended impact of IBCM. Themes were developed and reviewed iteratively and checked for consistency and appropriateness, amending where necessary. Themes included: inputs, activities, outputs, outcomes, and aims. Transcripts were compared for differences and similarities in how IBCM was operationalized, barriers and facilitators encountered during implementation, and the desired outcomes and aims of each program. Interviewees were sent a copy of the manuscript to validate accurate representation of their IBCM program and their feedback was incorporated.

RESULTS

Conceptualization of IBCM

Description of IBCM

Several experts first heard the term 'Integrated Bite Case Management' in relation to rabies control in Bali, Indonesia in 2011 (52). However, most learned of IBCM from a program in Haiti (14) or through their own experience. All programs used the term IBCM, except for three where their intervention was referred to as either 'a One Health approach to bite management' (Chad) (10); 'clinic-based surveillance' (Madagascar) (27); or a 'One Health approach' to guide PEP recommendations (Brazil) (31).

Experts described IBCM in a way that combined its key components (activities) with the role it plays (outputs/outcomes):

> "IBCM at its simplest is the ability to provide a proper risk assessment, usually in the context of the exposing animal, in a way in which the outcomes of the risk assessment can impact the human treatment decision." (Expert #1, International-level)

These reported components (activities) were mostly consistent and aligned with the WHO definition of an IBCM program. They included: 1) reporting a bite or exposure event, 2) performing a risk assessment, 3) triggering an investigation for any bite deemed high-risk, 4) conducting an animal investigation, 5) observing animal for 10-14 days (to confirm a healthy animal) or collecting samples and diagnostic testing (from dead/euthanized animals), and 6) sharing feedback and investigation results across sectors (**Figure 1**).

Although a consensus emerged about the required components of IBCM, there was still some uncertainty about the definition. Specifically, experts had varied opinions about whether IBCM must always be initiated by a bite event, or determine treatment decisions or be used to specifically enhance surveillance. Therefore, while most interviewees perceived their work as being IBCM or similar to IBCM, some did not consider their program to formally be IBCM (for example, in Southern Brazil where the objective was not to strengthen surveillance, but to better manage PEP).

Participant's concept of IBCM evolved over time and with experience. Most international-level experts viewed this approach as "passive public health surveillance" initiated by any suspect rabid animal – not only from bites:

> "I used to think it was integrated BITE case management. Whereas, my attitude now is it's the full investigation of a suspect animal, whether it's for a bite or just a dog in the community behaving strangely." (Expert #3, International-level)

Purpose of IBCM

Participants consistently identified several key roles of IBCM (outlined in the outcomes section of our conceptualized logic model, **Figure 2**). These roles were emphasized differently for each program and not all roles were relevant for every program. These roles were to: a) enhance surveillance through improved case detection, thereby enabling evaluation of control and prevention measures; b) directly and formally connect the health sector to the veterinary sector; c) inform PEP administration, aiming to improve patient care and increase adherence; d) better manage limited resources through judicious use of PEP; and e) advocate for community and stakeholder support and funding for rabies programs, and guide their implementation.

Experts agreed that countries with endemic dog rabies should all have a surveillance program, but opinions differed on when IBCM should be incorporated. A few experts viewed IBCM as an advanced surveillance strategy specifically meant for countries with a well-established control program who were close to elimination. Others argued that IBCM is fundamental and required at all stages (e.g., endemic, emerging, elimination and post-elimination) within routine surveillance:

> "IBCM is needed as a country scales up its rabies elimination efforts and as an early intervention to try to bring down human deaths. It's needed in countries where 1) you have a lot of human deaths and you need to do a better job getting PEP to the people at risk and 2) as you really start to take elimination seriously, it's needed as a foundational system for evaluating the efforts that are going into vaccinating dogs. Then it's important in the end-game, post-elimination phase to continue to evaluate the risk to people bitten." (Expert #3, International-level)

TABLE 2 | Comparison of operationalized IBCM programs.

Country	Region of IBCM program	IBCM start and end dates	IBCM funding source	Mobile application used	Hired team vs local govt staff	Where bite events are reported	Who does risk assessment	How investigation is triggered	Who does animal investigation	Rapid Diagnostic Tests used
Chad	4 regions*	2016-2018	Research	✗ No app	Local govt + hired hotline staff	Hotline	Govt health workers + hired hotline staff	Hotline staff call investigator	Govt animal health staff	✓ Yes
Kenya	Makueni county	To start 2021/ 2022	Research	✓ App	Local govt + hired field/ hotline staff	Health facilities + hotline	Govt health workers + hired field / hotline staff	App notification &/ call/SMS to investigator or same person	Govt animal health staff + hired field staff	✓ Yes
Madagascar	Moramanga District	2016-2019	Research	✓ App	Local govt + 1 hired field staff	Health facilities	1 hired field staff at 1 clinic*	Call/SMS to investigator	Govt animal health staff + Govt health worker	✗ No
Malawi	Districts of Blantyre, Zomba and Chiradzulu	2018 - present	Research, Donor	✓ App	Local govt + hired field/ hotline staff	Hotline + health facilities	Govt health workers + hired field/ hotline staff	Call/SMS to investigator	Govt animal health staff + hired field staff	✓ Yes
Tanzania	20 districts in 4 regions*	2018 - present	Research	✓ App	Local govt + 2 hired field staff	Health facilities	Govt health workers	Call/SMS or group chat message to investigator	Govt animal health staff	✓ Yes
Brazil	State of Rio Grande Do Sul*	2015 - present	Govt	✗ No app	Local govt	Health facilities	Govt health workers	Call investigator	Govt animal health staff	✗ No
Guatemala* *TBC*	TBD	Pilots started in 2020	Donor, Govt	✓ App	Local govt + hired field staff	Health facilities + community outreach*	Govt health workers	App notification &/ call/SMS to investigator	Hired field staff or local vet students*	✗ No
Haiti	7 of 10 departments	2013 - present	Donor, Govt	✓ App	~15 hired field staff	Health facilities + community outreach*	Hired field staff	Same person assesses risk then investigates	Hired field staff	✗ No
Peru* TBC	Regions of Arequipa and Puno	Pilots started in 2019	Donor, Govt	✓ App	Local govt + >20 hired field staff	Health facilities + community outreach*	Govt health workers	App notification &/ call/SMS to investigator	Hired field staff	✗ No
India	State of Goa	2018 -present	Donor, Govt	✓ App	~20 hired hotline & field staff	Hotline + health facilities	Hired hotline staff	Hotline staff notify field staff	Hired field staff	✓ Yes
Indonesia	Province of Bali	2011 - present	Donor, Govt	✗ No app	Local govt	Health facilities	Govt health workers	Call/SMS to investigator	Govt animal health staff	✗ No
Philippines	Province of Albay	2017-2021	Research	✓ App	Local govt + 3 hired field staff	Health facilities	1 hired field staff per clinic	Call/SMS to investigator	Govt animal health staff	✗ No
Philippines	Provinces of Romblon & Oriental Mindoro	2019 - present	Govt, Research	✓ App	Local govt	Health facilities	Govt health workers	Call/SMS or group chat message to investigator	Govt animal health staff	✓ Yes
Vietnam	5 provinces*	2019 - present	Donor, Govt	✓ App	Local govt	Health facilities	Govt health workers	App notification &/ group chat message to investigator	Govt animal health staff	✗ No

**IBCM programs in Guatemala and Peru have started training and pilot studies, but implementation was delayed due to COVID-19. IBCM is not being used yet and both countries are still relying on passive surveillance to find rabies cases. *Chad's IBCM program was implemented in 4 administrative regions: Logone Occidentale, Ouaddaï, Hadjer Lamis, and Chari Baguirmi (21). Tanzania's IBCM program is implemented in 4 regions: Mtwara, Mara, Lindi, and Morogoro (13). Brazil does not have an official IBCM program, but similar protocols are implemented in 3 states in the South Region: Paraná, Santa Catarina and Rio Grande do Sul. Vietnam's IBCM program is currently implemented in 5 provinces in Central and Northern Vietnam: Phú Thọ, Bà Rịa-Vũng Tàu, Nghệ An, Lạng Sơn and Đắk Lắk and will be expanded to a further 4 provinces in 2022, which includes Southern Vietnam. Information collected through interview data.*

Operationalization of IBCM

There was considerable variation in how IBCM was operationalized across settings, which were diverse in terms of their economic and epidemiological contexts (**Table 1**).

The most important prerequisites considered necessary for IBCM were the identification of a designated person/team responsible for investigating animals; and health facilities (hospitals, clinics, etc.) where bites are reported and PEP is

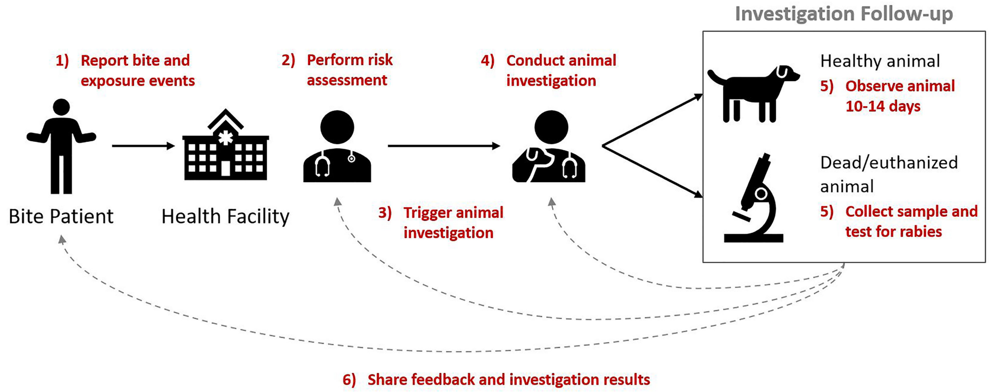

FIGURE 1 | Key components of IBCM. Annotated in red are the six components (activities) that comprise the IBCM approach with arrows and numbering indicating the sequential order of these components.

administered. Several experts further mentioned the importance of stakeholder and community engagement prior to implementation.

The key input that differed between programs was who was identified and trained to carry out activities (**Table 2**). Three categories of workforce were identified: fully hired, a combination of hired and local government, and fully local government. Other inputs that varied were the use of rapid diagnostic tests (RDTs) and mobile applications for reporting/ data management.

Program activities were similar amongst each workforce category. IBCM programs with a fully hired workforce—meaning their primary job responsibility was rabies/IBCM and their salary was paid by external funding—relied on the same team or person to conduct most or all of the IBCM components:

> "We have trained surveillance agents … They all have the app on a tablet and go to the sentinel hospitals in their area weekly to check for bites … also through word of mouth. Then they go out and investigate the dog bites and report them." (Expert #10, National-level)

In contrast, programs with a fully local government workforce trained existing capacity to complete IBCM activities. This involved health workers (nurses, doctors, etc.) conducting risk assessments and alerting their animal health counterparts (animal health workers, veterinarians, etc.) to investigate biting animals. Programs with a combined workforce trained a hired team and used local government staff—sometimes volunteer medical/veterinary students—to conduct risk assessments or animal investigations.

Almost all IBCM programs used paper-based or electronic registers from health facilities to collect bite data. These were typically from district and regional-level hospitals supplying PEP, but in some countries from rural community-level clinics with PEP access (e.g., Philippines). In addition to registers from health facilities, some programs used hotlines and/or trained local community health workers to report bite events. This was done particularly to enhance surveillance in rural areas with low PEP-seeking behaviors or limited access to PEP.

The mechanism to trigger animal investigations varied from calling/messaging the investigators, using hotline staff to notify them, and/or group chats or submitting data into mobile apps that send notifications to investigators.

Barriers and Facilitators to IBCM Implementation

Reported barriers and facilitators could be placed under five main categories: risk assessment; PEP provisioning; animal investigation; the use of IBCM to facilitate One Health collaboration; and data reporting and mobile technology (**Table 3**).

Risk Assessment

IBCM programs with a hired workforce designated to perform risk assessments generally experienced fewer challenges than those with a local government workforce. Health workers were often stretched by busy workloads and other responsibilities/ priorities. Some commonly reported barriers were: high volume of patients; added workload without compensation; high staff turnover; feeling that IBCM is not their responsibility or lacking interest; frustrations from work duplication (already have a reporting system); no accountability or lack of supervisory support; and reluctance to change/adopt a new way of working. Hiring staff addressed many of these barriers since rabies was their primary responsibility. Though this usually required additional funding from research grants or donors, challenging sustainability.

Some programs aimed to use risk assessments for more judicious use of PEP, typically in locations with frequent shortages or high expenditure on rabies biologics. These programs often experienced the challenge of ensuring health workers could perform risk assessments to a sufficiently reliable and effective level for PEP to be withheld or discontinued. While some health workers were proficient using WHO protocols to assess wound severity (Category I, II, and III), others required

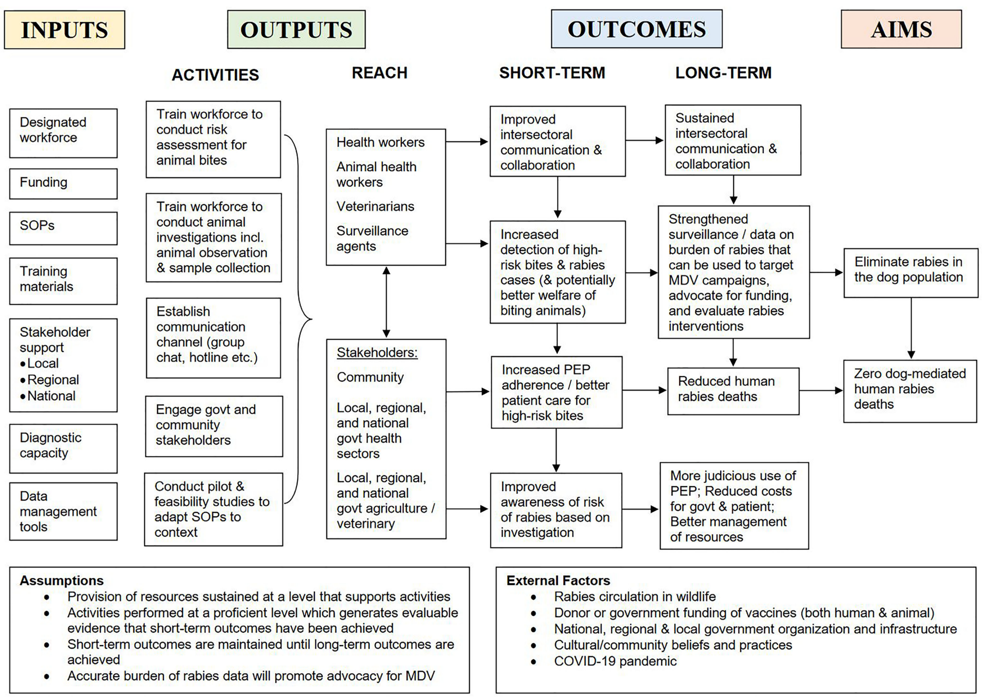

FIGURE 2 | Logic model of IBCM. Representation of the relationship between resources (inputs), activities, outputs, short- and long-term outcomes, and aims of an IBCM program.

multiple training sessions. The majority were not familiar with the clinical signs of rabies in animals or the importance of assessing risk *via* the status of the biting animal:

> "The main issue is that it's been difficult for the nurses to perform high-risk assessments … a lot of the cases they post in the peer support chat are not high-risk based on our definition. And when you look at the protocols of the Department of Health, they actually have a lot more criteria on what is considered high-risk, which concentrates on the nature of the wound rather than the animal status." (Expert #15, Local-level)

Other significant challenges regarding judicious use of PEP included there being no legal basis for determining PEP decisions from risk assessments; and health workers' hesitancy to withhold or discontinue PEP, even where there was no apparent risk. Hard copy protocols, routine training and communication, and use of apps that automatically assign the animal case definition, supported health workers' ability to more accurately determine low-risk vs high-risk bites and make treatment decisions.

PEP Provisioning

Most experts expressed that the level of PEP provisioning influenced health-seeking behavior, adherence, and the number of bite patients that present to health facilities. Free PEP policies reportedly increased accessibility and adherence to vaccine regimens. However, the provision of free PEP could also lead to much higher patient throughput and sometimes an excessive workload for those performing risk assessments. Furthermore, demand for PEP frequently remained high even when the risk of rabies was very low or even zero.

Typically, health-seeking and PEP adherence is much lower where patients pay for PEP or where travel costs are high due to limited PEP accessibility and availability. These settings often have a lower throughput of patients, with a larger proportion of those presenting as very high risk, as patients make their own assessment of risk. A few experts said that despite limited access to PEP being an issue, IBCM was often easier to implement in these settings:

> "Of the people presenting for post-exposure prophylaxis, we're probably looking at about half of them being bitten by dogs that we would consider to be high-risk, and likely to be rabid. We don't see a lot of patients, but the patients that we see have a high chance of being really at risk for rabies." (Expert #5, International-level)

Experts reported that the most-at-risk regions for rabies were rural areas where people had poor access to health services and may seek out traditional healers after being bitten. This also may affect the performance of health facilities supplying PEP as sentinels for high-risk bites. Some experts stated that improving PEP access could use up substantive rabies funding

TABLE 3 | Barriers and facilitators to implementation of IBCM programs.

	Risk Assessment	PEP Provisioning	Animal Investigation	One Health collaboration	Data reporting/mobile technology
Barriers	**Govt health workers:** Work Capacity • Busy workload • High patient volume • Many responsibilities • Other priorities • Work duplication Human & Financial Resources • High staff turnover • Not compensated for additional IBCM work Compliance with guidelines • Felt rabies not their job • Reluctance to change or adopt new routine Performing risk assessment • Limited knowledge of rabies incl. WHO Categories & signs of rabies in animal • Focus on wound severity not rabies signs in biting animal Judicious use of PEP • Hesitancy to discontinue or withhold PEP • No legal basis for risk assessment to inform PEP	**PEP accessibility:** Costs • High out-of-pocket costs of PEP • High travel costs to closest clinic w/ PEP Access • Most vulnerable populations don't have access to health services **PEP availability:** • Frequent stockouts • Not available in many rural/ remote areas **Excessive PEP use:** • Free PEP policy can lead to excessive PEP-seeking behavior • Indiscriminate PEP use can cause excessive costs/ stockouts • Same or more demand for PEP even when low or zero risk of rabies **Local beliefs:** • Seeking care at traditional healers	**Govt animal health workers:** Compliance with guidelines • Investigations late or not conducted • No follow-up • Reluctance to change or adopt new routine • Felt rabies not their job • Other prioritizes • Not motivated to investigate until death Human & Financial Resources • Lack of personnel • High staff turnover • Not compensated for time or travel costs Training l • Lack of formal trainingl • Not comfortable handling animals **Conducting animal investigation:** • Cannot find or identify biting animall • Not able to get sample **Diagnostics:** • Few diagnostic lab(s)l • Must ship samples far without cold chain	**National-level:** Governance structuresl • Govt ministry not structured for One Healthl • Lack of ministry cooperationl • Unbalanced sector power Policy l • Rabies neglected/not priority • Lack of funding • Difficult integrating IBCM into national policy **Regional / Local-level:** Compliance with guidelines • Health sector not notifying about bites • Investigation results not considered for PEP decision Stakeholder Engagement • Lack of local ownership • No prior intersectoral communication • Difficult to change routine behavior • Difficult to establish and maintain communication Required Skills • Limited experience with data management	**Data reporting:** • Lack of data submission by both sectors • Feedback not provided to other sectors • Investigation case not formally closed **Mobile technology:** App issues • App development timely and many iterations • App not feasible in all settings – e.g., high illiteracy/no written language • Limited network coverage • App must be updated and re-downloaded User Issues • Lack access to smartphones/tablets • Reluctance to use app • Required additional proficiency training
Facilitators	**Workforce:** • Hiring staff to perform risk assessments • Consistent feedback and communication **Training & Materials:** • Apps that automatically assign case definition • >Provision of hard copy protocols • Routine training	**Free PEP policy:** • Increased accessibility • Increased PEP-seeking • Increased PEP adherence **Community Outreach:** • Educating local traditional healers to report bites	**Workforce:** • Hiring staff to conduct animal investigations **Training & Materials:** • Hook and straw sampling techniques • Rapid diagnostic tests • Safely handling animals **Communication:** • Prepare stakeholders for swift rise in case detection	**Communication:** • Consistent feedback with all involved sectors • Establishing hotline to facilitate One Health link **Collaboration:** • Involving all sectors in discussions/decisions • Using existing One Health programs and networks • Employing veterinary staff within public health system	**Communication:** • Consistent feedback/ reminders to report **Mobile technology:** • Real-time data monitoring • Remote data access • Quick way to establish a surveillance system • Overcome language barriers

with diminishing returns. Alternatively, they felt that investment in mass dog vaccination, could decrease the incidence of rabies in source populations.

Animal Investigation

Barriers to conducting animal investigations using a government workforce were similar to those reported for risk assessments. Oftentimes, when an investigation was triggered, they were not conducted, were conducted too late, or there was no follow-up after the 10-14 day observation period. Factors included: required time/travel/resources (fuel, transport etc.); lack of personnel; high staff turnover; prioritizing diseases considered more economically important in livestock and poultry (e.g., foot-and-mouth disease, avian influenza, lumpy skin disease); lacking formal training as a veterinarian; and/or not feeling comfortable handling animals. Commonly, the person designated to investigate the animal had a background and responsibilities unrelated to animal rabies control and did not feel rabies was their job:

> "The problem is that these inspectors can be veterinarians, biologists, environmental engineers or what they call 'technicians' without formal training … When they're biologists, they spend most of their time doing vector surveillance … If they're environmental scientists and engineers, they're more interested in water or restaurant inspection … When there is a veterinarian, usually there is more focus on rabies." (Expert #9, National-level)

Hiring program staff resolved many of these issues, but was not always an option due to funding constraints. Hired investigators also reportedly felt more confident handling animals and collecting samples, although hands-on training improved these skills for government investigators. Both hired and government staff experienced similar challenges during investigations, including not being able to find or identify the biting animal or collect a sample because the animal was already killed, buried, or decomposing on investigation.

A few countries had only one diagnostic laboratory with limited capacity in terms of equipment, staff, and quality control. Samples sometimes had to be shipped long distances, without cold chain or costs covered, often limiting sample submission to nearby areas. IBCM programs implemented in locations with established diagnostic capacity had a significant advantage. Furthermore, programs that trained field staff to use hook and straw sampling techniques simplified procedures and facilitated the collection, storage, and shipping of animal samples.

Some experts stated that rapid diagnostic tests (RDTs) are not reliable and pose complications to protocols for treatment decisions in the case of false negative results. Moreover, RDTs are not yet recommended by WHO and OIE, thus there is no guidance available for practitioners. Other experts found RDTs to be a facilitator for implementing IBCM, encouraging investigators to collect samples by providing immediate results to report to the health sector and communities:

> "The vets applied rapid test kits, which are not validated yet, but it was very good to give the vets something at hand to empower them. The veterinary system is usually less financed than the human health services. If you want to apply 'One Health' you should empower them to bring them closer to the human health services. Otherwise, it's a mismatch between roles … This test balanced it out a bit by giving the vets something to motivate the human health services to communicate with them." (Expert #7, National-level)

Protocols usually stipulated that all samples tested with RDTs should be confirmed with laboratory diagnostics and that PEP decisions should not depend on RDT results. Experts stated that RDT results generally matched laboratory results.

Experts noted that motivation to investigate commonly increased only after a human death or several animal cases in their area. Moreover, IBCM typically increased detection resulting in a swift rise in cases, which could be a disincentive, especially when approaching elimination. Sometimes animal investigators would be blamed for rising cases, which discouraged their future reporting. Experts articulated the importance of preparing leadership to expect increased case numbers on introduction of IBCM and explain that this provides better guidance for control.

The Use of IBCM to Facilitate One Health Collaborations

Barriers to collaboration between sectors were found at the national, regional, and local level. Government ministries were rarely structured to facilitate intersectoral collaboration and faced challenges getting sectors to work together. Typically, sectors had unbalanced power, with the human health sector having more resources in terms of funding and influence. The priority placed on national rabies programs varied between countries, and was often neglected by both sectors. Using pre-existing One Health programs as a resource for IBCM activities was reported as a significant advantage. Also, programs that involved all relevant sectors in joint discussions, decisions, and training experienced more success:

> "You need to have the buy-in of both the health and veterinary sectors, from the national- to the local-level. Because if they don't think it's important then we cannot force them to implement IBCM. They have to have a better understanding of why it is important so that it translates to actual work. If the National Program doesn't believe in it, then it is pointless to push it further. But if they recommend IBCM as a policy, then it should go down the line … from national, regional, to local government." (Expert #2, International-level)

Experts talked about the difficulty of creating local ownership and changing routine behaviors for information sharing at the individual level up to the ministerial level. There were barriers to getting the health sector to report high-risk bites to their animal health counterparts and to consider investigation results when making treatment decisions. Establishing and maintaining a line of communication between sectors was challenging in many settings. In a few instances, local government staff had limited data management skills which made it hard to link human bite cases to animal investigations.

To overcome these challenges, experts emphasized the importance of providing regular feedback and establishing a rapport between human and animal health workers, IBCM staff, and other stakeholders. Additionally, experts reported the need to consider local context and adapt protocols accordingly. Innovations, such as using hotlines as the link between sectors and the community, or developing different protocols for urban and rural settings, encouraged participation. Lastly, a few countries facilitated One Health collaboration by employing veterinary staff within the public health system (e.g., Brazil) - a strategy used in many high-income countries (e.g., United States).

Data Reporting and Mobile Technology

Experts reported a common barrier was lack of data submission from all involved sectors. Many times, the protocols for the risk assessment and all steps of the investigation (e.g., quarantine, follow-up, sample collection) were completed, but results were not reported, nor was feedback provided to other sectors.

The use of mobile applications for data reporting and management created both challenges and opportunities. App development initially took a great deal of time and many iterations. Yet once finalized, apps enabled IBCM protocols to be standardized by providing a template for questions and procedures for the risk assessment and animal investigation. Experts said the app allowed real-time data to be accessed remotely by program managers. This facilitated rapid identification and response to high-risk cases, as well as the timely provisioning of information to stakeholders and communities:

> "The apps allow the technical expertise to sit anywhere in the world and real-time monitor cases, [human] vaccinations, dog vaccination programs … In the past it would take 2-3 years to get all of this paper data, enter it, and start to learn anything from your system. This ability to real-time evaluate and monitor what's going on and make adjustments is invaluable." (Expert #1, International-level)

While programs using mobile apps experienced issues with network coverage and internet access, these barriers were overcome with a feature allowing data to be saved. Not all program staff had access to smartphones where the app could be downloaded, which required some programs to purchase tablets or work phones. In addition, experts mentioned there was reluctance from some staff to use the app. Older staff in particular experienced difficulties using this technology and often required additional proficiency training. Changes in technology meant apps frequently needed to be re-downloaded/updated or risked becoming obsolete.

Most experts felt apps were a solution to many issues, while others saw them as an added complexity to training and implementation. In many settings, mobile apps helped overcome language barriers and could be tailored to local contexts (language, geo-hierarchy etc.). Yet one expert said using a mobile app would be difficult in their setting due to high illiteracy in official and local languages.

Impact of COVID-19

IBCM programs encountered COVID-19-related obstacles on several levels. Some program start dates were postponed, and most training for ongoing programs were postponed or canceled in 2020 and 2021. Moreover, allocated time and resources (personnel, diagnostics, vaccine storage, surveillance efforts, supply chains, funding) were re-focused on COVID-19:

> "I think the main impact that COVID-19 is going to have on rabies in [country] is that it's going to hide the problem. Because the surveillance system stops - it's now focused on COVID … most of the resources for the lab go to COVID. And let's say on a regular basis rabies is really neglected - now it's going to be even more neglected." (Expert #11, National-level)

Increased pressure on the health sector meant many health workers were extremely busy and did not report high-risk bites as frequently. Local travel restrictions limited in-person animal investigations or prevented them entirely. Experts in some countries observed drops in animals tested due to decreased surveillance efforts. Several IBCM programs experienced declines in bite patient presentations, typically for 2-5 months at the start of 2020 lockdowns. Experts similarly described the cancellation or disruption of dog vaccination campaigns in 2020 and/or 2021 resulting in lower vaccination coverages. Some areas are already seeing rising human and dog cases, further necessitating the need for IBCM.

Lastly, the pandemic affected peoples' livelihoods and caused income losses, making healthcare less affordable. Experts speculated that people are not feeding community dogs as frequently, leading them to roam farther for food and creating a more favorable environment for rabies transmission. People were reported to have also been abandoning pets due to costs and fear of COVID-19 transmission, potentially increasing stray dog numbers. In order to accurately measure the impact of the pandemic, enhanced surveillance such as IBCM is needed.

DISCUSSION

The main finding from this study was the variation between IBCM programs reported across epidemiological and geographical settings. Specifically, the interviews highlighted the diversity within experts' conceptualization of the definition and roles of IBCM. This range of perspectives demonstrates that there are different ways of organizing IBCM within health systems and that it is not a one-size-fits-all approach. While there was consensus among experts and the wider literature about the required components (inputs and activities) of IBCM (**Figure 1**) which aligned with WHO guidance (2), these components were operationalized differently between programs. Moreover, experts' perspective of the purpose of IBCM often differed and by implication, so did the desired outcomes of each program. In practice, IBCM was tailored to meet the demands of the local context and level of rabies control in place. Experts were well versed about how IBCM operated in their settings and what outcomes they wished to achieve by implementing this One Health approach. But experiences did not necessarily translate across contexts, affecting perceptions about the function, motivation for, and implementation of IBCM. Nonetheless, despite differences in operationalization and desired outcomes, programs shared many similar experiences with the challenges they faced and progress in overcoming them.

Contextual features of each location—which can be described broadly as epidemiological or non-epidemiological (53)—contributed to differences in desired outcomes and barriers and facilitators to implementation. The epidemiological context (e.g., human deaths, incidence in dog population) partially influenced how much rabies was prioritized at the national and local level. However, most variation in terms of the success of IBCM and the impact achieved was due to the non-epidemiological context. This includes features such as: social and economic (e.g., health-seeking behaviors, GDP, HDI); cultural (beliefs, attitudes, and practices among policymakers, practitioners, communities); geographical (e.g., urban vs. rural); service and organization (e.g., motivation, willingness to change); policy (PEP provisioning); financial (e.g., funding); political (e.g., level of decentralization, distribution of power among sectors/stakeholders); and historical (e.g., presence of rabies). The issue of sustainability was at the core of many barriers to implementation, which the literature acknowledges as a major hindrance for other evidence-based interventions (54). Using an existing government workforce for program activities was typically seen as more sustainable. Yet, experts found it difficult to incentivize or motivate human and animal health workers to change their way of working and complete extra work without supplemental pay. The overall success of programs appeared to be influenced by practitioners' and stakeholders' viewpoint of the added value of IBCM relative to the extra time and effort required from them and ultimately the degree to which funding was allocated.

Most programs operated in settings with endemic dog rabies, weak or nonexistent surveillance, and scarce funding. Thus, the primary desired outcome was to establish a cost-effective surveillance system to rapidly identify high-risk human exposures and potential rabid animals. One exception was the IBCM-like approach used in three southern states in Brazil, where dog rabies has been eliminated and strong surveillance already exists. Instead of surveillance, their aim was to reduce indiscriminate use of PEP following a shortage. Judicious use of PEP was considered a pivotal role for some IBCM programs (e.g., Bali, Haiti, Philippines), while being of minimal importance to others (e.g., Goa, India). In general, countries where frequent PEP shortages occur and/or governments pay for PEP placed more emphasis on its judicious use. Though, that was not always the case. Both India and the Philippines have high numbers of patients receiving government supplied PEP for healthy animal bites. Yet, in India, where they affordably manufacture human rabies biologics, there was no aim to reduce unnecessary PEP. Furthermore, certain country contexts made implementing IBCM challenging. For example, in India where unowned and fairly homogeneous-looking dogs limited the ability to trace animals. Program success was also hindered in extremely resource-poor countries (e.g., Chad, Madagascar) due to inadequate infrastructure and more pressing priorities. Alternatively, some contexts made implementing IBCM more straightforward, such as countries in Latin America (e.g., Brazil, Peru) with substantial budgets for mass dog vaccination, and a strong history of One Health in action facilitating successful rabies control.

This study underscores the complexity of IBCM which stems from the interaction of its many components as they relate to the context or system where they are implemented (55). These findings demonstrate the importance of transferring the evidence base of the IBCM approach to inform adaptations when implemented in a new context to ensure effectiveness (56). Tools such as the ADAPT guidance provide a framework and checklist, facilitating the streamlining of these processes and reducing research waste (57). To prevent misunderstanding of the concept of IBCM, future guidance should include an explicit program theory (58), articulating how IBCM is expected to contribute to a chain of intermediate results and ultimately to expected outcomes (via a Theory of Change, similar to **Figure 2**). This has the potential to illustrate to stakeholders how implementing IBCM can be useful in their own context and might help overcome challenges specific to their setting. Furthermore, guidance should be expanded to include clear examples of how IBCM has been operationalized in various settings and how activities, outcomes, and aims might differ accordingly. As well as, guidance should be provided for practitioners on the use of RDTs, and for health workers receiving patients to cover assessment of the history and signs of rabies in biting animals, which is not integrated into WHO guidance on post-exposure prophylaxis. Lastly, IBCM programs should use standardized WHO tools and practices (2)—tailored to local understandings—for interpreting rabies risk to improve data quality and comparability of the burden of rabies and transmission pathways between settings.

As a relatively new approach, IBCM exemplifies the challenges faced when implementing One Health (59), with lessons that could be applied to other complex zoonotic diseases. Integrated One Health approaches are vital for tackling both endemic and emerging zoonoses, and for antimicrobial resistance (60, 61). Strengthening surveillance systems in LMICs for endemic diseases, such as rabies, builds foundations to address emerging zoonotic diseases like COVID-19 (62). Formalizing One Health approaches through intersectoral government bodies, including joint budgets and policies, can help to overcome institutionalized/structural barriers (61, 62). Yet, like IBCM, varying perceptions about the concept of 'One Health' make it difficult to standardize the operationalization of these approaches in both high-income countries and LMICs (63, 64). These challenges are amplified by the lack of sustainability of funding and infrastructure that are exacerbated in LMICs. This study emphasizes the need for more implementation research to improve and understand IBCM program delivery and policies (65). In recent years, such studies have helped strengthen the gap between knowledge and real-world action for a variety of neglected tropical diseases (65, 66). Future research exploring the knowledge-practice gaps of implementing IBCM could improve the cost-effective roll out of IBCM and provide a potential example for other One Health interventions.

There are some limitations in this study. One-hour interviews provided limited time to discuss perspectives of and experiences with IBCM. For broader comprehension about the implementation and adaptation of IBCM, more in-depth qualitative research is required (e.g., ethnographic participant observations, development of program theory) (54). Caution is required regarding interpretation over the study's reliability, as it was not designed to be representative for IBCM programs or specific regions/countries. Most participants had a background in veterinary medicine and/or research, and representation from the medical sector was lacking. Future research including the perspectives of clinicians and public health experts would be beneficial, however, it should be noted that there is relatively little involvement of medical professionals leading One Health programs for rabies. The logic model of IBCM (**Figure 2**) serves as a template, but does not fully describe the complexity of IBCM and how that relates to different contexts. Moreover, the programs included varied in maturity, from a decade in Bali, Indonesia (2011) to programs still in development, limiting direct comparison. Nevertheless, this study covered a wide scope of perspectives, work experience, epidemiological contexts (from elimination to high endemicity), and geographies. Hence, the study is a valuable step towards discovering lessons about this approach and for understanding how One Health can be operationalized to achieve dog-mediated rabies elimination.

We conclude with preliminary recommendations to support the design and implementation of IBCM programs keeping sustainability in mind. As the only endemic zoonosis with an official elimination goal set by the WHO, rabies should be prioritized and funded to support hiring of staff and implementation of control programs (15). A One Health approach to surveillance should be implemented in all endemic countries at any stage of their control program as this is the most targeted way to identify rabid animals. Many existing reporting systems for rabies are not fit-for-purpose for surveillance and provisioning of PEP. Surveillance systems are often siloed and do not consider the risk of the biting animal or recognize the value of risk assessments, leading to uninformed administration of PEP. In response, IBCM has been developed as a cost-effective approach to address these weaknesses (18). However, current structures within governments, policies, and ways of working pose barriers to introducing IBCM. To successfully and sustainably implement IBCM, there needs to be consideration for how governments and policy can be updated to better facilitate multisectoral, interdisciplinary approaches generally (67). It is imperative that each program is tailored to the context of the country/region where it will be implemented, with careful consideration of context during development, implementation, and evaluation (53). Lastly, the joint involvement and ownership of government authorities from all relevant sectors, local stakeholders, and the community is essential for the effective development and adoption of IBCM protocols (68, 69).

DATA AVAILABILITY STATEMENT

Data supporting the conclusions of this article will be made available on request due to privacy/ethical restrictions.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the College of Medical, Veterinary & Life Sciences Ethics Committee at the University of Glasgow (Ref No. 200190081). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CS collected, analyzed and interpreted the data, and drafted the initial manuscript. KH and NRC supervised data collection and methodology, contributed to the analysis and interpretation, and revision of the manuscript. SM and RM contributed to the interpretation of the results and oversaw the writing and revision of the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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The Impact of the First Year of the COVID-19 Pandemic on Canine Rabies Control Efforts: A Mixed-Methods Study of Observations About the Present and Lessons for the Future

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Achieving zero human deaths from dog-mediated rabies has been set as a global target for 2030. However, the COVID-19 pandemic has disrupted essential health services across the world, with disproportionate impacts on Neglected Tropical Diseases. Through a mixed-method study using stakeholder questionnaires and in-depth interviews, we examined the scale and nature of disruption from the first year of the pandemic to rabies control programs, and reflected on lessons for the future. Study participants included practitioners and policymakers working in government, academia, international organizations, and the pharmaceutical industry across 48 countries, mainly in Africa and Asia. Mass dog vaccination, essential to rabies control, was most heavily impacted and in 2020, was carried out as planned in just 5% of surveyed countries. Access to post-exposure prophylaxis (PEP) also decreased due to fear of COVID-19 infection and difficulties in reaching health care centers. Dog vaccination and PEP delivery suffered from disruptions to the importation and distribution of vaccines. School closures affected rabies awareness activities and, when public events moved online, they could not reach the most disadvantaged groups. Surveillance, already weak, was severely disrupted by movement restrictions which, together with reduced demand for PEP, exacerbated under-reporting. Participants reported growing complaints around free-roaming dogs, with numbers likely to have increased in some settings. In some countries, dog rabies outbreaks and human rabies cases were already ascribed to the pandemic, but further impacts are likely still to be realized. Meanwhile, decreased demand for PEP from COVID-19 constraints could lead to reduced procurement in future. In the wake of post-COVID-19 demands on health services, there is an opportunity for veterinary services to show leadership in progressing the Zero by 30 agenda, particularly in scaling up mass dog vaccination within and across countries, as well as potential to make better use of community-based vaccinators. Countries must further secure stable procurement of dog and human vaccines, classifying them as essential goods prioritized for import and where needed, through sharing of stocks. Dedicated telemedicine services also show promise, for example through fostering participatory disease surveillance, including Integrated Bite Case Management, and delivering up-to-date instructions on the closest sources of PEP.

Keywords: COVID-19 pandemic, dog-mediated human rabies elimination, post-exposure prophylaxis, One Health, mass dog vaccination, rabies dog rabies control

1 INTRODUCTION

Rabies is one of the oldest human diseases of animal origin known to humankind (1). All mammals can be infected with the rabies virus but, domestic dogs – because of their widespread presence in most areas of the world and their proximity to humans – are usually the main source of human infections. Worldwide, nearly all human rabies cases are caused by a bite or scratch from a rabid dog (2).

In 1885, the history of rabies reached a turning point when the vaccine developed by Louis Pasteur saved the life of the young Joseph Meister, the first person known to have survived this incurable and fatal disease. More than a century of progress in laboratory research, field epidemiology, studies in the socio-ecology of rabies, and applied research to improve the delivery of human and dog vaccination has led to the elimination of dog-mediated rabies in many areas of the world, mainly in the Global North (3) but also in Central and South America (4). At present, an estimated 59,000 people still die of rabies every year, especially in Asia and Africa (5).

In 2015, the World Health Organization (WHO), the World Organisation for Animal Health (OIE), the Food and Agriculture Organization of the United Nations (FAO), and the Global Alliance for Rabies Control (GARC) developed the first comprehensive global strategic plan for the elimination of dog-mediated human rabies deaths by 2030 ("Zero by 30") (6). This strategy applies the One Health approach, which acknowledges the interlinkages between human, animal, and environmental health and aims at developing integrated strategies for efficient use of resources and, ultimately, shared benefits. It aims at supporting countries in the development of national and regional plans for rabies elimination that include the following key areas of action: expanding and consolidating mass dog vaccination, increasing access to affordable post-exposure prophylaxis for exposed individuals, and closely engaging with local communities to ensure their uptake. Collecting reliable data through effective surveillance is fundamental to assessing the burden of rabies, targeting high-risk populations, and monitoring progress towards elimination (7). In 2018, these institutions further joined forces in leading the United Against Rabies Forum, a platform for global, regional, national and local stakeholders to coordinate and accelerate responses to dog-mediated rabies (8).

As a Neglected Tropical Disease (NTD), rabies also falls within the scope of the WHO's 2021-2030 roadmap for the elimination of 20 too-long ignored, yet fully preventable, diseases of poverty that disproportionately affect remote, rural, or underserved communities in the Global South (9). In such a crucial moment – the beginning of a decade-long, intense effort – the emergence of SARS-CoV-2 has swamped societies and put national health systems under immense pressure (10). As NTD interventions are implemented at the community level, these control programs have been particularly affected. According to a model-based assessment of the impact on the achievement of the 2030 goals for seven NTDs (not including rabies), most programs are expected to be able to recover from a 1-year interruption. However, longer delays as we are witnessing, will inevitably necessitate more intensive remedial strategies and will increase their burden (11). Of all NTD interventions, programs that relied on mass treatment/preventive chemotherapy were reportedly the most frequent and severely affected by the pandemic (12).

With regards to rabies, there is limited literature on the effect of the pandemic. In the Peruvian city of Arequipa where transboundary spread led to the re-emergence of rabies in 2015, modeling suggested that reduced dog vaccinations and decreased surveillance during the pandemic lead to an observed rise in cases and spread to a nearby city (13). Modelling from Haiti suggested that restarting dog vaccination in 2021 compared to 2022 would avert almost 300 human rabies deaths and prevent over 6,000 human rabies exposures over the next five years (14). Concerns were reported about chronic shortages of human rabies vaccines in Pakistan being exacerbated by the pandemic (15). While in November 2020, the first rabies death was recorded in Bhutan since 2016 (a 3-year-old girl) - stressing the need for timely post-exposure vaccination, even during these exceptional times (16).

Drawing from the observations and experiences of people directly engaged in rabies control programs around the world, this study provides a global, cross-sectoral assessment of the scale and nature of COVID-19 disruptions to current and planned efforts to eliminate dog-mediated human rabies.

2 MATERIALS AND METHODS

2.1 Study Design

This mixed-method study consists of a quantitative and qualitative survey, and qualitative interviews.

The survey questionnaire, in English, comprised 26 closed-ended questions (1 Likert scale, 7 single-choice, 8 yes/no, and 10 multi-choice) and 35 open-ended questions (25 calls to expand and comment on the previous close-ended question and 10 independent questions). The questionnaire was drafted by DN, an anthropologist, reviewed for content and intelligibility by the rest of the team, and prepared in Google Forms. The topics covered were mass dog vaccination and veterinary services, access and delivery of post-exposure prophylaxis, surveillance, awareness activities, and issues intersecting human and veterinary health. Because of the range of respondents and varied progress in rabies control across settings, the questions were intentionally generic. For comparison of the early COVID-19 scenario with the pre-pandemic situation, a precise timeline (the past 5 years) was given only for the three questions about the number of animal cases, animal bite patients, and human rabies deaths in 2020 surveillance data. For other events and activities which may have occurred irregularly and at different times, the responses were taken to refer to the last time(s) these occurred before being affected by the pandemic. The full completion of the questionnaire took 20 to 40 minutes, depending on the detail provided in answer to open-ended questions.

Respondents were further invited to a subsequent, in-depth interview aimed at 1) clarifying and expanding on the survey answers, 2) exploring the nuances from each local context, and 3) allowing the interviewee to bring up unaddressed issues.

2.2 Data Collection

Using convenience sampling, the invitation to the survey was emailed to a list of global, regional, national, and local rabies stakeholders from the network of the United Against Rabies Forum and rabies practitioners known by the study authors. No exclusion criteria were applied. Recipients were asked to forward the invitation to colleagues in their sector and/or country. Responses were accepted for five weeks, from early February to early March 2021. The only personal information requested was the work sector and the email address.

Respondents who expressed interest in being interviewed were contacted, in chronological order, to schedule online, one-to-one meetings. The criteria used to schedule interviews were 1) availability within the first week of March 2021, 2) being at ease discussing in English, and 3) diversity of countries and sectors (although we do not claim country and sector representativeness). Upon permission, all the interviews were audio-recorded. Interviews lasted from 45 to 120 minutes.

2.3 Analysis

All the received questionnaires were included in the analysis, with some adjustments. Four questionnaires were not country-based, but provided a broader international perspective (e.g. from the United Nations Children's Fund and the pharmaceutical sector). We excluded these from the country-based analyses, instead of using their inputs for qualitative analysis. Second, Taiwan (Province of People's Republic of China) was evaluated separately from mainland China, given major epidemiological differences. Third, Bhutan, Brunei Darussalam, and Taiwan all provided high numbers of questionnaires relative to their populations (respectively, 13, 5, and 2). We therefore aggregated quantitative responses from these states while keeping qualitative responses disaggregated for further analysis.

Surveyed countries were divided into categories based on the presence of dog-mediated rabies (9) and country investment in dog rabies control (**Figure 1A**). Countries with routine national dog vaccination programs maintained for at least five years were classified as having rabies control in progress, but dog-mediated rabies not yet eliminated.

The harmonized data was consolidated and cleaned for comparison across the responses. Figures and maps were created using the R computing language (v4.0.02). The interviews were initially transcribed with Amberscript®, an online software for automatic transcription, then checked manually, anonymized, and edited into verbatim transcripts.

The qualitative answers from the questionnaires and transcripts were analyzed with Nvivo12®. Codes were created (and defined) inductively, applied, and adjusted iteratively during several rounds of data reading. Several rounds were necessary to maximize reflection, minimize errors (because only one author, DN, conducted the qualitative analysis), and develop themes across both qualitative data sources (qualitative survey answers and interview transcripts).

2.4 Ethics

This study was deemed to constitute routine public health surveillance that did not require independent ethics review, but followed ethical standards and guidelines as outlined by WHO (17). Participants were informed prior to starting the questionnaire as to the purpose of the survey and that data would be used for a scientific publication. Respondents agreed to participate in this study when undertaking the questionnaire and consent was collected for scheduling and starting interviews. Informed consent was obtained for the collection and analysis of audio-recorded information. Recordings were retained by WHO.

3 RESULTS

3.1 Overall Impact on Rabies Activities

We received and accepted 103 questionnaires and, at the end of the data analysis process described in 2.3, we worked on 82 questionnaires from 48 countries. Most responses came from endemic countries (n=62, 76%) across Africa and Asia (n=65 altogether, 79%) (**Figure 1A**), and people working in the national government, academia, international organizations, and the animal health frontline (n=72 altogether, 88%) (**Figure 1B**). Nineteen people were interviewed; 15 interviewees talked about individual countries, while four provided comparative information about two to four countries.

According to the study participants, the pandemic hit rabies control activities hard both in endemic and in-progress countries, but two main differences were observed. First, the impacts were easier to assess in the in-progress countries than in the endemic countries where surveillance systems were frailer. Second, in endemic countries which were just beginning their journey to the

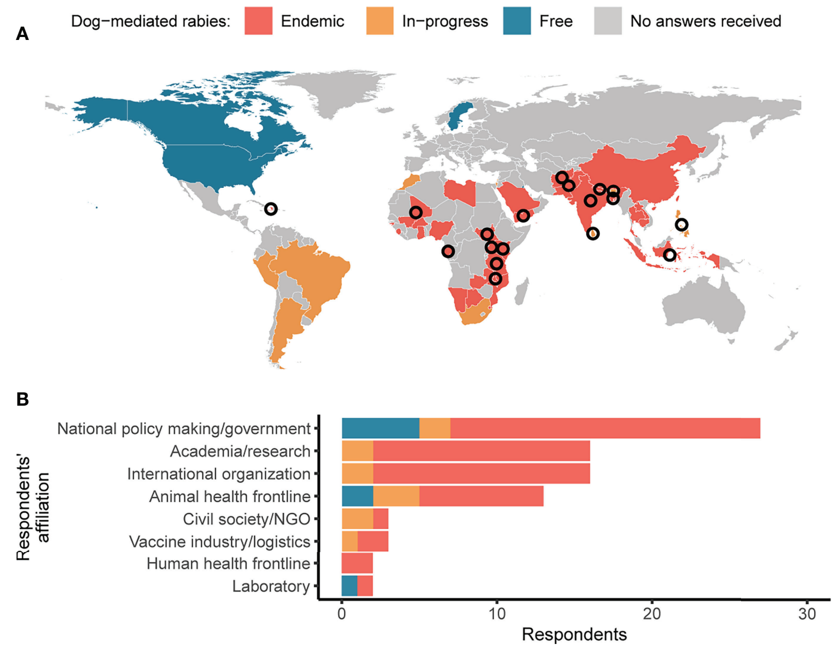

FIGURE 1 | Survey responses and interviews across different countries and sectors. **(A)** Map of survey responses received from countries with endemic dog-mediated rabies (red), with rabies control programs in-progress (orange) and that are free from dog-mediated rabies (blue). Countries not visible on the map at this scale include Brunei Darussalam, Israel, Kuwait, and Singapore. Interviews were carried out with respondents from countries indicated by the circles. **(B)** Survey responses by sector, with shading as per the map.

Zero by 30 goal, "the momentum that was gaining was lost," while in-progress countries experienced an unfortunate step back.

The question on whether funds for rabies control were reduced or diverted to COVID-19 response divided respondents; 42 (51%) replied negatively and 39 (48%) positively. While respondents in endemic countries and in-progress countries were roughly equally distributed, seven out of the eight respondents from rabies-free countries observed no disruption to their national rabies budget. Respondents across the three country categories estimated a 25% to 50% reduction in funding. Five of those who reported no budget cuts specified that the financial resources allocated to rabies control were so low and unpredictable even before COVID-19, that no visible change occurred because of the pandemic. One respondent noted that, besides being intentionally moved to COVID-19 control, rabies funds also declined because of the government's decision to suspend the garbage collection tax (which local authorities usually use for dog vaccination, among other purposes) to reduce pressure on families during the pandemic.

In most interviews, respondents from all sectors reported that the first resources to be cut or re-directed to the management of COVID-19 – or of other co-occurring emergencies – were those for the procurement of animal rabies vaccines and the implementation of dog vaccination campaigns. Dog vaccination campaigns often rely on unpredictable financial support from external donors or civil society, and these suffered from a sudden shift in priorities when the pandemic started. Where rabies control relies on government support, funding was often concentrated only upon delivering post-exposure prophylaxis to bitten individuals, with other rabies control activities neglected. In some countries, participants observed that, both in the human and the animal health sectors, the rabies budget was not intentionally cut, but rather remained unspent due to the impossibility of performing rabies control activities because of lockdowns and movement restrictions. It was unclear whether these savings would remain available for rabies activities in the future. One participant from the pharmaceutical sector claimed that all energies and funds were focused on the manufacturing of the COVID-19 vaccine. From a global perspective, many countries reduced the quantity of human and dog vaccines they procured during 2020, as well as their forecast for 2021 and beyond.

The return to a pre-COVID-19 situation was not expected to happen before 2022, assuming that rabies would receive at least the same amount of political and financial commitment as prior to the pandemic. The recovery will also depend on other co-occurring disease outbreaks reported by respondents (e.g. African swine fever in the Philippines, screw-worm fly disease in Yemen), food security issues, the production and distribution of COVID-19 vaccines, and the strategic planning of all the human and animal vaccination campaigns interrupted by the pandemic. The effectiveness of post-COVID-19 rabies control efforts will also depend on how much countries truly adopt a One Health approach. One respondent observed that the pandemic had neither a positive nor a negative impact on the discussion about One Health at the policymaking level, because "it's just COVID era."

3.2 Disruption to Rabies Activities

Each of the three pillars of the integrated strategy for dog-mediated human rabies elimination was affected by the pandemic, although to differing extents.

In the first year of the pandemic, dog vaccinations were carried out as planned in just two countries (4%) (**Figure 2A**). Disruptions included delays of at least six months, prolonged duration, increased costs and failure to reach targets, with the cancellation of vaccination in some areas that were planned for. Several respondents pointed out that vaccination campaigns can be planned only at specific times of the year (mainly depending on school holidays and season), causing further delays should they restart. One rabies-free country decided not to perform the usual targeted vaccination of dogs in areas at risk of incursions.

In the few countries that have mass dog vaccination campaigns along their borders (i.e., India and Bhutan; Thailand, Lao PDR, and Cambodia), this activity was completely cancelled due to pandemic travel restrictions, mandatory quarantines, and increased costs. In Eastern Africa, an important meeting on cross-border rabies control coordination planned for April 2020, in preparation for countries applying to Gavi's vaccine investment strategy, was cancelled. In South Asia, the international cooperation program to be launched by FAO was suspended.

Access to post-exposure prophylaxis was impacted by the pandemic in 79% of the surveyed countries (**Figure 2B**). According to one respondent, vaccine demand halved in South Asia. In an endemic country with chronic shortages of post-exposure vaccines where bite victims must travel to the neighboring country for access, the closure of borders reportedly had a dramatic effect. Nevertheless, other endemic countries – 6 out of 32 (19%) – reported no negative changes compared to pre-COVID-19.

Awareness activities for children, mainly carried out at schools, survived the first year of the pandemic in only two countries (4%), both of which were endemic (**Figure 2C**). In the experience of a large NGO that runs rabies control programs in several Asian and African countries, the worst impact of the pandemic was on education.

3.3 Reasons for Disruption

3.3.1 Mass Dog Vaccination

About a quarter (n=13/59) of respondents who detailed the reasons for disruption to dog vaccinations observed only one cause, while most reported multiple; two (n=12, 20%), three (n=11, 19%), four (n=13, 22%), or five (n=9, 15%).

Most respondents (n=46, 56%) – across all country categories – claimed that restrictions on the movement of personnel were the main hindrance, especially when high-risk COVID-19 areas coincided with endemic rabies areas (**Figure 3A**). Additionally, it was difficult to organize vaccination campaigns in adherence to local COVID-19 safety guidelines (n=30, 37%), particularly campaigns usually carried out by Non-Governmental Organizations (NGOs) rather than the government. Other reasons for disruption included dog owners being afraid of gathering for dog vaccinations (28, 34%) and, especially in in-progress countries, dog vaccines being limited or unavailable (n=24, 29%) and vaccinators being quarantined or reassigned to COVID-19 response (n=21, 26%). Other issues mentioned by respondents included: budget constraints; animal officers losing their contracts due to staff cuts; government veterinarians moving to the private sector due to a lack of budget for salaries; children – those who often bring dogs for vaccination – being forbidden to leave their house; and, people being afraid of dogs spreading COVID-19 at vaccination points. Indeed, one respondent talked about the new challenge of respecting "double distancing; human-human and animal-human." Interestingly, some participants observed that at the beginning of the pandemic, people's fear of dogs' vulnerability to COVID-19 increased the demand for vaccination, including rabies. Finally, the need for mobile phone applications to record dog vaccination participants, personal protective equipment, hand sanitizers, extra personnel to manage queues, and, in one example, isolation cabins, all increased the cost of dog vaccinations.

Dog vaccination was affected differently in rural versus urban settings. Some respondents said that, especially early in the pandemic, concerns about the impact of COVID-19 were higher in urban than in rural communities. Echoing this view, another participant observed that "COVID-19 may be bad, but rabies is far scarier." So, in rabies-aware communities, rural dog owners were willing to participate in dog vaccinations, also thanks to persuasion from community leaders. Yet, vaccinators – often travelling from towns – were reluctant to work in rural areas fearing poor social distancing. Besides these concerns, the main challenge was the difficulty or costs for urban vaccinators to reach rural communities due to travel bans, towns being quarantined, and safety risks when travelling to remote areas. In one endemic country, because of the risk of hijackings, central static points replaced mobile clinics. Several participants shared their enthusiasm for dog vaccination programs based on local, lay animal vaccinators – meaning trained community animal health workers – instead of external professionals. It was argued that this would reduce transport-related challenges and costs and, in the current situation, COVID-19 spread, while increasing sustainable community engagement. Only one respondent said that, despite the challenges, rural areas were prioritized anyway, because of the high numbers of cases.

In urban settings, vaccinators were easily available and rabies awareness remained as high as usual, but people's fear of coronavirus eroded community support and COVID-19-related logistical issues made dog vaccination challenging. For example, central static points were difficult to organize in towns due to the lack of large spaces where social distancing could be guaranteed. Moreover, stay-at-home orders and limits on gatherings were often strict in cities, reducing the number of dogs that could be vaccinated each day. Nevertheless, some positive aspects were reported. For example, as people spent more time at home, they had the time to bring their dogs for vaccination and, one respondent noted, they even took advantage of dog vaccinations to have a reason to go out. In the most affluent neighborhoods, static drive-through clinics were set up to vaccinate dogs without owners leaving the car and respecting social distancing. Additionally, vaccination services in

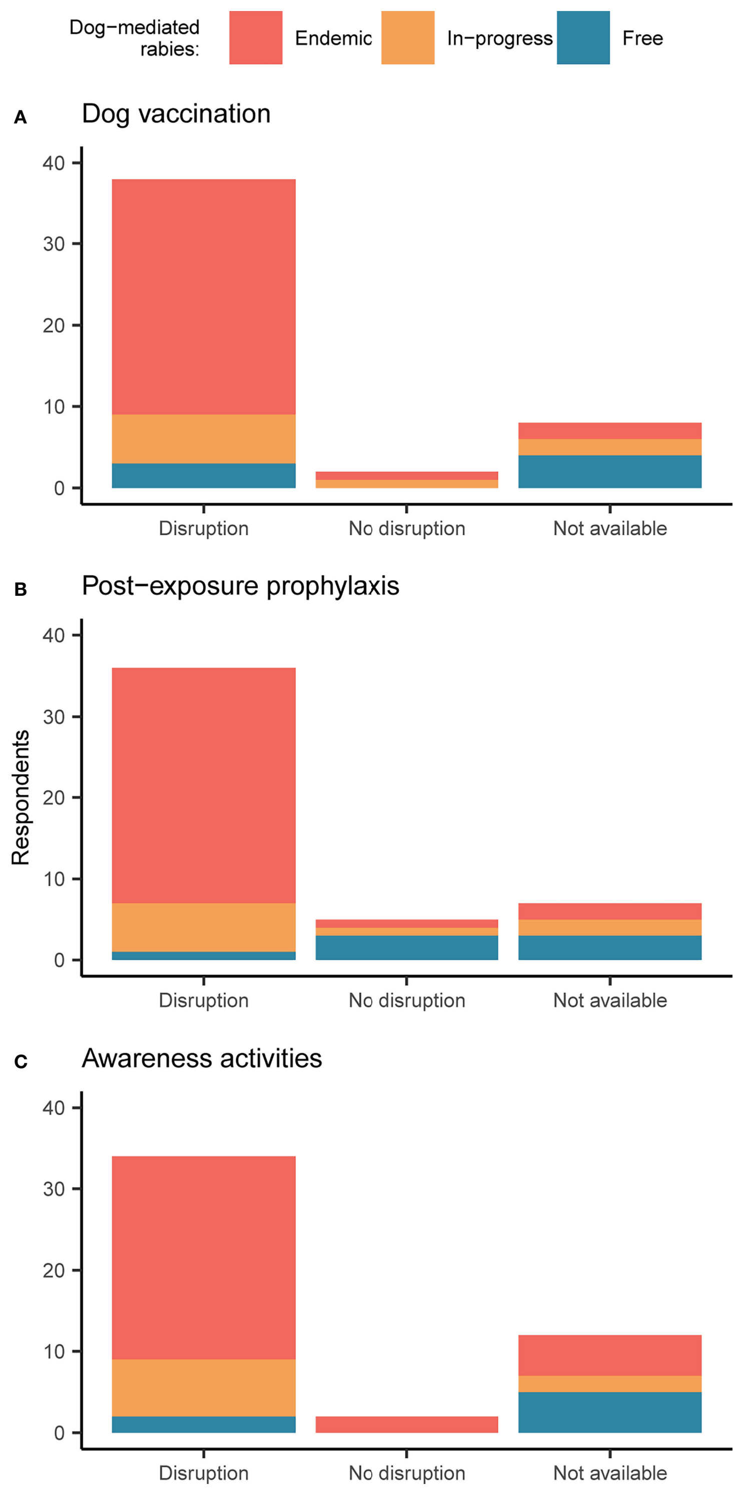

FIGURE 2 | Impacts of COVID-19 on rabies control activities. Respondent perceptions regarding impacts on **(A)** mass dog vaccination, **(B)** post-exposure prophylaxis, and **(C)** awareness activities targeting children. Bars are shaded according to responses by country, with endemic countries in red, countries with rabies control in-progress in orange, and countries that are free from dog-mediated rabies in blue. If multiple responses were available for a country, these were aggregated, and in the rare cases where there was no consensus, information was considered not available.

FIGURE 3 | Reasons for disruptions to rabies control and prevention activities. Respondents' stated reasons affecting **(A)** mass dog vaccination, **(B)** health-seeking behavio, **(C)** provisioning of post-exposure prophylaxis, and **(D)** rabies surveillance. Bars are shaded according to responses by country, with endemic countries in red, countries with rabies control in-progress in orange, and countries that are free from dog-mediated rabies in blue. Results are presented individually (by study participant) rather than aggregated by country. As indicated by the asterisks in the figure, "COVID-19 safety" refers to the difficulty for organizers in adhering to pandemic control guidelines, while "Fears" refers to concerns about leaving home and crowding.

private animal clinics – where available – were strengthened in some instances, even though only well-off dog owners could afford them. Finally, catch-vaccinate-release – which is used in some countries with many unowned dogs and where people have issues handling these dogs – was easier to perform than before the pandemic, because of reduced traffic and pedestrians.

The pandemic affected the production and supply of animal rabies vaccines. One-third of respondents reported delays (n=28, 34%) and reductions (n=25, 30%) in import, both caused by the slowdown of international trade and delays at customs, which impacted procurement orders by governments (also through the OIE Vaccine Bank), private suppliers and NGOs. In-progress countries especially reported issues with in-country vaccine distribution (n=25, 30%). Seven respondents (9%) from endemic countries observed a decrease in vaccine production, mainly because of budget constraints. Rabies-free countries faced less severe delays in vaccine importation. As a lesson for future emergencies, one respondent stressed the importance of classifying human and dog vaccines as essential goods when prioritizing import. Several participants also emphasized the need for the government, private distributors, and NGOs to coordinate and share their stocks nationally and internationally, to ensure doses do not go unused or expire – as two participants reported happening during the pandemic. One respondent lamented that, before and during the pandemic, dog vaccines in urban areas remained unused, because only people in rural areas care about dog vaccination.

3.3.2 Post-Exposure Prophylaxis

One-third (n=15/50) of respondents reported changes in health-seeking behavior for post-exposure prophylaxis, attributing only one reason for this, while most respondents identified two (n=12, 24%), three (n=13, 25%), or four (n=7, 14%) coinciding causes.

People's fear of attending clinics because of the risk of COVID-19 infection was the most reported cause (n=34, 41%), in endemic and in-progress countries (**Figure 3B**). Other obstacles included difficulties in reaching clinics because of reduced public transportation – and reluctance to share private transportation – (n=22, 27%) and reduced financial means (n=15, 18%) and, in parallel, ease of access to local remedies (n=9, 11%). According to, respectively, 24 (29%) and 14 (17%) of the participants, bite victims delayed going to clinics and interrupted their vaccination schedules.

For urban residents, strict stay-at-home orders and sudden clinic closures hampered access to PEP, but bite victims in rural areas were disproportionately affected by the pandemic. In some countries, even before COVID-19, post-exposure vaccines were available only in major towns, which became harder and more expensive to reach during lockdowns and under movement restrictions. In one endemic country with an ongoing refugee crisis, where people in refugee camps often need airlifting to find a clinic with post-exposure vaccines, the pandemic exacerbated inequalities in access to care. When lockdowns started and public transportation decreased, access to healthcare worsened for rural migrants who got stuck in the cities where they were working, but where they could not benefit from Universal Health Care schemes. Additionally, respondents reported that a lower awareness about the need for medical assistance among people in rural areas was a factor contributing to bite neglect and the use of traditional healing during the pandemic.

From the perspective of the health provider, there were disruptions in the delivery of post-exposure prophylaxis. One-third (n=17/59) of respondents reported one reason for this, while most observed two (n=14, 24%), three (n=15, 26%), four (n=4, 7%), or five (n=6, 10%) reasons for disruption to the provision of health care to bitten individuals.

Shortage of human vaccines – both because of supply issues and financial constraints, in almost equal measure – was identified as the main obstacle to the provisioning of post-exposure prophylaxis by 54% (n=44) of respondents, mainly in endemic countries (**Figure 3C**). Postponement of Gavi's planned vaccine investment strategy for rabies was a major concern for some respondents, worried about the need to address chronic shortages in the poorest countries. The second reason, mentioned by 49% (n=40) of respondents, was staff shortages – both because of quarantine and illness and, to a lesser extent, redeployment – and was mostly reported from in-progress countries. Other obstacles were closure or conversion of clinics (n=21, 26%), especially in in-progress countries – in one of which, all primary health care services were closed for five months – and, mainly in endemic countries, the availability of post-exposure vaccines only in the private sector (n=16, 20%). In a war-torn, endemic country, where post-exposure vaccines used to be available in private clinics, none could be found during the pandemic.

In two in-progress countries, toll-free numbers and telemedicine were implemented to assist people at home, preventing them from visiting hospitals when unnecessary. In some endemic countries, media reported on stories of bite victims who delayed seeking medical assistance, to increase public awareness about adequate bite management and denounce the lack of vaccines in health care facilities. Especially during lockdowns, it was difficult for people to know where to find open clinics and to travel to them, especially because, due to the fear of COVID-19, people tended to avoid big hospitals, preferring smaller clinics that were either closed, had no vaccines in stock, or could not administer cost-saving intradermal regimens. One interviewee stressed the value of a hotline through which bite victims, especially from rural areas, can be directed towards the most convenient health care facility with vaccines in stock.

3.3.3 Awareness Activities

Figure 2C showed the impact of the pandemic on awareness activities for children, resulting from prolonged school closures and stay-at-home orders. In one endemic country, the usual sponsorship for in-school rabies education was cut. In another, where rabies prevention is part of the national curricula, online lessons were organized, but were usually accessible only to urban children with a computer at home and a good internet connection. Whether through online or in-person lessons, one respondent observed that the experience acquired in teaching COVID-19 prevention to school children may be useful for rabies as well.

The 2020 World Rabies Day events for the general public were also affected. Half (n=41) of survey respondents reported disruption. According to 21 people (26%), in-person activities were held but with lower attendance, while 11 (13%) said that in-person activities were completely cancelled. One-third (n=26) shared a more positive experience, with new or re-organized online events. According to one respondent, the fact that events were online improved attendance by health workers. Another maintained that "the COVID-19 lockdown resulted in many – especially veterinarians – looking for a reason to do something meaningful" and this became evident on World Rabies Day 2020.

A respondent from the Philippines, recalling the strong vaccination hesitancy that started after a problematic dengue vaccine campaign in 2017, stressed the importance of investing adequate resources in the design of health communication campaigns, both for COVID-19 and human and dog rabies.

3.4 Surveillance

3.4.1 Disruption to Surveillance

One-quarter (n=14/61) of respondents who detailed the reasons for disruption to surveillance mentioned only one reason. Most people observed two (n=16, 26%) and three (n=16, 26%) co-occurring causes, while some reported four (n=11, 18%) or five (n=4, 7%).

The main hindrance – across all country categories – were restrictions on the movement of the field surveillance staff (n=43, 52%), which led to cases being missed or investigated late (**Figure 3D**). Other reasons, especially in in-progress countries, were lack of staff (n=35, 43%) and the difficulty of carrying out surveillance in adherence to COVID-19 safety guidelines (n=24, 29%). Other issues were budget cuts (n=21, 26%) and scarcity of equipment for sample collection and testing (n=20, 24%).

Qualitative data offered additional insights. The rabies surveillance workforce decreased because of staff cuts, salary reductions, and personnel being moved to food security tasks. Several respondents described a vicious cycle: as access to and delivery of post-exposure prophylaxis declined, so fewer dog bite cases were reported, bite reports were not sent to investigators, who could not perform diagnostics or investigations, resulting in incidents remaining unaddressed. Furthermore, the transport of samples from remote areas was slower than usual. As observed by one participant, this apparent drop in animal bites and demand for post-exposure prophylaxis could lead to a downward adjustment in the procurement of human rabies vaccines for the next few years.

Most respondents (n=45, 55%) claimed that laboratory capacity was not reduced or diverted, especially in either endemic or rabies-free countries. In contrast, this was a much-lamented issue by respondents from in-progress countries. A few respondents noticed the positive impact of the pandemic in building and expanding laboratory capacity – in terms of equipment and staff skills – that had the potential to provide future benefits for rabies diagnostics.

3.4.2 Trends in Animal Cases, Animal Bite Patients, and Human Rabies Deaths

One-third of respondents did not express their opinion about trends in animal cases, bites to humans, and human rabies deaths during the first year of the pandemic. Those who responded often highlighted the weakness of the surveillance system even before the pandemic, and the high risk of declining trends not being genuine. Additionally, one participant noted that the effects of the pandemic on these trends would be felt only in 2022 and beyond, because drops in dog herd immunity (arising from disruptions in dog vaccination campaigns) would take time to become evident.

The question on animal rabies cases depicted a very fragmented scenario, with 21 (26%) respondents reporting a decrease, 18 (22%) an increase, and 17 (21%) a stable situation. In contrast, animal bites to humans and human rabies cases seemed to have decreased according, respectively, to 27 (33%) and 26 (32%) of the respondents. It was observed that this may be caused either by an actual reduction in contact between people and free-roaming dogs during lockdowns, or reduced presentations by bite victims to health facilities. Importantly, one participant pointed out that, if stay-at-home orders really protected people from exposure to bites, this hardly applied to rural and impoverished urban areas, where a fully indoor lockdown was not always possible. Another mentioned the risk of rabies misdiagnosis due to health providers being under excessive stress. Eighteen (22%) and 19 (23%) participants claimed that, respectively, animal bites to humans and human rabies cases remained stable and only nine (11%) and eight (10%) respondents reported increases from surveillance data.

That said, from two in-progress and one endemic country, we collected accounts of human rabies cases directly linked to the pandemic. In Bhutan, a young girl bitten in her village by an unknown dog, was not brought to the hospital by her parents as her wound was minor and strict movement restrictions were in place. Her village on the border with India was both rabies endemic and had high COVID-19 transmission. Cross-border mass dog vaccination campaigns routinely performed pre-pandemic were interrupted when the border was closed in Spring 2020. In the Philippines, a man gave up his search for an open bite clinic in the town he travelled to and went back to his village, where he subsequently developed rabies and died. In Uganda, "the lack of resources [for dog vaccination] has always been a problem, but nothing has contributed to the escalation of the rabies problem like COVID-19" and the doubling of the cost of dog vaccines that followed. Since December 2020, a human fatality and recurrent canine rabies outbreaks have been ascribed to suspended dog vaccinations. Similar reports came from Tanzania, where no dog vaccines were available to respond to a sudden rise in dog rabies cases.

3.5 Changes in the Dog Population and Human-Dog Interactions

About one-third of respondents – across all country categories – reported increased numbers in dogs (n=32, 39%) and a worsening in dogs' health (n=29, 35%) (**Figure 4A**). Several added that, due to the shortage of food from eateries and people who feed dogs in the community staying at home, some community dogs had become feral, formed packs, and expanded their range. Another third (n=24, 29%) observed no changes. Some respondents reported a decrease in the dog population (n=16, 20%) – particularly in in-progress countries – and increased dog aggressiveness (n=13, 16%). This trend was explained as a result of starvation, lower reproductive rates, and dog owners restraining their free-roaming dogs at home during lockdowns.

Regarding people's interactions with dogs, most respondents (n=48, 59%) noticed an increase in dog abandonment that they considered to be due to financial constraints caused by pandemic-driven job loss and fear of animals spreading COVID-19, in equal measure (**Figure 4B**). Other reasons for abandonment were natural calamities affecting dog owners (e.g. typhoons and floods) and behavioral changes in pet dogs during lockdowns. No matter the reason, increased abandonment was observed especially in endemic countries. One-third (n=27, 33%) of participants reported a rise in complaints about the presence of free-roaming dogs and subsequent requests for interventions (e.g. sterilization, removal, culling, etc.) to local authorities. Another third (n=20, 24%) noticed, on the contrary, an increase in people feeding free-roaming dogs, out of personal initiative or persuasion from the government, religious organizations, or animal welfare associations, especially in urban areas of South Asia. Nevertheless, one respondent commented that "dogs are worshipped, so people feed them, but that's all they do." Several respondents, across all country categories, observed increased dog adoption and some expressed their concern about the possibility of this resulting in high abandonment in future. In one European rabies-free country, the illegal import of dogs was particularly high in 2020, probably because of the increased demand for dogs to buy or adopt. While in one endemic country, a respondent mentioned an increase in dog meat consumption.

The question about how the media reported free-roaming dogs during the pandemic revealed divergent responses (**Figure 4C**). Half (n=41) of respondents noticed no changes. The other half observed, in almost equal measure, more cases of cruelty towards dogs (n=14, 17%), more attacks by dogs on humans (n=12, 15%), and on livestock (n=10, 12%), and a more caring attitude towards dogs (n=10, 12%). Attacks on humans and livestock occasionally triggered frustration and retaliatory measures against dogs. One respondent remarked on the key role played in their country by the media in keeping public attention to rabies high, but also pointed out the importance, and the struggle, of maintaining engagement despite emerging issues, such as anti-microbial resistance.

4 DISCUSSION

This study explored how the first year of the COVID-19 pandemic affected canine rabies control and dog-mediated human rabies elimination efforts in 48 countries, mostly in Asia and Africa. Given the broad range of issues, and the descriptive nature of the data, we summarize and discuss our key findings through the exposition of three overarching themes identified during data analysis. Our aim is to reflect and share ideas as to how the experience of the pandemic can support rabies stakeholders in improving their strategy to be more efficient, resilient, and sustainable in the future (18).

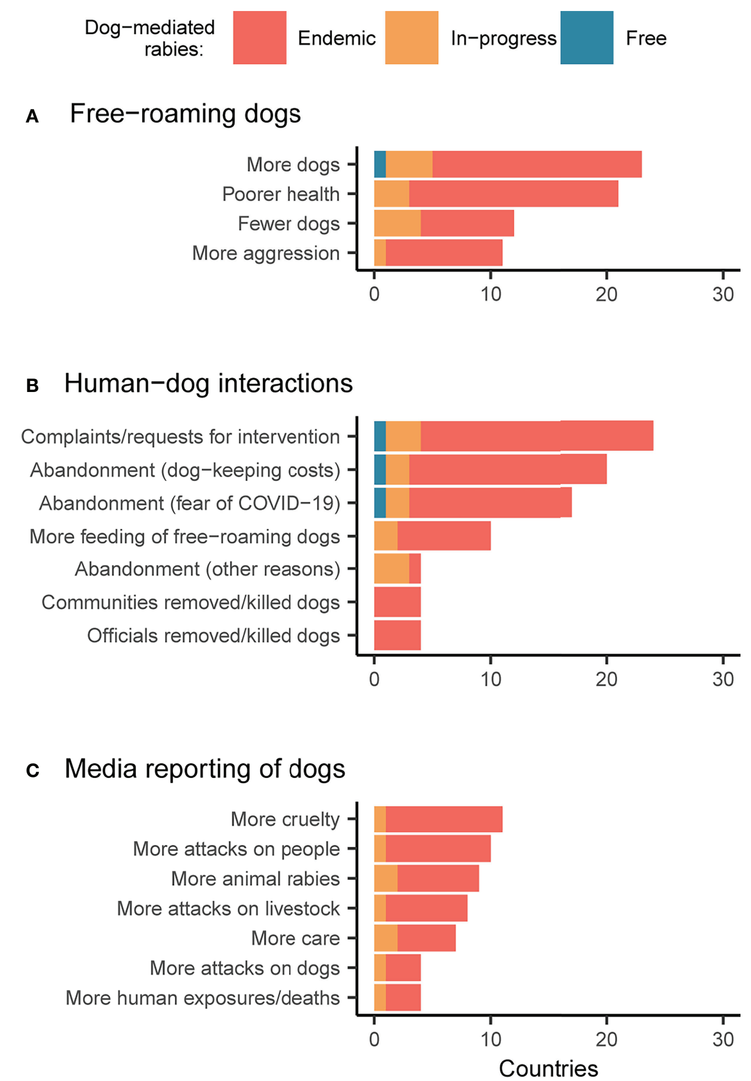

FIGURE 4 | Changes in dog populations during the pandemic. Respondent perceptions regarding changes to **(A)** free-roaming dog populations and behavior, **(B)** human-dog interactions, and **(C)** media reporting on dogs (comparing the pre- and during- COVID-19 scenario). Bars are shaded according to responses by country, with endemic countries in red, countries with rabies control in-progress in orange, and countries that are free from dog-mediated rabies in blue.

4.1 Rabies Does Not Circulate in a Bubble

The different impacts of the pandemic across different components of rabies control illustrate how an integrated One Health approach is needed for effective control and prevention of rabies. Yet, it also shows how this can make the strategy vulnerable to social, political, economic, and ecological disruptions.

The first indicator of this interconnectedness is the fact that – especially with regards to dog vaccination and surveillance – multiple co-occurring reasons caused their disruption during the first year of the pandemic. This demonstrates how crucial an integrated, whole-of-society approach to rabies is, needing active engagement with all relevant stakeholders.

The way COVID-19 has influenced the human-dog relationship (not only in the surveyed countries, but around the world) (19) and the possible short- and medium-term consequences of this on rabies transmission and control provide a further example. During the first year of the pandemic, free-roaming dogs likely increased, mainly due to abandonment because of decreased family income, misconceptions about animal transmission (widely documented early in the pandemic) (20), behavioral issues in "lockdown dogs" (mainly studied in the Global North) (21, 22), and natural calamities affecting dog owners.

Surveillance data indicate an apparent decline in animal bites and human rabies cases in many countries during the first year of the pandemic, although this did not occur everywhere (see the case of South Africa in **Box 1**). This decline may be explained by decreased contact between people and free-roaming dogs during lockdowns and school closures. Nevertheless, an alternative explanation is provided by under-reporting, which would be consistent with literature reporting increased dog bites – especially in children – since the beginning of the pandemic and especially during lockdowns (27–29). If this is the case, bites not captured by surveillance in 2020 are likely to have mainly occurred among those who could not spend lockdown periods indoors (e.g. people living in the streets, in informal settlements, in itinerant pastoralist communities) or who live in remote or rural areas with poorer access to health care. If, as feared by some (30–32), dog abandonment increases post-COVID-19, there may be increased risks of bites and, particularly where dog vaccinations have been discontinued, increased rabies exposures.

The growing dog population trend mainly observed in endemic countries has worrying implications for the near future. In a post-COVID-19 scenario, when countries will have competing priorities and limited financial resources, it is to be expected that dog vaccination will not receive the necessary attention. The gap in implementation may also lead to other approaches being adopted, for example dog culling, which is sometimes perceived as a rapid and accessible form of rabies control. However, indiscriminate dog culling is known to be ineffective because vaccinated dogs may be killed, people may hide or move their unvaccinated dogs to protect them, and community trust and engagement can be severely affected. In some endemic countries, dog culling was already being undertaken (routinely or sporadically) before COVID-19 and, during the pandemic, further requests were reported. Increasing numbers of hard-to-catch unowned dogs will only make vaccination efforts more time-consuming and expensive. Many communities in rabies endemic areas also depend on livestock for their livelihoods. If dog attacks on livestock have increased during the pandemic, people's perception of dogs and dog-related issues, including rabies control, may also be affected.

As exemplified by the transboundary movement of rabid dogs across the Bhutanese-Indian border and the increased illegal import of dogs in Sweden in early 2020, countries cannot work alone towards dog rabies control and elimination. Key areas for future collaboration that emerged from this study are cross-border dog vaccination programs (33) – acknowledging rabies as a border security issue – and sharing of dog and human vaccines between neighboring states during emergencies.

4.2 Communities Need Local Solutions

Movement restrictions were the primary reason for disruptions to dog vaccination, surveillance, and rabies awareness campaigns in 2020 and were the second most reported challenge for health-seeking by bite victims. Even though other factors co-occurred, the distance between vaccinators and dogs, investigators and bite incidents, educators and communities, and bite patients and life-saving vaccines proved harmful and, in some cases, lethal.

With dog vaccination, the possibility of entrusting properly trained community vaccinators (34) and the use of thermotolerant vaccines and locally-made passive cooling devices (35) emerged as potentially game-changing strategies for future rabies control. These strategies were recommended by the participants in this study not only to address pandemic disruptions, but to overcome broader challenges, such as the limited workforce. In many endemic countries, with insufficient veterinarians and weak cold chains, local vaccinators are an essential resource and can increase capacity and reach into communities, while reducing staff and transport costs, engaging communities and ensuring timely vaccination of new dogs.

The pandemic also highlighted the dependence of most rabies endemic countries on the import of both dog and human vaccines. Centralized vaccine production allows for stricter adherence to international quality standards, but there are major benefits to regional or national self-reliance for vaccine production if quality assurance, safety, and effectiveness can be achieved (36). In-country distribution challenges could, again, be minimized by the distribution of thermostable vaccines and shared supplies between countries in emergencies. Meanwhile, support from the OIE Vaccine Bank (37) and Gavi, the Vaccine Alliance (38), remains essential. Moreover, actions that are being taken to improve the inequities in vaccine supplies highlighted by COVID-19 should be co-opted in the longer term to benefit rabies control and prevention as well.

Participatory disease surveillance (39), which involves close collaboration between at-risk communities and human and animal health surveillance authorities, can also potentially alleviate the obstacle of centralized surveillance staff being unavailable or unable to quickly reach communities. Research on rapid, efficient but cheap diagnostic tests to be used directly in the field, to bypass the problem of sample shipment to central laboratories, is progressing (40–42).

In-person rabies awareness activities, both for the general population and schoolchildren, were disrupted by stay-at-home orders and school closures. Online events were organized to replace them and, in some countries, proved more successful than in-person activities in engaging with human and animal health workers. Yet, online activities could not reach children with limited computer or internet access, especially in rural areas but also in impoverished urban neighborhoods. Paper-based materials may be more reliable – for use both in schools and at home – and a better investment to simultaneously reach children with fun activities, and their relatives, with basic information on

BOX 1 | One Health Teams with Strong Veterinary Capacity Made the Difference in South Africa.

In South Africa, a rabies endemic country, a significant increase in the number of confirmed dog and human rabies cases was reported in 2020 and 2021 (23–25). The uneven geographic distribution of these cases demonstrates the reach that veterinary-led rabies programs need to have into communities (26). In the areas where close-knit One Health teams existed in pre-pandemic times and veterinary services remained in place despite COVID-19 and pressures from other animal disease outbreaks, surveillance and response mechanisms were quickly activated: mass dog vaccinations were strengthened, availability of post-exposure vaccines was ensured, and targeted awareness campaigns were promptly organized. This allowed rabies outbreaks to be quelled. In the areas where mass dog vaccination had been patchy, "veterinary services fell apart during COVID-19" (26) and dog rabies outbreaks were much harder to control, despite the considerable resources invested. Together with the changes in people's health-seeking behaviors that occurred during the pandemic, this resulted in human losses. South Africa shows that countries can cope with a 2-year reduction, or even interruption, of rabies control activities if they have a functioning One Health system and routine mass dog vaccination in place.

both dog vaccination and post-exposure prophylaxis. Adding key hotline numbers could enhance these materials for communities and be displayed in key locations (e.g. schools, health care centers, meeting halls, etc.).

4.3 Rural and Urban Settings Require Different Approaches

Rabies control and prevention were impacted by the pandemic in different ways depending on their rural or urban location. Despite variation among countries and the impossibility of drawing generalizable conclusions, the differences are worth discussing.

In rural communities where rabies awareness and engagement with local leaders tend to be high, dog vaccination was most severely affected by travel bans and COVID-19 safety standards. This supports the inclusion of community-based vaccinators in rabies control plans, and the continuation of strategies that ensure sustained community engagement and empowerment. In urban areas, the lack of large spaces was the main impediment, but innovative vaccination methods were piloted. Furthermore, the catch-vaccinate-release method worked particularly well because of quieter roads and, whenever possible, private vaccination was often strengthened. It seems that cities are well suited for (and perhaps require) multi-method vaccination strategies, with methods adapted to suit particular groups where they are most effective (e.g. catch-vaccinate-release out of peak hours, fixed and roaming static points during the day – 43, etc.) and – when available - with engagement with the private veterinary sector.

In relation to post-exposure prophylaxis, urban residents mainly faced the problem of finding open clinics, whilst rural residents also had to deal with travel restrictions, limited public transportation, and reduced financial resources, leading to underestimation of risk, delays in access to care, and use of ineffective remedies. Not only in exceptional times like the pandemic, but also in normal circumstances, the set-up of dedicated animal bite and rabies hotlines seems advantageous on several fronts. First, phone calls are a fast and cheap way for bite victims to receive immediate and standardized risk assessment (assuming network services/coverage), and up-to-date instructions on their nearest source of post-exposure prophylaxis. Second, hotlines work as a starting point for Integrated Bite Case Management, with immediate benefits of increasing detection of animal and human rabies cases, and targeted distribution of post-exposure vaccines. Third, hotlines provide an additional communication channel for participatory disease surveillance. Fourth, they can potentially strengthen the One Health approach to rabies, if jointly managed by staff from both the human and animal health sectors. However, further research is necessary to explore the feasibility and costs of this promising intervention (44).

4.4 Limitations

This study has several limitations. First, it was based on a convenience sample of known stakeholders engaged in rabies control efforts. Even though we received a relatively high number of responses that were quite well distributed across countries and work sectors, our convenience sample is not representative and does not allow for reliable generalization. Second, the fact that the questionnaire was only in English limited the participation of non-English speakers. Third, for most countries (n=33, 69%) only one opinion was collected, while for the others we had several questionnaires and interviews available. Fourth, in the questions that asked respondents to compare pre- and during-COVID-19 scenarios, the recall bias is to be considered. Fifth, due to chronically poor rabies surveillance and variable levels of dog vaccination in most rabies endemic countries, the 3-tier classification system that we used is intended as tentative. Sixth, even though the manual cleaning of the survey answers was carried out systematically and the entire process was repeated twice to minimize error, it involved some subjectivity. Similarly, coding was done by only one person, and reflexivity and subjectivity were an integral part of the process.

5 CONCLUSIONS

Although difficult to quantify, the impact of the first year of the COVID-19 pandemic on rabies and rabies control efforts appears significant. All the components of the current One Health-grounded strategy to eliminate dog-mediated human rabies were affected, but dog vaccination was the most severely disrupted. Considering the number of years necessary for dog vaccination programs to mature and scale up, it is recommended that, as soon as possible, efforts should be reinstated and intensified. Areas of particular attention, and possible innovation, include:

- Mobilization of political will and resources towards achieving the Zero by 30 goal, especially in the animal health sector;
- Creation of a rabies-specific budget that can be dedicated to long-term animal and human vaccine procurement;
- Classification of animal and human vaccines as essential goods
- Design of mechanisms to support national and international animal and human vaccine sharing among rabies stakeholders;
- Identification of the most cost-effective, local, and sustainable methods of meeting the needs and challenges of different communities, particularly in having their dogs vaccinated, for example through the use of community-based vaccinators;
- Implementation of cross-border dog vaccination campaigns;
- Development of telemedicine systems such as hotlines to increase fast and hassle-free access to PEP, support Integrated Bite Case Management, and build participatory disease surveillance;
- Design of practical rabies awareness packages that target children and their parents.

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:

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ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BA-R, DN, KH and SB: Conceptualization. DN, KC, KH, SB, and SC: Study design and methodology. DN: Investigation. DN and KH: Analysis. KH and RS: Visualization. DN: Writing – original draft. BA-R, KC, KH, SB and SC: Writing – review & editing. All authors read and approved the final manuscript.

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Determinants of Rabies Post-exposure Prophylaxis Drop-Out in the Region of San-Pedro, Côte d'Ivoire

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Abstract: Despite the fact that death from rabies is 100% preventable with a course of post-exposure prophylaxis (PEP) treatment, canine rabies still causes about 59,000 human deaths worldwide annually, half of which are occurring in Africa. In Côte d'Ivoire, rabies remains a threat partly due to the high drop-out rate of the life-saving human PEP treatment among people exposed to dog bites. Each year, half of the victims starting treatment, do not complete the course. The current study therefore assessed the determinants for drop-out of the life-saving treatment among people exposed to rabies in the department of San-Pedro in Côte d'Ivoire.

Methods: A mixed-methods approach was used, including questionnaires, observation, individual interviews and focus group discussions, to gather socio-demographic and economic data from 235 participants about possible reasons for abandoning treatment. The study population consisted of patients and medical and veterinary health professionals who were selected using stratified sampling and purposive selection from a database available at the Rabies Center of San Pedro.

Result: The drop-out of PEP treatment was related to perception bias and a habit of low attendance of health care and vaccination centers in the population. Quantitative analysis shows differences between rural and urban areas and an association with age when it comes to treatment completion. The dropout rate was most significant among patients who, in case of other illness, did not routinely see a doctor or go to vaccination centers. The rate of abandonment was higher among those who believed that dog-related injuries could be easily treated at home, and who believed that a person with rabies could be cured without completing the preventive treatment. Insufficient provision of health information on rabies and logistic constraints related to the practical organization of treatment, including the long distance to the anti-rabies center and weaknesses in the patient follow-up procedure, did not contribute to the completion of PEP.

Conclusion: Established determinants for drop-out provide a framework for effective design and implementation of rabies control strategies to accelerate rabies deaths elimination efforts. In particular, access to PEP and community knowledge about rabies need to be improved and integrated in the health system and education system, respectively.

Keywords: rabies, post-exposure prophylaxis, treatment completion, One Health, Côte d'Ivoire

INTRODUCTION

Canine rabies causes approximately 59,000 human deaths per year, primarily in Africa and Asia (1). This is of particular concern because deaths from rabies infection are 100% preventable with the timely administration of human post-exposure prophylaxis (PEP) in the early stages of exposure to rabies (2). Rabies prophylaxis consists of wound management and the administration of several doses of rabies vaccine (3) at the earliest moment after exposure, and before any symptoms appear, to avoid death and disability (see different treatment regimen below). However, achieving compliance remains a challenge in the African region, thus limiting rabies elimination efforts. In Côte d'Ivoire, according to the National Institute of Public Hygiene (INHP), the number of people exposed to rabies per year is approximately 12,000. Nearly 50% of those who begin prophylactic treatment in vaccination centers do not complete it (Republic of Côte d'Ivoire INHP, 2019), while only 12% of the canine population is vaccinated (4). Official deaths from human rabies are around 20 people per year. However, there are likely more unreported deaths in the communities (Republic of Côte d'Ivoire INHP, 2019). Most deaths occur in children under 15 years of age (50%) and the majority is in rural settings (60%) in the southwestern and northern parts of the country, with Bouaké and San-Pedro topping the list (Republic of Côte d'Ivoire INHP, 2018).

In San-Pedro, the post-exposure vaccine management is the responsibility of the INHP's rabies vaccination service. This service is concerned with non-adherence to the treatment protocol. Each year, nearly 500 cases of exposure are notified and treated by INHP-personnel, according to the national protocol of post-exposure care for rabies. The vaccination process is organized over a 21 or 28-day period with protocols ranging from 4 doses for the Zagreb Regimen to 5 doses for the Essen Regimen. Costs for the Zagreb protocol are 32000 FCFA (51,92 USD) and for Essen 40000 FCFA (64,33 USD). This means that each dose of vaccine is 8000 FCFA (12,86 USD) (Republic of Côte d'Ivoire INHP, 2019). As mentioned above, about half of the bite victims who start PEP, abandon the treatment after the first few doses, which may have fatal consequences. The underlying reasons for the poor adherence are unclear in the Ivorian context.

The abandonment of PEP is not unique to the African context. In China, for instance, despite the affordability of the treatment and the proximity of the center to the patients, victims consult the vaccination center too late, as PEP must be administered within a set time frame to avoid death or lifelong disability (5). A low level of knowledge about rabies in the population has been frequently cited as the main cause. Studies carried out in Côte d'Ivoire also mentioned that a lack of awareness leads bite victims to abandon the PEP before completion. In the city of Abidjan, studies identified lack of knowledge as a factor for patients abandoning treatment (6). However, the major factor for treatment abandonment is "a lack of financial means" (7, 8). In 2016, a strong partnership was formed through the Rage-GAVI project to estimate the burden of rabies in Côte d'Ivoire (4), which included national actors such as the National Institute of Public Hygiene (INHP), the Ministry of Livestock and Fisheries (MIRAH), the Swiss Center of Scientific Research in Côte d'Ivoire (CSRS-CI), as well as international partners [Global Alliance for Vaccines and Immunization (GAVI) and the Swiss Tropical and Public Health Institute (Swiss TPH)].

The research to estimate rabies cases was carried out in the areas of Bouaké and San-Pedro, two cities with a high rabies burden (4, 8, 9). At the time, the Zagreb and Essen treatment protocols were in use. As it is generally known that costs prevent victims from completing the vaccination regime (10), the rabies centers in San-Pedro and Bouaké offered the new Thai Red Cross vaccination protocol free of charge alongside the traditional protocols, during the project period. This was expected to reduce the dropout rate (11). The Thai Red Cross protocol requires four appointments over a period of 28 days. Each time two doses are administered. In comparison, the existing Zagreb and Essen protocols, require 3 visits to administer 4 doses and 5 visits for 5 doses, respectively.

A the end of the intervention, the rate of non-adherence had decreased for all three protocols, reducing the dropout rate from 50 to 35% (9, 11). A large majority (78%) of the patients chose the Thai Red Cross protocol offered free of charge. Astonishingly however, the dropout rate, was significantly higher among those who used the free vaccine protocol than among those who chose a fee-based protocol.

In the face of the dropout that occurred during the period when the vaccine was free in San Pedro, the results of previous studies alone, the majority of which fall within the field of epidemiology, are not sufficient to explain the lack of adherence to treatment. The underlying reasons for low adherence to PEP in San Pedro appear to be differences in the way patients relate to exposure and use of the health center in case of illness in general.

Therefore, a more holistic approach is needed to understand non-adherence to PEP. Our study in social science, which is part of a long-term research partnership between human and animal health and a transdisciplinary One Health approach, therefore aims to describe and analyze the social determinants of non-adherence to PEP treatment that occurred during the period of free health care in San Pedro.

METHODOLOGY

Site and Period of the Study

The social science study took place from February to April 2019 in the rural and urban areas of the five (5) sub-prefectures of the San-Pedro Department, namely San-Pedro, Grand-Béréby, Dogbo, Doba and Gabiadji. San-Pedro is the fourth largest city of Côte d'Ivoire and located in the extreme southwest of the country, bordering Liberia to the west and the Gulf of Guinea to the south. The San-Pedro Department belongs to the Bas-Sassandra district (**Figure 1**). The department covers an area of 7,072 km^2, with an estimated population of 562,000 inhabitants, of which 140,000 live in towns and 422,000 in rural areas.

San-Pedro has port activity, agriculture, agro-industry and tourism. The inhabitants are also involved in small and medium-sized enterprises, of which trade and handicrafts are the main activities. This locality is governed by state authorities and traditional authorities such as village chiefs, notables, village elders and religious authorities.

In the area rabies is endemic and has a high prevalence. At the time of the study the department had approximately 800 dogs according to official data with an estimated dog vaccination coverage of only 7% (4). The main activities with which these animals are associated are guarding and hunting. Although many dogs have known owners, many stray dogs are left on the streets. Attached to the sub-prefecture of San-Pedro, a rabies center services animal bite and scratch victims from all five sub-prefectures.

Type of Study and Tools for Data Collection

This cross-sectional study collected both qualitative and quantitative data. For the quantitative component, a questionnaire was developed to collect data on socio-demographic and socio-economic characteristics of the bite victim. Questions were answered by the victims themselves or by their guardian at the health center. Other data collected included information on the etiological understanding of rabies, vaccine prevention, therapeutic itineraries, and perceived challenges of vaccine management. Additional qualitative data were collected through semi-structured individual and group interviews. Interview guides were developed and administrated to patients or their guardians and to health professionals. The objective of the qualitative interviews was to discuss aspects in depth, including the motivations for therapeutic behavior, that the questionnaire alone did not cover.

Target Population

The study covered two types of stakeholders: community members and professionals in the human and animal health sectors. People in the first category were composed of dog bite and scratch victims and their guardians who had visited the rabies center between June 2017 and March 2018, during the free vaccination period. During that period, 35% of patients did not complete their treatment.

People in the second category were composed of personnel from the National Institute of Public Hygiene (INHP), the Ministry of Animal Resources and Fisheries, the local committee for rabies control, and the public and private veterinary services. With them, we discussed how management and control of rabies in the department were organized.

Sampling Method and Data Collected

Respondents for the questionnaire survey were selected using a stratified random sampling technique to select a representative sample. Data for 409 patients (and their guardians) was available at the INHP rabies center to which we had access through the consent of the GAVI project. After sample size estimation (12) for quantitative survey studies, a sample size of 199 victims was determined. Some information on socio-demographic characteristics of the patients was already included in the case files such as sex, age, place of residence, occupation, vaccine type used, number of visits to the center and contact details. Based on this information, we used proportional stratified sampling as explained by N'Da (13) to then randomly select the 199 individuals needed for the study. The stratification process considered place of residence, sex, social class, and type of vaccine used. In cases where patients could not be contacted, they were replaced with other patients with the same characteristics.

During the recruitment process, which included the examination of the victims' files at the rabies center to select a sample representative of the whole pool of patients, we faced several challenges. Since this study commenced 2 years after the project, contact details for several selected patients had changed. Furthermore, patients who abandoned treatment turned out to be more likely to decline to participate. Replacements were made from the original database each time, until the pool of drop-out patients was exhausted. Thereafter the required sample size was reached by including victims who completed the treatment. The lead author visited the selected households to administer the questionnaire using the software "ODK collect" on a tablet (http://dl.acm.org/citation.cfm?id=2369236; https://getodk.org/). On a few occasions, when the respondents did not want to meet in person, the questionnaire was administered via phone.

Less than a third (28%) of the respondents were victims themselves, due to the fact that most dog bite victims are underage and interviews were therefore conducted with their parents/guardian.

Our respondents were composed of women and men with different backgrounds, religious faiths, educational levels, marital status, ethno-national identities, from different household types and engaged in different economic activities.

To gain further insights into the obstacles victims face to complete the treatment, qualitative methods were used to collect data on health seeking and medical treatment habits in general, including treatment of bite wounds and access and affordability of care (14). Informants for the individual interviews were selected from both categories, drop-outs and people who had completed the treatment, using a convenience sampling technique. If they consented, the interview was held immediately

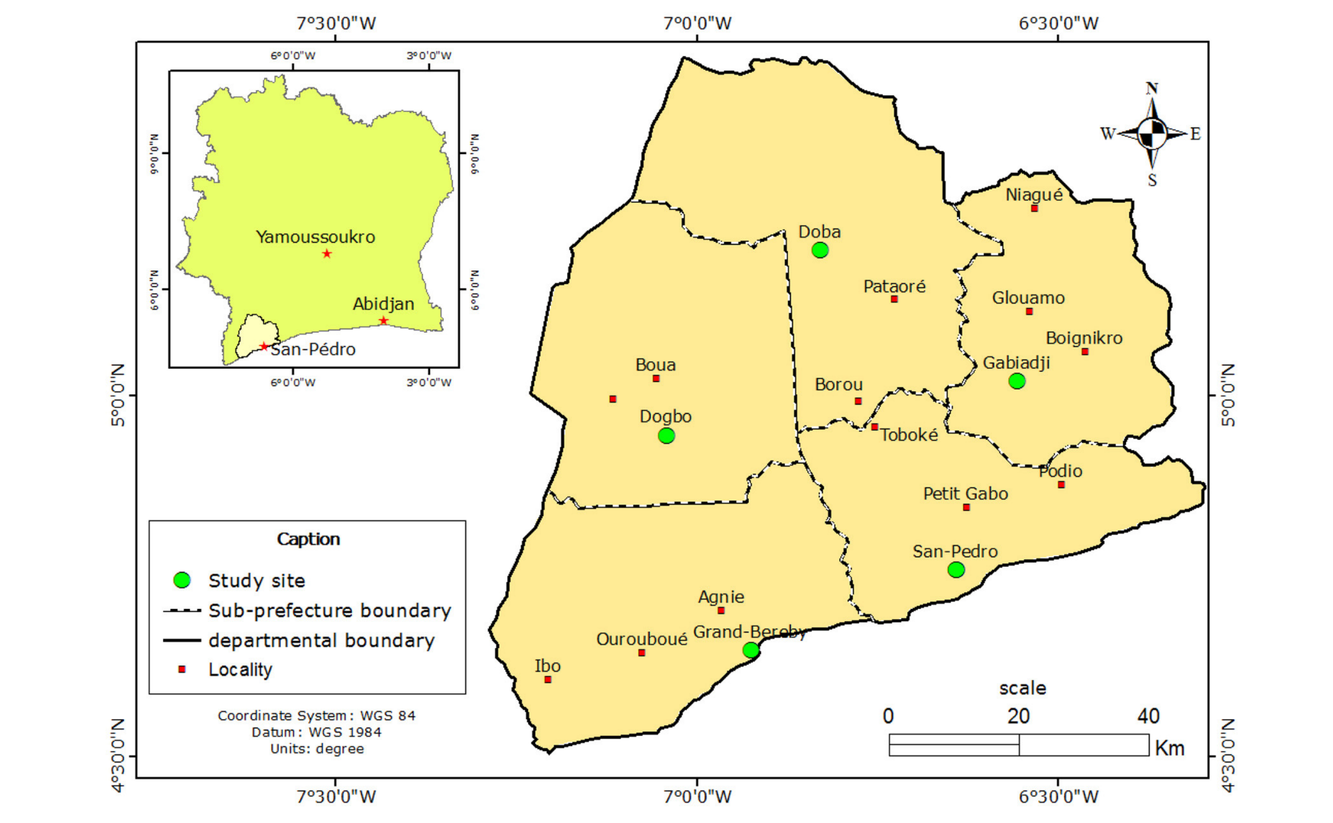

FIGURE 1 | Study site (*N* = 5), cross-sectional study conducted from 14 February to 28 April 2019 in the department of San-Pedro, Côte d'Ivoire.

after the questionnaire. After 25 individual interviews with patients or their guardians, including 15 who had abandoned and 10 who had completed their treatment, the interviews were stopped, as saturation was reached. From the institutional side, two human health professionals, two animal health professionals and a representative of the local rabies control committee in San-Pedro were interviewed. The interview guide covered questions about awareness campaigns and care practices, as well as any difficulties encountered for both activities.

Two focus group discussions (FGD) with six participants each were conducted at the rabies center of San Pedro to deepen the understanding of reasons for children to abandon treatment: One FGD was conducted with the parents or guardians who had accompanied the child victim to the meeting, the second FGD—held in parallel—was conducted with the children themselves. Bite victims were selected after the administration of the questionnaire if they were willing to participate. The objective of the FGDs was to assess the children's knowledge of rabies and to compare their level of knowledge to their parents'.

Ethical Dimension and Conduct of the Study

This study was covered by the ethical approval of the GAVI Project: ethical approval (N/Ref: 072/MSHP/CNERkp) from the National Ethics Committee of Côte d'Ivoire. The agreement of the Northwest and Central Ethics Committee of Switzerland was also obtained [Ethics Committee of North Western and Central Switzerland (EKNZ) Basec 2016–00,220]. At the research site, we sought survey authorization from the Ministry of Higher Education and Scientific Research, the INHP, the Directorate of Veterinary Services, the regional prefect and the sub-prefects. During our visits to households, we presented these authorizations and gave an oral explanation of the purpose of our presence before the start of the interviews in order to obtain the participants' informed oral consent. Thus, only those who agreed were interviewed.

Data Analysis

The quantitative data was analyzed using STATA 14.2 (StataCorp; College Station, USA) and R software version 4.1.2 (Core-Team. R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*, 2019).

Descriptive analysis was carried out with different socio-demographic characteristics related to the respondents. This was followed by general linear regression (GLM) modeling with binomial distribution to investigate associations between treatment completion (yes, no) and variables on socio-demographics of the victim. Models were selected based on backward elimination from an initial null model that included all variables. A likelihood ratio test (LRT) was used to test each variable and remove those with the highest *p*-value at each iteration from the maximal model until only statistically significant terms remained. Model fit was assessed by plotting the coefficients and inspecting the residuals against the fitted values. To determine the influence of statistically significant variables, LRTs were used. The package "MASS" was used in R (15).

Analysis looked at the association between treatment completion as dependent variable, and the characteristics of the bite victim as independent variables. These explanatory variables were "sex", "age bracket", "education level", "religion" and urban or rural "zone", "district" as well as "number of people in the household" and household "income".

For the qualitative data, content analysis was conducted. In an iterative inductive process, the transcribed interviews were coded, and themes identified in relation to the research questions (16). We have selected quotations to deepen and illustrate aspects of the quantitative results. To analyze and interpret the findings, we drew on the following theoretical texts: the concept of cultural capital (17), which stipulates that practices of care are influenced by all the know-how available to the individual as a result of the socialization at the family and environmental level and the conceptual framework five A's of access (availability, accessibility, affordability, adequacy, acceptability) for access to care (14) with a particular focus on accessibility and affordability of care.

RESULTS

Socio-Demographic and Economic Characteristics of Patients and Respondents

A total of 199 individuals participated in the study from all 5 sub-prefectures of the San-Pedro department in Côte d'Ivoire. A summary of the respondents' socio-demographic characteristics is shown in **Table 1**. Most suspected rabies patients were male children (75.3%) of which 65.2% were in school. The average age was 10 years and as a result, most of our respondents (72.3%) were the guardians of the underage bite victims. Only 27.6% of those interviewed were themselves victims (adults). Respondents' education levels were as follows: primary school education level (29.8%), secondary school education level (29.8%), higher school education level (20.2%), no school education level (20.2%). The average monthly household income was 100 000 FCFA (160.84 USD). Participants resided in the sub-prefectures of San-Pedro (83.9%), Gabiadji (6%), Grand-Béréby (5%) and Doba (2%). The majority of participants lived in urban areas (85.4%) compared to 14.5% from rural areas. They practiced Christianity (69.4%), Islam (21.6%), other religions (4.5%) or no religion (4.5%). Treatment completion was 81.9 percent and drop-out rate was 18.1 percent.

TABLE 1 | Summary of the socio-demographic characteristics of victims only and of all respondents.

Socio-demographic characteristics		Frequency	Percentage (%)
Sex of victims	Female	49	24.62
	Male	150	75.38
Education of victims	No formal education	23	11.56
	Primary	130	65.33
	Secondary	38	19.10
	Higher	8	4.02
Status of the respondents	Victims	55	27.64
	Parents	144	72.36
Education of respondents	No formal education	40	20.20
	Primary	59	29.80
	Secondary	59	29.80
	Higher	40	20.20
Place of residence of victims and respondents	San-Pedro	173	86.93
	Gabiadji	12	6.03
	Grand-Béréby	10	5.03
	Doba	4	2.01
Zone of victims and respondents	Urban	170	85.43
	Rural	29	14.57
Religion of victims and respondents	Christian	138	69.35
	Muslim	43	21.61
	None	9	4.52
	Other	9	4.52
Victim's vaccine completion	Complete	163	81.91
	Drop-out	36	18.09

Factors Associated With Treatment Drop-Out

Patient Profile

The first model of our analysis showed that only two characteristics of the victim are associated with treatment completion and discontinuation: age and zone of residence (rural or urban). The odds of abandoning treatment were significantly higher when the victim was between 25 and 29 years of age. Moreover, urban residents were more likely to complete the treatment than their rural counterparts (**Tables 2**, **3**). According to the qualitative data, this may be due to distance (see below).

Knowledge of Rabies Etiology Among Respondents and Patients

Data from the questionnaire showed that a total of 94% of respondents had heard of rabies as a dog disease while 6% did not know it was a disease affecting canines. The disease was known under different terms and in general, people who lived in urban areas and had received formal education called rabies primarily "rabies". Those who lived in rural areas and had little formal education called rabies "canine disease" or "canine madness", which is often a direct translation from local languages (Baoulé, Malinké) into French.

Respondents generally knew that humans could contract rabies and dog bites were identified as the main mode of transmission (74.7%). They mentioned aggression (40%), drooling, hair loss (30%), wasting (10%) and excessive barking (15%) as the main symptoms of rabies in dogs. In humans, symptoms were completely unknown (58.8%) or poorly known (associated with diarrhea, drooling, wasting, hair loss and vomiting, 27.7%; aggression, 13.4%).

As far as knowledge about the severity of rabies is concerned, the study found that 81.9% of respondents did not consider it to be a serious disease, neither for humans nor animals. While 27% said that it is impossible to cure a dog with rabies, 20.6% said the opposite. The remaining 52% did not know whether a dog could be cured of rabies or not. For humans, 29.71% were certain that rabies could not be cured, while almost half of respondents (43.43%) were certain that a rabies patient could be cured. The remaining 26.86% were unable to respond due to lack of information.

The interviews revealed little information about the knowledge on the signs of the disease, but some identified rabies as a dog disease, not a human disease. However, they had difficulties giving accurate descriptions about symptoms of the disease. The focus group of patients' parents on knowledge of rabies etiology showed low knowledge of the severity of rabies. Respondents seemed to be aware of dogs as vectors of the disease, but were not aware of the outcome of the disease.

There was an indication that people from urban areas had more knowledge or access to knowledge regarding the urgency to seek treatment as statements show:

Q1: *"Well, people said, especially my neighbor, to quickly go with him to the hospital that if I waited, he was going to be like the dog, too. Otherwise, I do not know much about the disease."* (Mother of non-compliant patient, 40s, no formal education, urban resident, IDI).

Q2: *"Well, what I know is that if a dog bites you, it can make you sick. If you don't go to the hospital to get antibiotics or a tetanus shot, it can give you tetanus. But, if you go and get at least one vaccine, you are safe."* (Father of non-compliant patient, 40s, secondary school level, urban resident, FGD).

The focus group conducted with exposed children revealed that they had a much higher level of knowledge on rabies than adults. More than half of children had an accurate description of rabies symptoms, compared to adult patients and parents from another FGD.

Three-thirds of children identified rabies as a disease transmitted by dogs and cats and were clear about the mode of transmission and the consequences of infection.

Q3: *"[...] dogs transmit rabies. When they bite someone, if they don't go and get vaccinated there, one day they can go crazy and act like a dog."* (Patient, 9 years, primary school level, urban resident, FGD).

TABLE 2 | Model results of association between post-exposure rabies treatment drop-out and socio-demographic and economic characteristics of rabies victims in rural and urban areas with 95% Confidence Interval (CI) and *p*-value.

Variable	Odds Ratio	2.5% CI	97.5% CI	*p*-value
Model 1*				
Age (years)				
0–10	1	NA	NA	NA
11–15	16.68	0.541	46.661.399	0.345
16–18	0.743	0.039	44.377.313	0.786
19–25	33.801.620	0.717	148.844.944	0.107
26–29	67.998.961	159.181.655	308.483.962	0.009∞
≥30	0.621	0.16447036	18.871.908	0.434
Zone				
Rural	1	NA	NA	NA
Urban	0.3653479	0.14204669	0.9753399	0.03857*

∞*Significant p-value at the 5% level.* *Modeling the victims' characteristics associated with treatment abandonment. NA, not applicable.*

TABLE 3 | Likelihood ratio test χ^2 values of all significant variables in the final generalized linear model on the probability of abandoning treatment.

Variable	χ^2	*p*-value
Zone (Urban area)	4.032	<0.044
Age (years)		
26–29	11.1	<0.049

Perception Related to Rabies Exposure and Post-exposure Treatment

Interviews and data from focus groups revealed different types of perceptions of dog attacks and vaccine treatments. The first perception was commonly expressed by parents of non-compliant patients living in urban areas whose education ranged from non-formal to secondary education. These respondents perceived dog bites and scratches as ordinary injuries that could easily be treated at home. The attack of a person by a dog was presented as a natural occurrence that had no cultural significance. Some participants compared a dog bite to an insect bite, which they felt should not be interpreted in any other way, nor associated with the spiritual world.

Q4: *"When a dog bites a person, it is not something spiritual. Otherwise, people will say that when a mosquito bites you it is a curse or a blessing. In any case, it doesn't mean anything."* (Compliant patient, 30s, secondary school level, urban resident, IDI).

Other participants interpreted dog bites as a mystical act. For instance, they considered dog attacks as an omen. An omen is considered positive, when the aggression has a positive connotation in favor of the aggressed (delivery of a spell, good news in progress) or negative when the aggression is not in his or her favor (a spell cast on the victim, an upcoming misfortune etc.). This perception of a dog aggression as a mystical act led study participants to say that they were destined to suffer dog aggression.

Q5: *"When a dog bites you, it can be a sign of misfortune or blessing. So, if it bites you, you must pray to God. A dog does not bite everyone and not in vain."* (Father of non-compliant patient, 40s, primary school level, rural resident, IDI).

Nevertheless, whether the respondents considered dog attack wounds as ordinary wounds or as an omen, they went to the health center for the same reason, namely wound care. In the case of both groups, care was more focused on managing the wounds for prompt healing than on any illness that the bite may cause. People from both groups did not, however, deny that being bitten by a dog is a real danger to the victims. The role of prophylactic treatment was described as supplementary wound care:

Q6: "*We were going to the hospital to treat the wound. But his wound healed before the vaccine was finished. So, we didn't go to finish the vaccine anymore."* (Father of non-compliant patient, 40s, secondary school level, urban resident, IDI).

Treatment Habits

The data of patients' therapeutic itineraries after exposures revealed a plurality of itineraries. Almost half of the patients had used the vaccination center as their first point of contact for treatment. However, only 10% went immediately. The rest (90%), waited for hours, days, and weeks without seeking treatment before going to the rabies center. The reasons for this delay presented three types of health care seeking behavior. Almost three quarters decided to seek treatment at home first, before going to the rabies center (74%), while 25% used community health centers first. The remaining 1% used traditional healers.

Q7: *"We went late to the vaccination center because we were treating her at home. As the wound became bigger, we went to see the local doctor and he asked us to quickly go to the place where they treat dog bites, because if we don't go it's not good."* (Mother of non-compliant patient, 50s, no formal education, urban resident, IDI).

However, nearly half of the patients who used home treatments, also received vaccination doses at the same time and almost all patients (80%) practiced home care at one point for healing. Patients living in rural areas used mostly a herb, "*sékou touré*" *(eupatorium odoratum L.*), that usually grows on the side of the road, mostly in villages, and cassava leaves *(manihot esculenta)* as well as the cassava itself for wound care. "Black powder" concocted by traditional practitioners was purchased and used with hot water. In contrast to rural residents, urban residents used disinfectants, salt and bleach, as well as tablets purchased from local shopkeepers. Remedies varied by area of residence. However, the use of hot water remained common practice. The logic associated with this use of complementary or alternative care was based on trust placed in these remedies and the experiences of use.

Q8: *"Plants are medicines. One plant can cure several diseases. Now, if you know [...] the plants, why waste all your time in the hospital instead of healing yourself [with the plants] [...] It's for the wound. These are things that everyone knows. In all my life, I have never seen hot water aggravate a wound. On the contrary, our parents applied it on us and we didn't die."* (Father of non-compliant patient, 50s, high school level, rural resident, IDI).

Q9: *"In any case, that's how it is with us. Before we run to [the health facilities], we take care of ourselves first. Otherwise, why should our parents have taught us these things?"* (Mother of compliant patient, 30s, secondary school level, rural resident, IDI).

Hence, most respondents showed some attachment to complementary treatments such as self-medication, street care or traditional treatments when cases of illness occurred. For the care of certain conditions such as malaria, some respondents did not necessarily consult a health center. Instead, they used traditional treatments and bought drugs in the informal market. In general, patients did not associate wound management with vaccination centers, even though patients were used to receiving one injection at health centers at the beginning of any wound treatment to prevent tetanus.

An important finding of our investigation of health care seeking habits is that the majority of the patients who dropped out from the vaccine treatment, generally only used health centers for what they perceived as serious diseases. People would go to the health center right at the onset of an illness for diagnosis or at the later stage of a disease for intensive care when other treatments had failed. just over half of the participants reporeted health centers as their preferred place for emergency treatment (55.7%).

Organization of Rabies Vaccination

Accessibility and Transportation Costs

An important observed factor of access to PEP is the location of the immunization center. It is located centrally in the regional capital, San-Pedro, and patients are distributed throughout the department of San-Pedro and its five sub-prefectures (San-Pedro, Béréby, Dogbo, Gabiadji, and Doba). The distance between San-Pedro and Dogbo is approximately 102 km, 55 km for Grand-Béréby, 104 km for Doba and 45 km for Gabiadji. Many of the villages of the mentioned sub-prefectures are located even further away from San-Pedro than the main town and are often in remote areas that are difficult to access.

During visits to the vaccination center, most patients used intercity minibuses communal cabs or motorcycles. The duration of the trips often varied between 1 h, 2 h, half a day or even a whole day, depending on the area of residence, the means of mobility and the state of the roads. However, particularly in rural areas, that roads were impassable during rainy season.

The results on geographic accessibility show that for the population, costs and exhaustion linked to the distance constituted an important obstacle for patients to access care. For most patients from remote areas, the duration of the treatment and frequency of visits requested were a hindrance to care completion.

The average monthly household income was 100000 FCFA (160.84 USD) for four people per household. The cost of travel to and from the vaccination center per individual was estimated at between 0.85 USD and 1.70 USD for the neighborhoods closest to the vaccination center and about double for the more distant neighborhoods. Individuals from remote areas paid between 5.10 USD and 20.40 USD. These travel costs were doubled if the patient had to be accompanied, which was often the case due to the high number of underage bite victims. In general, patients were accompanied by at least one relative when visiting the center.

This means that even for people who live close, transportation costs correspond to the daily income of one person. For those living further away however, the return-fare to the rabies center for the patient and a person accompanying the bite victim amount to a monthly income of one person. We can conclude that transportation costs and indirect costs linked to the loss of income played an important role in the decision to discontinue the treatment.

Pre-exposure Management of Rabies

Data collected from the patient and parents of patient's interviews revealed that most respondents (94%) had heard about rabies through various channels. The most frequently mentioned was at school. Respondents reported having received information about rabies during their primary and secondary school years. They also said that they had not attended any sensitization events for the prevention of rabies, except from some information received during their visits to the rabies center. They had hardly ever attended an awareness campaign to inform themselves about the seriousness of dog bites.

The data collected with health professionals revealed difficulties in the management of rabies in San-Pedro. They identified the problem of funds as a constraint to the implementation of general awareness activities for the population. Although outreach campaigns are organized, at least during the Word Rabies Day, they are generally directed at community leaders who in turn are responsible for sharing the information received with their communities. How this is done, with what means and to what extent is unclear.

Patient Follow-Up in the Post-exposure Management

As mentioned above, the GAVI project offered free vaccination from June 2017 to March 2018. However, our results showed that 95.98% of the people who visited the rabies center for treatment had not been informed in advance, i.e., before their arrival at the center, of the availability of free vaccine. This suggests that the project has not managed to communicate successfully to the population on the availability of the free vaccine.

Q10: "*I didn't know there was a free vaccine. It was when I arrived that I knew. So I agreed to use it, because I didn't have much money.*" (Patient, 20s, Primary school level, male, urban resident, IDI).

Furthermore, asked about their vaccination sessions at the rabies center, half of the patients reported that they had not been further sensitized about rabies, its severity or the need to continue their treatment to term (52%).

Q11: "*They didn't explain anything about rabies. When I arrived, he told me the price of the vaccine and then he filled out some paperwork and then when he vaccinated me, he gave me my booklet and told me to come back in a week.*" (Patient, primary school level, male, 30s, urban resident, IDI).

Regarding the management of patient's follow-up appointments for their next doses, the vaccination center keeps records in a patient file in which each patient's date of the follow-up visit is recorded. However, this information is not systematically used to follow up on patients. Patients are not contacted when they do not attend the scheduled appointments. Health workers informed us that the rabies center in San-Pedro lacks a social service that exists in Abidjan, and which is dedicated to follow-up and any social aspect of patient care. Hence, a follow-up system or device to remind patients of their upcoming appointments is lacking in the rabies center of San-Pedro.

DISCUSSION

Our research revealed perception bias of the severity of rabies and a habitual low attendance of health care and vaccination centers of the population. Quantitative analysis suggests differences between patients from rural and urban areas regarding treatment completion. Drop-out was also higher among those who believed that dog-related injuries could be easily treated at home and who believed that a person with rabies could be cured without completing the preventive treatment. We also found a lack of knowledge on rabies and challenges in the practical organization of treatment.

Our study shows that offering the vaccine free of charge has helped to bring the rate of PEP treatment abandonment down from 50 to 35%. This is in line with findings of a study in Chad which found the same effect when comparing dog owner-charged with free vaccination campaigns (18, 19). Despite some challenges of the implementation of the campaign in Côte d'Ivoire, results strongly suggest that free PEP alone does not ensure compliance.

This relates partly to affordability of the treatment which is not just related to the direct costs of the vaccine. Indirect cost of transportation, loss of study time and income also must be taken into consideration (10, 14). For most of the non-observant patients at school age, repeated absence at school was perceived as awkward and those working in the informal sector felt that they could not afford more days without income. How costs may be shared between the patient and the public health system has to be further reflected upon and goes beyond the scope of this article.

A holistic perspective when looking into health care seeking behavior in context of rabies treatment completion therefore, seems to be a more suited approach. This will enhance our understanding of the populations' care practices in case of dog bites that expose victims to rabies.

As a first step, we have to look at dog bites as wounds and ask how such wounds are generally treated. As the data show, health centers are only consulted when people judge the situation as severe. In our case, according to the study participants, a bite wound by a dog is often not perceived as severe. This has to do with a lack of knowledge, which corresponds to findings from studies from Côte d'Ivoire (7, 20) and elsewhere in Africa (21–23) and beyond (5). Yet our study population were all people who consulted the rabies center for care, where they received a consultation from health workers. Recent studies from Chad revealed that the knowledge of health workers needs to be improved (24) and for Côte d'Ivoire we can conclude that the way counseling is provided has to be improved. Furthermore, the qualitative data from both the victims and the health personnel also showed that the follow-up system has to be strengthened. According to the health workers, the rabies center in San Pedro lacks social workers or a system assisting with patient follow up after they received their first dose. In view of the dropout rate (35%) despite the free vaccine, such a service may be crucial to improve treatment completion.

However, the whole chain of events regarding wound treatment and follow up care should be considered. Numerous studies from the field of medical anthropology have highlighted that care practices in Africa are best conceptualized as a therapeutic continuum with different medical options of which the patient takes different combinations (25). For instance, as observed in our study, victims may first treat the bite wound at home before seeking medical care at the health centers, which is absolutely necessary to combat the rabies pathology, and later complement the treatment of the wound with herbal baths at home.

The sample of this study was based on patients from the list of the rabies care center, hence excluded people who had exclusively consulted traditional healers and only used home remedies. It is known from studies estimating the burden of disease for rabies, that many cases go unreported because they are treated at home (4, 25, 26).

During field work, a man said that his wife and son must have died of rabies after having been bitten by their rabid dog who left their house afterwards. For years, he had thought it had been witchcraft and consulted a witchdoctor to protect himself and the younger children (Rose N'Guessan, personal communication, San Pedro, 2020). Such and similar cases highlight that many deaths are never reported. It was by serendipity, that such a case was reported.

To save lives, it is crucial that the awareness of rabies is raised in a tailor-made manner in which cultural barriers are addressed. Moreover, traditional healers need to be sensitized. Because of shortcomings of the health service provision, part of the population is used to rely on traditional healers and their advice is crucial. Rather than seeing them as obstacles, if sensitized, they may help to transfer bite victims in good time to health facilities where patients can receive PEP.

The need to actively improve the connection between health centers and the communities they serve is evidenced by the fact that many bite victims are hard to reach, not only for health care provision, but also for research. As mentioned in the methods section, many victims who had abandoned the treatment were no longer reachable 2 years after the end of the campaign on the phone number they had provided. During this period, the Ivorian state requested all numbers to be registered with ID cards for security reasons, and if that did not happen, the number was blocked. This and other reasons linked to difficulties of replacing a lost or stolen phone due to poverty, have made contacting hard to reach people, difficult. Without the means to follow up on patients, health workers and researchers cannot establish if treatment abandonment has had fatal consequences. This could be addressed by registering a second phone number from a relative who has not been bitten by the dog. Furthermore, some of the patients that had dropped out, often refused to participate in the study. Because they had not completed the treatment, some may have feared some kind of reprimand for non-compliance.

Dog bites and scratches are treated like other ordinary wounds at home, and more than half of interviewed patients believe they can be easily treated with home remedies. More than half believe that if a patient does not follow their preventive treatment correctly and is later found to have rabies, they can still be treated and cured. This way of thinking leads to lack of interest in or neglect of preventive treatment.

Regarding the lack of knowledge that emerged as a factor of non-adherence in the analysis of our data, the literature shows that the therapeutic practices observed in patients are a reflection of what they know about their condition as a sick person or as a person at risk (27). We observed that the less respondents know that rabies is incurable, the more they abandon preventive treatment.

More than half of the respondents (72.4%) were parents of patients, most of whom had heard about rabies during their primary school years and had an educational level between primary and secondary education. The average age of these parents was 45 years. These factors accounted for "vague" and "erroneous" knowledge about rabies, which influenced their treatment practices. Purchasing power and the decision to treat which does not necessarily belong to the exposed person, forces children to abandon treatment even when they have good knowledge of the disease (28). Representatives of the rabies committee in San Pedro reported that a child who was bitten by a dog in one of the districts had asked her parents to be taken to a health facility, as she had been sensitized at school, but the parents took her to a traditional healer and she later died (representative of rabies committee in San Pedro, transdisciplinary workshop, Abidjan, 2020). That children often do not have a say in the treatment they receive has also been shown in Tanzania in a similar context (29). This shows that sensitization campaigns at schools are not sufficient to make sure all bite victims benefit from PEP. From our data, it seems that the sensitization strategy does not contribute effectively enough to making the population aware of the dangers associated with dog bites in order to increase adherence to vaccination.

In Côte d'Ivoire, a study in Abidjan also mentioned this factor of non-adherence to prophylaxis (6). Studies in Abidjan and in San-Pedro and Bouaké, described the lack of knowledge of rabies as an important factor shaping people's attitudes and practices (7, 20). In Abidjan it was found that low knowledge about rabies correlated with non-vaccination of dogs by household heads in Abobo (30). In Burkina Faso, a study showed that rabies was not known to most of the respondents (31). Moreover, in a Cameroonian study with a predominantly dog-owning population, rabies was attributed to a sexually transmitted disease (32), which did not contribute to the use of rabies vaccine by dog owners. In China, the lack of knowledge about rabies was noted as the main reason for inappropriate wound treatment and delayed PEP (5, 33).

Yet this was not found in all studies. Research conducted in Abidjan on the abandonment of PEP for rabies showed successively that the treatment drop-out was linked to the dog owners' refusal to cover the costs of the patients' treatment, as well as the lack of financial means for the purchase of vaccines (7, 8). Costs also seemed important in our study, particularly indirect ones, but our results show that the role of financial means may have been overestimated in other studies. Our study was conducted in a period during which PEP was offered for free. This allowed us to see a more nuanced picture about causes contributing to the dropout rates, where the lack of knowledge and general treatment habits of patients together rank higher. However, our study which was not conducted in the countries capital, also laid bare the difficulties linked to accessing health facilities from remote areas (accessibility) and afford PEP for rural populations (affordability) (14).

Our analyses show that low attendance at health and vaccination centers in patients' habits was a determining factor in non-adherence to prophylactic treatment. It was observed that patients abandoned the treatment, because they rarely attended vaccination and health centers when they had other types of diseases. In case of illness, and depending on the perceived risks of severity, as well as the habit of care by assimilation of the disease or exposure, they prefer to resort first to traditional treatments or self-medication. Some use health centers only once when they are ill to validate the diagnostic hypothesis before returning for traditional or self-medication treatment alone or in addition to the treatment recommended by the physicians. For others, return to health centers is only favored when the disease worsens, and they no longer have other options. The course of the disease guides and defines the therapeutic resources used (34). Self-medication and traditional treatments are very common in Africa (25, 35). In the absence of a well-functioning public health system with trained medical personnel, medical pluralism that alternative treatments in is parallel and intertwined health care practices are likely to persist. Therefore, underserved and isolated communities heavily rely on traditional healers and self-medication. As a result, health centers are only visited when traditional treatment fails and when there is no other option. In the case of rabies, this is, unfortunately, always too late.

This weight of therapeutic habits among patients is strongly linked to their level of health education. In our context, it therefore highlights the importance of therapeutic habit in the intention to adopt a new desired behavior, which in this case is compliance with prophylactic treatment.

The therapeutic education acquired by patients in their experience, either through the family, the groups to which they belong and the environment, is reproduced when cases of disease occur (17), especially when they have no knowledge of the diseases. Thus, the low attendance of health and vaccination centers in the patients' habits in general determines the non-compliance with prophylactic treatment. Our results also showed that the age group of 19–25-year-olds has a higher risk of not completing treatment which could be related to their lack of experience as young adults.

Results reveal a centralization of rabies treatment that proved to be a hindrance to PEP. Due to the requirements of conservation of the vaccines and the specific character of their administration, patients were therefore obliged to travel long distances to the health center on routes that were difficult to navigate at least four times within a period of 28 days. Thus, the long distances traveled by patients due to poor track conditions and unavailability of transportation led to the retraction of their vaccination decision. These factors, combined with the cost of transportation, which was sometimes considered excessive, especially since patients were always accompanied by at least one parent and belonged to households with a monthly income of only 160.84 USD, influenced the decision whether to use the center. Thus, the lower social categories will consider proximity in their choice of health center, unlike those who have a more comfortable social position. In the abandonment of rabies PEP and animal vaccination, a study conducted on the determinants of rabies post-exposure prophylaxis abandonment presented this factor as well (36). It was found that populations who resided far from the vaccination center took significantly longer to reach the vaccination center to start treatment. It was showed how accessibility and lower economic status were the main factors associated with delayed initiation of prophylaxis for rabies prevention (37, 38).

As far as the limitations of the current study are concerned, it is important to note that the initially desired sample size of 209 people was not reached. Only 199 people could be interviewed, which gave us 95% of our initial sample. With this subsample, instead of a dropout rate of 35% as found in the complete database of 409 patients, the study was conducted with a sample showing a dropout rate of only 18%. Therefore, there is a risk that the quantitative results are slightly biased and can only identify overall tendencies, but nevertheless complementing our findings from the qualitative data

CONCLUSION

Rabies should be controlled using a One Health approach including vaccinating 70% of the dog population (39). However, in view of notoriously low dog vaccination coverage in many low- and middle-income countries, this study has tried to elucidate factors that would help to improve the situation for dog bite victims. Non-compliance with prophylactic treatment in vaccination centers in San-Pedro and throughout Côte d'Ivoire by persons exposed to rabies remains a real obstacle to the success of the national strategy for rabies control in Côte d'Ivoire. This study identified the key determinants of non-adherence to PEP in San Pedro by using a holistic perspective when looking into health care seeking practices. A combination of low detailed knowledge and therefore misjudging the severity of rabies as well as reliance on home care and traditional medicine in the context of low, unaffordable health care coverage emerged as the main factors for abandonment of PEP. Lack of knowledge highlights the importance of communication and awareness raising about rabies in the different socio-cultural groups of the communities.

These results show that many efforts need to be made to achieve the goal of eliminating rabies by 2030. It therefore appears necessary at the institutional level for health workers to strengthen and operationalize the monitoring and awareness system. By setting up a patient awareness and follow-up service that can widely explain to patients and guardians the dangers of attacks by dogs, cats and rodents, and that can contact them for appointments would contribute to raising awareness of the danger of rabies and the importance of patient care. It is also necessary for health officials to reinforce the training of community health workers on the dangers of dog bites to enable them to be "equipped" to better refer patients to the rabies vaccination center. Equipping the local rabies control committee set up in San-Pedro during the GAVI project to organize awareness campaigns by group of neighborhoods, by school and from local radio stations is also necessary. Another action to be implemented will be to integrate a rabies program in primary and secondary schools to improve children's knowledge of rabies, even though our study reveals their lack of decision-making power in their care. It is also essential to bring the center closer to the population by creating representations in two other sub-prefectures. New intradermal vaccination schedules with fewer doses promoted by WHO will allow to reduce indirect costs and are likely to increase completion rates.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The study was reviewed and approved by the ethical approval of the GAVI Project (N/Ref: 072/MSHP/CNERkp) from the National Ethics Committee of Côte d'Ivoire. The agreement of the Northwest and Central Ethics Committee of Switzerland was also obtained [Ethics Committee of North Western and Central Switzerland (EKNZ) Basec 2016-00,220]. Oral informed consent was obtained by study participants.

AUTHOR CONTRIBUTIONS

RN'G: sociologist, carried out the study the general writing of the document. KH-T: social anthropologist and contributed to all stages of the project from conception to finalization. DA: socio-anthropologist, contributed to the academic supervision of the work, and contributed to the correction and development of data collection tools. ST: physician, her contribution consisted of providing guidance, and correcting the document. VK: veterinarian, guided this work, and contributed to the correction. AN: veterinarian, participated in the guidance of the work, and helped with the drafting of the manuscript. GN: medical entomologist and epidemiologist and contributed to the correction of the document. IK: professor of Sociology, supervised this work at the academic level, and contributed to the sociological orientation of this work and to the editing. KK: epidemiologist, contributed to statistical analysis, and the correction of the document. BB: Veterinary-epidemiologist and specialist in rabies control in Africa, the principal investigator of the project, and has contributed to all stages of the project. All authors contributed to the article and approved the submitted version.

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"When a dog bites someone": Community and service provider dynamics influencing access to integrated bite case management in Chad

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This study aims to identify factors on the community, the human health and the animal health provider level that determine access to Post Exposure Prophylaxis (PEP) and animal rabies diagnosis in the light of a future integrated bite case management (IBCM) approach for rabies control in Chad. The study was embedded in an overall project conducted from 2016 to 2018, to determine rabies burden and vaccine demand in West and Central Africa. Data collection took place during the projects closing workshops with stakeholders organized between August and September 2018 in the three study zones in Chad covering Logone Occidental and Ouaddaï province and parts of Hadjer Lamis and Chari Baguirmi province. A qualitative approach based on focus group discussion and in-depth interviews was used to get insights on access to care and animal investigation after suspected rabies exposure. A total of 96 participants, including 39 from the community (bite victims, dog owners) and 57 human and animal health providers (health center managers, chief veterinary officers, chief district medical officers, chiefs of livestock sectors) contributed to the study. Based on an existing conceptual framework of access to health care, several points of dissatisfaction were identified, in particular the unaffordability of human rabies vaccine for PEP (affordability) and the distance to travel to a health facility in case of a bite (accessibility). In addition, there are unfavorable attitudes observed highlighted by the importance given to traditional or local rabies care practices to the detriment of PEP (acceptability) and a low level of knowledge among Chadian communities regarding bite prevention, coupled with a very inadequate information and awareness system regarding the disease (adequacy). As for human and veterinary health services, both sectors suffer from insufficient resources for PEP on the human health and rabies diagnosis on the veterinary side impacting negatively on availability and accessibility of both these services. Action to improving provision of rabies health services and increasing knowledge about risk and prevention of the disease among the population need to be undertaken to implement IBCM, improve access to PEP and achieve the goal of eliminating dog mediated human rabies by 2030 in Chad.

KEYWORDS

rabies, Chad, One Health, PEP access, integrated bite case management (or alternatively IBCM)

Introduction

Rabies is a zoonotic disease caused by an RNA virus of the genus Lyssavirus and transmitted through the bite of an infected animal (1). It is a fatal disease once clinical symptoms appear (2). Despite the fact that effective tools for controlling this disease are known, including canine and human vaccination, more than 59,000 people die from rabies each year worldwide (3). The domestic dog is the main source of human exposure and the primary reservoir of rabies in developing countries (4). Post-exposure prophylaxis post-exposure prophylaxis (PEP) is the only way to avoid rabies after exposure, but often PEP remains difficult to access in low-income countries (5). A recent study revealed that improved access to PEP, potentially supported by free provision of human vaccine by GAVI, the vaccine alliance, could prevent more than 400,000 deaths in a timeframe of 15 years (6). Together with large-scale dog vaccination interventions, dog mediated human rabies cases could be eliminated in the near future as outlined in a global strategic plan by the tripartite [WHO, FAO, OMSA (former OIE)] and the Global Alliance for Rabies Control (GARC) (7). PEP and dog vaccination combined are highly cost-effective interventions, provided that human health and veterinary services collaborate well together (8). Investigation of the rabies suspicion status of a biting animal through an animal health professional can help to assess a patient's exposure risk and thus ensure adequate use of human rabies vaccine that is currently in short supply (9, 10). This risk assessment and advanced surveillance based on a One Health (OH) approach is called integrated bite case management (IBCM) (11) and has been proven to be effective in various settings including Haiti (12), Tanzania (13) and the Philippines (14). To describe effectiveness of IBCM the elements of access to health care described in the framework proposed by Obrist et al. (15) can be applied on community, human health and veterinary health levels, respectively. These seven elements are: (1) Availability: The existing health services and goods meet clients' needs; (2) Accessibility: The location of supply is in line with the location of clients; (3) Affordability: The prices of services fit the clients' income and ability to pay; (4) Adequacy: The organization of health care meets the clients' expectations; (5) Acceptability: The characteristics of providers match with those of the clients; (6) Compliance: health care providers adhere to best practices; (7) Adherence: clients adhere to recommendations. Figure 1 illustrates these elements in the context of IBCM.

In Chad, rabies is endemic, and despite numerous studies carried out in this country in recent years, control measures have not evolved. The Chadian dog population is highly dynamic and mostly free roaming (16). Nearly half of all households (45%) own dogs and the dog to human ratio is 1:8 (17). Less than 2% of the Chadian dog population is vaccinated and access to PEP for dog bite victims is as low as 8.5% (18). The population of Chad is confronted with problems of inadequate human and veterinary health structures. There are recurrent challenges of insufficient human and veterinary health personnel as well as difficulties in accessing health care in general and rabies prevention in particular (19). It has to be noted that PEP in Chad includes only active vaccination, passive vaccination with rabies immunoglobulin (RIG) that according to WHO recommendations would need to be applied for all category 3 exposures (11) is virtually not available in Chad.

From 2016 to 2018, a project to estimate the burden of rabies and PEP demand in West and Central Africa was conducted with financial support from GAVI (20). In Chad, this funding has enabled the extension of canine rabies surveillance to four administrative provinces through establishment of decentralized diagnostic units (21), collection of data on bite exposure on household level and access to PEP on health facility level (18). The results of the household survey showed that the incidence of dog bites ranged from 2.1 to 9.5 per 1,000 people per year depending on the region, and almost half of the bite victims were under 15 years of age (18). However, due to many reasons including socio-economic, geographical and cultural factors, only 33% of households seek care in medical facilities in case of a bite (18). Traditional care is seen to be practiced widely and traditional healers were consulted more often than veterinary services (22). Assessments of knowledge, attitudes and practices of health and veterinary workers also revealed numerous problems in the organization of care for people exposed to rabies (23, 24).

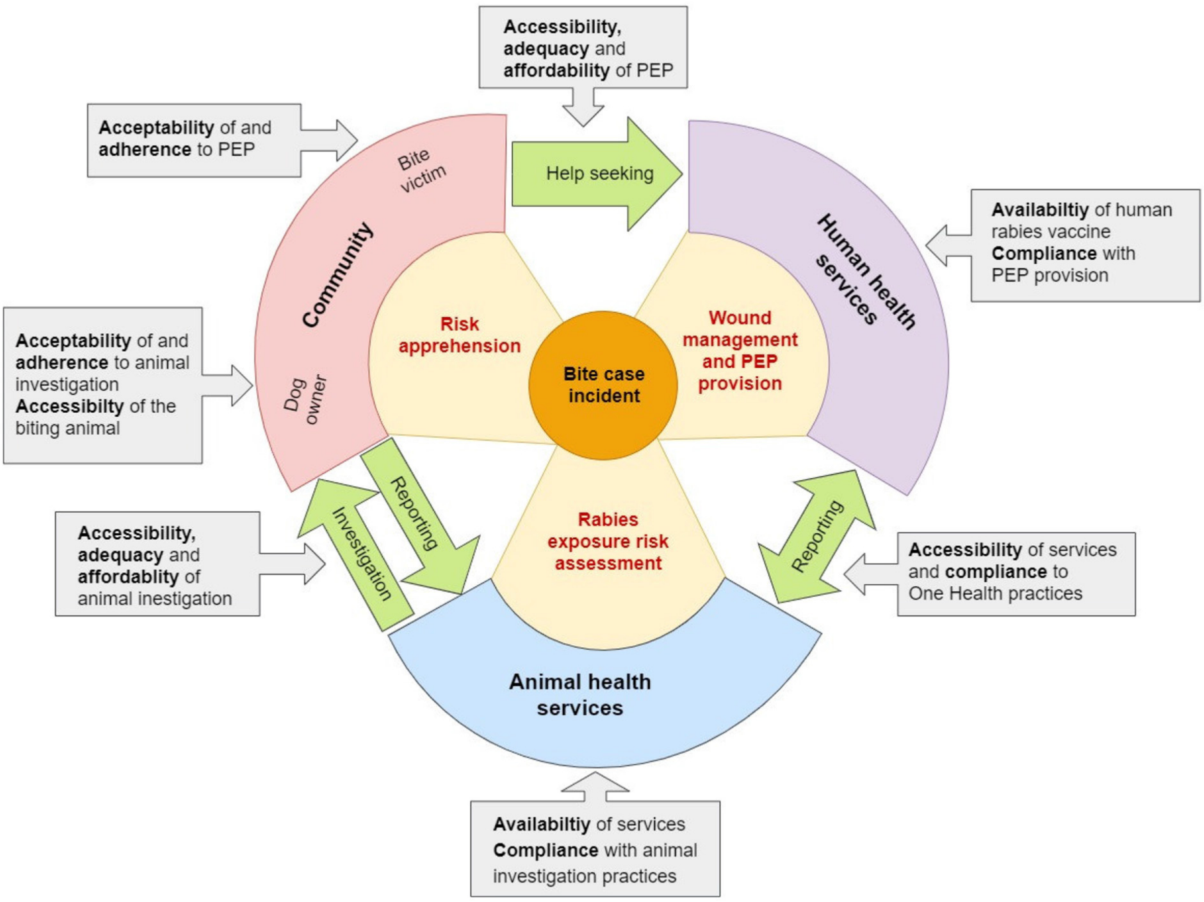

FIGURE 1
The integrated bite case management (IBCM) access cycle with the various access elements described by Obrist et al. (15) (gray boxes) influencing on different levels of the cycle (community, human health and animal health services). Red text in the cones describes the roles of the primary actors involved in IBCM. Green arrows highlight the communication actions that need to be undertaken between the three levels.

With regard to rabies service delivery, human and veterinary health personnel were not used to collaborate for the management of bite victims, prior to the GAVI project. During the project a OH approach was piloted in the study regions with the aim to improve communication between the human and animal health sector (20). Despite this effort, among overall 1,540 bite cases reported during the health facility survey, only 20% had been the subject of effective collaboration between human and veterinary health services (18), which illustrates the persistence of communication difficulties between the two sectors even after reinforcement through an OH project.

The findings from the various quantitative studies of the GAVI project allowed us to assess rabies risk and the need for PEP on the national level and to identify a lack of access to appropriate health and veterinary facilities with well-trained health and veterinary workers (18, 21, 23). However, the purely quantitative data did not allow us a deeper understanding of the mechanism of PEP access and implementation of joint case management by human and veterinary health services. Therefore, we identified the need to complement the quantitative data with a qualitative data collection to better explain the barriers to seeking rabies care. The aim of this qualitative study presented here is to identify factors affecting access to IBCM in Chad on community and service provider level across the various elements of the health care access framework described above (15). The combined approach for data collection on both the demand (community) and the provider side (animal and human health institutions) helps to understand the dynamics between the two and identify points of leverage to improve the access cycle (Figure 1). Together with the already published results of the quantitative studies, the findings of this complementary qualitative study will help to plan future rabies control activities in Chad, in particular implementation of IBCM in public veterinary and human health facilities and well-targeted public awareness campaigns.

Methodology

Study background and aim

The qualitative study presented in this paper is part of a large-scale research project implemented in Chad, Côte d'Ivoire and Mali, to estimate the burden of rabies and vaccine demand in West and Central Africa, funded by GAVI described in Léchenne et al. (20). In Chad, this research project lasted from January 2016 to September 2018 and was implemented in three study areas belonging to four administrative provinces (see chapter Study areas below). As described in the introduction the project included quantitative data collection on household level (bite case survey) (18), health facility level (bite case reporting and PEP follow-up) (18) and veterinary facility level (animal rabies reporting and diagnosis) (21). The qualitative study presented here was initiated to complement these quantitative studies. The data collection was conducted from August to September 2018 on the occasion of the GAVI project closing workshops in the selected provinces. Data was collected through Focus Group Discussions (FGD) and in-depth interviews (IDI) with community representatives and representatives of the institutional level (see chapter Selection of study participants below). The objectif was to get a parallel insight from the community and the care provider level on the factors influencing access to PEP and implementation of IBCM in both these backgrounds. We chose this approach because access to PEP on the human side and appropriate rabies investigation on the animal side is shaped by the balance between availability and demand. If demand for a particular service is low, this service becomes unprofitable for a provider and is therefore not supported and vice versa. Therefore, we understand the mechanism of PEP access as a circular process that can only be understood through a holistic view integrating all concerned levels of this mechanism. Presenting findings from the demand side (community level) and the service provision side (institutional level) together, helps to identify negative or positive dynamics influencing the access cycle (Figure 1).

Study areas

The study areas were the same than those chosen for the overall GAVI-project: The Ouaddaï province (OU), the Logone Occidental province (LO) and the rural area around N'Djamena within a radius of 100 km around the city covering parts of the province Chari Baguirmi (CB) and Hadjer Lamis (HL). These four areas were selected to cover different demographic characteristics of the country. According to data from the 2009 general population and housing census (25), Ouaddaï has a population of 997,257 and is a predominantly Muslim region, with the largest ethnic groups being the Maba and Massalit. Ouaddaï comprises four health districts: Abéché (urban center); Adré, Abdi and Abougoudam (rural districts). The Logone Occidental region has a population of 961,503, the main ethnic groups are the Ngambaye and Christianity is the dominant religion (25). This province is also divided into four health districts which are: Moundou (urban center); Beinamar, Laoukassy and Bénoye (rural districts). The third study area of rural N'Djamena is covering two health districts of the Chari-Baguirmi province (Mandélia and Dourbali) and two health districts of the Hadjer-Lamis province (Massaguet, Mani). The two district cover about 700,000 inhabitants or 50% of the population of the two provinces combined (25). In these regions live the Baguirmien, the Arabs, the Barma and the Kotoko, and the Sara-Ngambaye ethnic groups. Each study area covered four health districts.

Selection of study participants

Based on our experience from the quantitative data collection within the GAVI-project and our research aim, we identified six stakeholder groups from which to collect in-depth qualitative data: bite victims (BV), dog owners (DO), health center managers (HCM) and chief veterinary officers (CVO), chief district medical officers (CDMO) or district hospital chiefs (DHC) and chiefs of livestock sectors (CLS). Drawing on respondents from the quantitative survey, sampling was carried out in a purposive manner to include informants from the different groups identified and from all health district of the study areas. The following sampling strategy was used in order to cover a wide spectrum/range of situations encountered in the quantitative study.

- A selection of four dog owners by study area (1 per health district) having reported a bite incident during the extended animal surveillance period (21); two residing near (<5 km) and two residing far from a veterinary station (>5 km).
- A selection of four bite victims by study area (1 per health district) encountered during the health facility survey (18); two residing near (<5 km) and two residing far from a health center or hospital (>5 km).
- Two health center managers by health district in all study areas, amongst them one manager whose facility had recorded many (>10) and one whose facility had recorded very few bite victims (<10) during the health facility survey (18).
- Two chief veterinary officers by health district in all study areas, amongst them one whose facility had recorded many investigated dog bite cases (>10) and one whose facility had recorded very few investigated dog bite cases (<10) during the extended animal surveillance period (21).

- All chief district medial officers of the health districts covered by the GAVI-project or in case of absence the district hospital chief.
- All chief of livestock sectors corresponding to the health district zones covered by the GAVI-project.

The recruitment of participants was done with the help of the quantitative data basis (health facility survey and animal case reporting) and according to the inclusion criteria mentioned above. Identified participants were contacted by phone or through the help of the respective local project collaborators (veterinary officers and health center managers). They were informed of the purpose of the study in order to obtain their free consent to participate. Upon agreeing, they were informed of the date and place of the IDI and FGDs. Data was collected in three major cities: Abéché, N'Djaména and Moundou. The timing of the data collection was coupled with the 2-day closing workshops of the GAVI-project held in these three cities between August and September 2018. Since the window of opportunity for the data collection was very narrow, participants that were not available on short notice on the respective dates for either the FGD or the IDI in each area could not be replaced. This led to overall fewer study participants than initially planned (Table 1). Participants from the surrounding villages and provinces received 10,000 CFA francs each as an incentive for transport, while those living in the respective city where the workshops took place received 5,000 CFA francs.

Collection tools and methods

FGDs were used to understand collective perceptions and attitudes toward bite case management and PEP seeking, while IDI were needed to understand the personal motivations behind health-seeking and reporting (for bite victims and dog owners) or service provision (for participants of health and veterinary services). The interview and focus group guide for victims and dog owners was structured along the following axes: knowledge about rabies, attitudes in case of a bite and obstacles to obtaining appropriate treatment. The interview and focus group guide for health and veterinary services was structured around the following main themes: quality of the services currently offered, demand of these services by the population, needs of the services for effective rabies control, perception of the OH approach. The focus group and interview guides by participants group can be found in the Supplementary material S1.

Field data collection was entirely carried out by the student in charge of the research (lead author), assisted by a student from the Afrique One-ASPIRE consortium, in Master's degree at the University of Korhogo in Côte d'Ivoire (second author). The senior coordinator of the GAVI study (last author) supervised data collection. All IDI and FGDs were recorded by a Dictaphone and with the prior oral consent of the respondents.

TABLE 1 Description of participants by stakeholder group and data collection tool.

	In-depth interviews				Focus group discussion DO and BV		Focus group discussion HCM and CVO		Focus Group Discussion CDMO/DHC and CLS	
Study areas	DO	BV	HCM	CVO	No. FGD	No. participants	No. FGD	No. participants	No. FGD	No. participants
Ouaddaï	2/4	1/4	1/4	1/4	1/1	8/8	2/2	14/16	1/1	8/8
Logone Occidental	3/4	3/4	1/4	1/4	1/1	6/8	2/2	12/16	1/1	6/8
Hadjer Lamis and Chari Baguirmi	4/4	4/4	0/4	0/4	1/1	8/8	1/2	07/16	1/1	6/8
Total	9/12	8/12	2/12	2/12	3/3	22/24	5/6	33/48	3/3	20/24

Both target numbers and the actual achieved numbers are provided by group and tool (achieved/targeted).

Data collection was conducted in French and sometimes in local languages.

For the FGDs, participants were separated into community level (DO and BV), the primary health proivders (HCR and CVO) and the administrative level (CDMO, DHC and CLS). FGDs took place in a quiet location on the premises of the workshop location. Group sizes ranged from min six to maximum eight participants depending on the actual presence of invited people. Table 1 depicts the number of participants by stakeholder group and data collection tool and also provides achieved vs. targeted numbers. On the community side, nine IDI with dog owners, eight IDI with bite victims and three mixed FGDs were conducted. Concerning the institutional side, four IDI (two with HCR and two with CVO) were conducted. Unfortunately, in the third study area no IDI for this stakeholder group could be done. However, participation of HCR and CVO in FGD was good with overall five mixed FGDs conducted and a total of 33 participants. With CDMO, DHC and CLS only FGD were conducted. Since the targeted number was fewer than for HCR and CVO only one FGD per study area took place.

The fact that the initial targeted number of participants was not reached has to do with challenges linked to mobility during the rainy season. At the time of data collection in August, not all participants were able to attend the data collection location at the district capital. However, data saturation was reached before the initial study size was obtained allowing for a comprehensive analysis.

Data analysis

Translations into French were double checked by both research facilitators. The transcribed texts were imported into the Nvivo12 software version, and after several readings, the text corpus was coded according to the themes of interest in the study. Taking into account the themes addressed, the main information on the socio-cultural and organizational factors that influenced the perceptions of the populations and providers for the control of rabies through the OH approach, was analyzed with the content analysis method. The content analysis method followed an inductive approach where the main themes identified were derived from the data. The analysis was performed by the first and second authors of this manuscript. They both have acquired ample experience in rabies research during several former projects in Chad and were members of the local GAVI project team. Translations of quotes into English were slightly edited to increase readability.

Ethical aspects

Ethical approval for the overall study, covering research activities in the three GAVI-project countries (Mali, Côte d'Ivoire and Chad), was granted by the Ethics Committee of Northern and Central Switzerland (EKNZ). The specific research activities of the GAVI-project in Chad received research approval from the Chadian Ministry of Public Health (MSP) (No. 1569 / MSP / SE / SG / DGAS / 2016) and the National Bioethics Committee of the Chadian Ministry of Higher Education (No. 298 / PR / MESRS / SG / CNBT / 2016). Oral consent was obtained from each participant before data collection.

Results

Characteristics of the participants

On the community level, 39 dog owners and bite victims participated in the study, 31% (12) in Logone Occidental, 28% (11) in Ouaddaï and 41% (16) in the study area around N'Djamena. There were more men 82% (32) than women 18% (7). Of the participants 67% (26) were Christians and 33% (13) were Muslims. The majority were engaged in agriculture and livestock rearing 67% (26), 21% (8) were wage earners, 8% (3) were traders and 4% (2) were students. Regarding the level of education of the participants 49% (19) had no formal education, 12% (5) had primary education, 26% (10) had secondary education and 13% (5) had higher education.

On the institutional level, 57 providers participated in the study, 35% (20) in Logone Occidental, 42% (24) in Ouaddaï and 23% (13) in rural N'Djamena. There were 84% (48) from the human health and 16% (9) from the veterinary health sector. The age of the respondents ranged from 35 to 62 years, with an average age of 47 years, and there were 55 men and two women. The respondents were composed of 53% (30) nurses, 22% (12) doctors, including two veterinarians, the other 25% were heads of veterinary posts (usually livestock technicians) (4), senior livestock technicians (3), technical health officers (4), senior health technicians (3) and one pharmacist.

Access determinants on community level

Knowledge of the population about rabies

Participants in the FGDs and IDI described rabies as a dog disease transmitted to other dogs or to humans by bite. The signs commonly cited for animals were fury, aggressiveness and altered behavior; as for human rabies, participants indicated that rabid people barked like dogs.

> Q1: "*When I was in Moundou, there were many cases. All the rabid dogs, when they bite another dog there, some week later the dog there also becomes rabid. It's a disease that attacks the dog. It becomes furious, its eyes become red and it becomes aggressive. It doesn't even recognise its owner. Everything it comes across, it will bite, and when it bites a person, the disease will develop in the person who will perhaps end up dying. But I was a kid when I saw the rabies victims*

> *taken to hospital. I was told that they screamed like dogs, too, but I didn't see that. But I know that rabies is a disease that kills.*" (IDI: DO, male, CB)
>
> Q2: "*Before it disappeared, it didn't have this behaviour. But when it was bitten by another dog and it disappeared for 11 days, as soon as it came back it changed. It doesn't recognise anyone, it bites everything, even the wood. That's why we had to kill it.*" (IDI: DO, male, LO)

Some participants incorrectly stated that the disease that the dog transmitted to humans was tuberculosis. The same participant believed that a deranged mind or madness was the result of rabies transmission.

> Q3: "*Some people say that a dog can also transmit tuberculosis to a man. Also a deranged mind, they say that it is the dog that gives that.*" (IDI: BV, male, HL)

Overall, the rabies knowledge of the participants from the community side seems to be quite good, but this has to be interpreted with care, since all participants had prior experience with a bite or a rabies case and were in contact with the GAVI project that included awareness campaigns. Accounts from the care provider side showed that lack of knowledge in the community, especially about rabies risk even in case of a minor wound, is hindering access:

> Q4: "*Many people do not come to the health services to ask for the vaccine. I have had enough cases in my area. People have died from rabies, because there are some people who are bitten by the dog and indeed the dog is rabid. They treat themselves at home with traditional care. Afterwards, the wound is healed, and they think they are cured because the wound is healed. They don't know that there is the virus in the blood and after some time, [...] as soon as the virus attacks the brain, they come and get to us, that such and such a day our relative was bitten by a dog and by then it is already late. They can't understand that this is what causes a lot of health problems.*" (Extract from FGD: CVO, male, LO)

Attitudes and practices of the community

When asked about practices in the event of exposure to bites, participants report positive actions such as the washing of the wound, recourse to medical structures to receive treatment and the need to put the animal under observation. However, the FGDs showed that the majority of respondents are not particularly aware of any mode of rabies prevention or post-bite prophylaxis. They simply adhered to the principle of going to a medical facility to seek treatment after exposure instead of consulting traditional healers. Some respondents were aware of the need to take a biting animal to the veterinary station to ascertain whether it is ill with rabies as opposed to killing it immediately.

> Q5: "*When the child was bitten, we washed the wound and also took the child to the hospital, he received injections. And the dog we took it to the veterinarian and the head of the post to examine the dog.*" (Extract from FGD: BV, male, CB)
>
> Q6: "*It's the medical treatment, which allows us to save many people bitten by rabid dogs. But for a person bitten by a rabid dog to be cured by traditional treatment, I have never seen that*." (Extract from FGD: BV, male, LO)

On the other hand, there are also people who do nothing at all after a bite case, either through ignorance or negligence. This is what this victim of a bite from DS Laokassy tried to explain in his words:

> Q7: "*She was a woman, the dog bit her but they did not get her the rabies vaccine and they did not take her to the hospital either, until the rabies broke out. That is when they brought her in and then the doctors could not save her. She is a person but she barks exactly like a dog like that, that's when I understood that it's rabies, apparently the virus has reached her whole body.*" (IDI: BV, male, LO)

The interviews also report the refusal of a victim's father to respect the referral instructions of the head nurse of the health center, which eventually led to onset of symptoms some weeks later and eventually the child's death. This refusal could be both a result of negligence and difficulties to travel the long distance to the facility with vaccine in stock.

> Q8: "*We have a child from the village who was bitten by a rabid dog. His father brought him to the Djarmaya Health Centre, the RCS (Responsible of the health center) transferred him to the Massaguet District, so that he could receive the vaccine. But the father said we want injections [against tetanus] and we are going to return to the village. Some 40 days later the child started to get sick, when he was given water, he said: 'No, I don't want to!' He refused to drink and he died.*" (IDI: BV, male, HL)

Because all participants from the community had sought help after a bite incident either at a human health or at a veterinary facility, attitudes and practices reported by them were mostly positive. However, the FGDs and IDIs on the care provider level revealed certain barriers related to attitudes and practices of the community. For example, instead of seeking help in a health facility and get PEP, some patients find the alternative or have a preference for traditional treatments. Providers report that traditional treatment is detrimental to the health of the population, especially because most of these traditional practices prohibit patients from seeking other treatment, especially bio-medical care.

> Q9: "*Whenever there is a bite case, if it is a child, the parents refuse, they oppose medical treatment. They say that they have already given traditional treatments and that the victim should not take even one tablet of Paracetamol. As soon as he takes it, he will die and we are obliged to accept it.*" (Extract from FGD: CVO, female, LO)

The health service providers felt that the reasons for using traditional health services are multiple: They are linked to the poverty of local communities (see section on affordability and geographical accessibility below), but interviews also reveal that socio-cultural constraints built around beliefs are a strong factor for taking resort in traditional alternatives. Some even see the spirit of witchcraft in rabies, which reinforces the belief that it should be cured by a traditional healer.

> Q10: "*There are many reasons for this [to consult a traditional healer], sometimes it is an economic problem, when the patients do not have the means to pay, so they take resort in traditional care. But sometimes it's a socio-cultural constraint, people give much more importance to traditional treatment than to modern treatment, especially in Beinamar. So sometimes we try traditional treatments and when it doesn't work, we go to the hospital. Sometimes, in the case of rabies, they think that a witch doctor has taken the person and that makes them reluctant [to consult a health centre].*" (Extract from the FGD: CDMO, male, LO)

Cultural and religious practices also influence access of dogs to veterinary observation and rabies diagnosis. For example, a relevant obstacle in some Muslim communities is the religious taboo of handling dogs and especially dog carcasses. On the other hand, the cultural practice of eating dogs in some Christian and Animist communities has a negative impact on animal rabies surveillance. Accounts from health providers show that killing and eating of a dog that has bitten someone is widely practiced.

> Q11: "*If people know that you have cut the head off a dog they will not eat with you.*" (Extract from FGD: CVO, male, OU)
>
> Q12: *When a dog bites someone, we want to put the dog under observation and if it dies, the analysis must be done at this level so that we understand. But people kill the dog and they eat up to the head, it is difficult.*" (Extract from FGD: HCM, male, LO)

According to both the population and service providers, there is a need to diversify sources of information and awareness, to inform and influence individual and community habits on rabies knowledge, attitudes and practices. Especially in a context where a large number of the population lives in rural areas, information carried by public media does not always reach remote villages.

> Q13: "*Because if the vaccine is available but the community doesn't know about it, it would be difficult. So maybe we need to inform more at the peripheral level. As for the rabies vaccine, when a dog bites someone, you don't automatically have to kill the dog and eat it. So you have to make people aware of this.*" (Extract from FGD: HCM, male, LO)

Affordability and geographical accessibility

In the opinion of the people interviewed, many people cannot seek care or follow treatment when they were bitten because of their socio-economic conditions. Most of the respondents said that human rabies vaccines are very expensive and thus virtually unaffordable to them, for example one would have to sell an asset such as an ox to get it.

> Q14: "*We want the human rabies vaccine to always be available in hospitals, because before there was no vaccine and if you could find it, it's expensive. Once, a bite victim travelled to Koumra (about 200 km away) to get a dose for 12,000 FCFA [about 20 USD]. So to treat a person bitten by a rabid dog, you either have to sell an ox or some other good to save that person.*" (Extract from FGD: BV, male, LO)

The population's view of the high cost of the vaccine for PEP is also echoed by health service providers.

> Q15: "*The cost of the vaccine here is extremely expensive. [...] Common people cannot spend more than 50,000 for one child and if he has three children, where is he going to find the money.*" (Extract From FGD: HCM, male, CB)

The lack of financial means and thus the unaffordability of PEP, forces certain segments of the population to fall back on traditional care as described above.

> Q16: "*It is poverty that leads people to give importance to traditional treatment, which prevents them from going to the health center.*" (IDI: HCM, male, LO)

A district medical officer explains that the government is well aware of this problem and that there is the willingness to provide free PEP on the institutional level.

> Q17: "*In fact, in human health, well before this project, the Chadian state had planned something for the care of people bitten by animals suspected of having rabies. Thus, free care was even instituted for these people. So, a certain amount of anti-rabies medication was made available to the health*

> *facilities, [. . .]. With the project, it is true that it allowed us to intensify the action and even if the project has come to an end, we are going to take up the results of this project to be able to do better in the future.*" (Extract from FGD: CDMO, male, OU)

In all three study areas, participants reported PEP access difficulties related to geographical barriers. Distance and transport difficulties is presented by the respondents as a factor that discourages patients from seeking post-bite treatment.

> Q18: "*To go and get this anti-rabies treatment, you need a means of transport, and maybe that's it. But even if you don't have transport you have to ask your parents, they don't have enough money either.*" (Extract from FGD: BV, male, LO)

The difficulties related to distance are also reported by the health providers. The results from the FGD and IDI among care providers revealed in fact that distance is affecting proper health seeking of the community in the first place, but also on adherence to treatment. According to health personnel, the long distances are a reason for people to prefer traditional care, especially for wounds considered not very severe. Even if they seek help, some patients only take two or three doses of vaccine and do not complete PEP, because of the distance. There are also patients who cannot seek help during the rainy season because some areas become inaccessible.

> Q19: "*For the bitten person to be able to travel 180 km to come and get the vaccine in the district poses a problem, because there is a barrier to transport and people are very reluctant to travel. Because for [the patient], it is not serious, he prefers to go and see the traditional healer and it is when it is bad that they come for the first time.*" (Extract from FGD: CDMO, male, OU)
>
> Q20: "*There are some patients who abandon the treatment, because they travel long distances to come and when they take [it] twice, they think that the person is already saved, and they don't comeback anymore.*" (IDI: HCM, male, LO)

Access factors on the institutional level

Adequacy of human and animal health services concerning rabies

Under the terms of law 09/PR of 19/05/2004, in its article 5, rabies was included in the list of notifiable infectious diseases subject to compulsory declaration throughout the territory of the Republic of Chad, but this law has not been reinforced and followed up. Rabies surveillance is currently neglected by both the human health and the livestock sector and the collection of data on animal rabies is therefore challenging. Veterinary health service providers also believe that it is due to the lack of knowledge of this law that the population is not reporting suspect cases.

> Q21: "*We have intervened in diseases that are legally notifiable, including rabies. But in livestock surveillance rabies was not included. Here, we are too interested in the economy, hence in animals that can be profitable, which is why rabies is neglected in surveillance.*" (Extract From FGD: CLS, male, LO)
>
> Q22: "*At the level of the Ministry of Livestock, we have lists of diseases. Rabies is one of the diseases considered contagious and that we must declare a case of rabies, [as stipulated by the] law 09 of May 2004. So for me, it is the lack of knowledge of the population that does not allow to pass on the information.*" (Extract from FGD: CLS, male, LO)

The preference of animal health services to deal with economically more profitable diseases and species is echoed by responses received by dog owners, who noted many points of dissatisfaction concerning the veterinary services:

> Q23: "*The veterinary services, they only vaccinate oxen and so every time they call for animal vaccination it is only for oxen. They have never talked about the dog, nor about dog vaccination. This means that we are not informed about this aspect of the problem.*" (IDI: DO, male, LO).

On human health side, the interviewees reported that rabies was not part of a national control program, nor does it appear in the monthly activity reports (in French: *rapport mensuel d'activité*, RMA) of health facilities. This means that rabies surveillance is currently not performed by the human health sector. In the FGDs, providers from the human health sector explained that this neglect is due to the lack of epidemiological information about rabies in humans, which would be important to apprehend the burden of the disease. Another reason for the neglect of rabies by the human health service is its zoonotic origin as a pharmacist explains. This is apparently leading to confusion about responsibilities between the sectors.

> Q24: "*There is no rabies control program in Chad. Even in the RMA rabies is placed as 'other case.' It is not written down as a case of rabies. But if a case of rabies is found, it is counted with the other diseases. This negligence comes from the fact that people do not understand the incidence and prevalence of this disease among the population.*" (Extract from FGD: CDMO, male, HL)
>
> Q25: "*Rabies does not appear in the monthly activity report. Normally rabies should also be a disease under surveillance like the other diseases. But unfortunately, we don't see this disease in the monthly report. I also believe that this is a reason why health professionals sometimes forget*

> *about this disease. I also see that rabies is a hybrid disease between the human and animal medical professional. So that's why this disease is sometimes forgotten. [. . .] But if it existed at the RMA level, I think that health professionals would have an eye for it.*" (Extract from FGD: HCM, male, OU)

Availability of PEP and animal rabies diagnosis

The health system in Chad is organized in a pyramid with three levels: the central level, the intermediate level and the peripheral level. The health center is the basic structure in the provision of care, and it is from the health center that the Minimum Package of Activities (MPA) is offered, consisting of preventive and curative activities. Bites cases are managed in these centers and should be covered by MPA. Health professionals explained that a few years ago, the Ministry of Health made rabies vaccines available to districts and health centers to treat bitten people free of charge, but for more than 3 years this free service has been suspended. This situation has led the managers of peripheral health structures to systematically refer bitten people to the larger cities like N'Djamena for PEP. In most human health structures, there are equipment shortcomings. The lack of a cold chain to store the vaccine was the major difficulty to supply vaccine to almost half of the facilities involved in the GAVI-project (authors own observation). This is also highlighted by the qualitative data.

> Q26: "*Yes, we have problems with the cold chain. In our health centre, we don't have a cold chain, that was our great difficulty. We often call the District to send patients who are bitten by rabid dogs to be vaccinated.*" (IDI: HCM, male, OU)
>
> Q27: "*Previously, it was difficult for us who are in the provinces [. . .]. Because for every case of a bite, it was systematic, we were obliged to refer the patient to N'Djamena to get doses of vaccine and systematically, we killed the dogs. Whether they were domestic dogs or well-known dogs, even here, it was systematic, there was no observation period.*" (Extract from FGD: CDMO, male, CB)

The quote above also refers to the lack of access to animal rabies diagnosis leading to the negative practice to kill a dog after a bite already mentioned in the result section on the community level. According to the heads of the livestock services, reasons for inexistent follow-up of biting animals are the lack of resources and insufficient facilities. This is leading to the fact that some tasks under the responsibility of the veterinary services are actually performed by human health wokers, which seems to frustrate the animal health workers.

> Q28: "*If we take Adré, there are more than 20 health centres, so with their own resources they [human health workers] are really helping us, whereas in the livestock sector there are only 4 veterinary posts. Even these veterinary posts lack the means to travel. Yes, especially in terms of resources, they have sacrificed themselves and sometimes [human health workers] replace us, they make observations, as if they were veterinarians and they take care of the bitten animal, for example.*" (Extract From FGD: CLS, male, OU)

Responses from participants from the human health sector underscore this lack of capacity of the animal health sector. Many health personnel think that veterinary services should be reinforced with facilities; equipment and training to be able to better do their work. One of the respondents even points out that this investment into better animal observation and rabies diagnosis will have a positive impact on the human health side through vaccine savings.

> Q29: "*There are many bites, and we are not sure. We are limited by the means of diagnosis. It's difficult to put the dog under observation, it's difficult to make a biological diagnosis to confirm whether the dog that bit the person is sick or not.*" (Extract from FGD: CDMO, male, CB)
>
> Q30: "*But if there are no trained people, even if there are buildings, we can't follow a dog. We treat people, so I think that these veterinary centres need to be provided with staff, enclosures and then we need to think about the animals' food. [. . .] Especially the cost of the vaccine, we can't give a person who isn't sick three doses, whereas if we observe the dog and the dog isn't sick, we stop, that makes it possible to save on the vaccine.*" (Extract from FGD: CDMO, male, HL)

Attitudes and practices of service providers

Among the participants, the compliance of health personnel to PEP recommendations was found to be adequate, except for the non-use of RIG due to unavailability. Similar to the self-reported attitudes and practices by the community, this result also needs to be interpreted with the necessary caution. The participating human health and animal health providers have received training before the GAVI project implementation and their compliance to the PEP scheme (ESSEN or Thai Red Cross, excluding RIG) was followed up during the project. The interviews indicate that health personnel advices the community to wash the wound with soap and water after exposure before coming to the health facility. At a health center a wound is usually treated with antiseptics and then with anti-biotics and anti-tetanus vaccine. Providers also explain that the first treatment with rabies vaccine is adviced before any possible results from the animal investigation by veterinary personnel.

> Q31: "*As soon as we receive a person bitten by a rabid animal, even if the animal is unknown, [. . .] we first apply an anti-septic washing and vaccinate it. We inform our veterinary colleague, [. . .] We have also made the population aware that as soon as a person has been bitten [. . .], he must*

> *carefully wash the wound with soap and water before taking it to the health centre. At the health centre, we will carefully treat the wound, provide care and vaccination.*" (Extract from FGD: HCM, male, OU).

On the veterinary level results of our study show that some livestock technicians are reluctant to handle samples taken from the dog's body due to the religious taboo to handle a dogs carcass mentioned above (Q11). This constitutes a barrier to diagnosis even if resources are available.

> Q32: "*For example, in Djarmaya, when there is a case, we automatically call the chief district medical officer and inform him of the veterinary agents, but they don't come. The one in Karal came twice, and then when we call him, he says that he is very far away and cannot come. The one from Pont Bélillé is often there, but his phone doesn't answer, they don't want to collaborate with us.*" (Extract from FGD: HCM, male, HL)
>
> Q33: "*The difficulty is getting the dog's head to the laboratory for diagnosis, as you said earlier. It's a bit difficult as in Abdi where they are. Also here, cutting the dog's head, they are not ready to do that. Either, he cuts badly or he cut it yesterday and it's today that he shows up saying 'I cut it yesterday' and he didn't even leave it in the fridge.*" (Extract from FGD: CVO, male, CB)

In regard to implementation of a OH approach, prior to the study the two sectors apparently operated independently, but the implementation of IBCM during the GAVI-project allowed for collaboration that was appreciated by both sectors as a favorable for rabies control. According to the providers, this collaboration allowed for the pooling of skills. However, in practice, difficulties were noted by the providers. Firstly, because of the very small number of veterinary structures compared to human health structures (see also Q35 above), which did not allow veterinary staff to collaborate effectively. Secondly, among the human health facilities, nearly half could not offer PEP, and victims were systematically registered and referred, because these facilities did not have a cold chain to store the vaccine (see also Q26 and Q27).

> Q34: "*Before the project, everyone managed their own area: the veterinarians managed livestock and the health workers managed health, so we didn't really mix, but now I think we speak the same language.*" (IDI: HCM, male, LO)
>
> Q35: "*Another difficulty in relation to care is the problem of collaboration between human health and veterinary staff. Here in our district, we only have one veterinary post in Krim-Krim, while at the human health level there are many HCs, so for the veterinary nurse to go round to put the dog under observation is a lot of trouble, so the work of the veterinarian with the community is a problem.*" (IDI: HCM, male, LO)
>
> Q36: "*Inter-sectoral collaboration, it's an approach that is really commendable. It allows us to put together skills to fight against diseases. When one sector works in a closed way, it is difficult. Because as we have seen with rabies, clinically we can take care of the patient, but the diagnosis poses a problem. If there is no collaboration, it will be difficult to implement the diagnosis to allow for proper management. As we have begun, we will stay the course so that all diseases can be properly managed for the health of the population.*" (Extract from FGD: CDMO, male, LO)

Finally, the participants highlighted the need to integrate the community in the OH approach. This nicely closes the cycle from community to human and animal health institutions and back to the community.

> Q46: "*But there is still a need to strengthen communication between the human health sector and animal health. In terms of the community component, we should also think about integrating community surveillance in the future, because this community surveillance will strengthen the identification of bite cases in households that can be referred to the health centers so that the patients can be treated.*" (Extract from FGD: HCM, male, CB)
>
> Q47: "*What is important is to advocate, to raise awareness, so that the highest authorities can allow us to have an ideal framework that will bring us all together, so that we speak the same language and truly commit ourselves to fighting this disease. Indeed, [...] the community aspect is very important. At this level, we must also take into consideration that in the future, we will have to sensitise the community to first of all know that the disease exists. [...] We have to make them understand that if the animal is with them, the animal must be protected, the animal must be vaccinated.*" (Extract from FGD: CDMO, male, CB)

To summarize the results we have adapted Figure 1 to show the challenges identified which influence on several access elements and on different levels of the access cycle (Figure 2).

Discussion

This study was conducted with the aim of identifying the main factors that constitute bottlenecks to rabies control in Chad following the OH approach. The results reveal numerous obstacles on community and institutional level, many of which are interdependent and therefore influence several access elements at a time. To provide a graphic overview of the study results we adapted Figure 1 to show the identified obstacles by level of actors and access element. The factors identified cannot be considered exhaustive and are only to a limited extent representative for the national Chadian context. This is

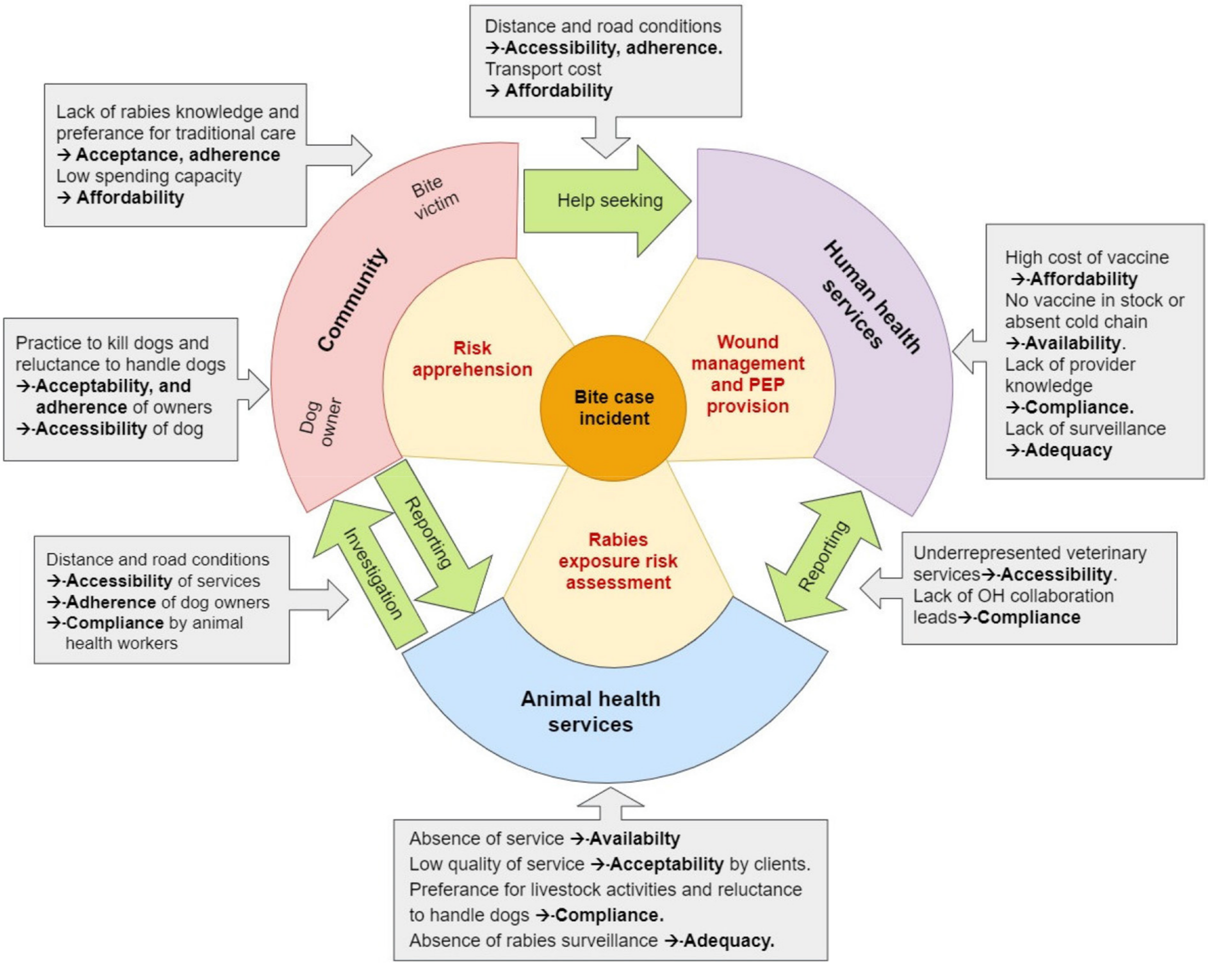

FIGURE 2
IBCM access cycle introduced in the introduction (Figure 1). This time the gray boxes highlight the barriers identified by level and linkage followed after an arrow by the corresponding access elements they influence.

because the recruitment of participants did not take into account criteria of gender, socio-economic status, cultural or educational background of community or institutional representatives. Access differences based on these criteria certainly exist, but could not be investigated through our study due to the limited methodological design. The results also need to be interpreted in the context of the GAVI-project that has certainly increased knowledge of rabies prevention and control on both the community and the service provider level during its 2 years period. Despite these major limitations we think that the results are valuable to give a rough overview of the major access barriers to be addressed in Chad for a future implementation of IBCM. The results also help to find explanations for the quantitative findings related to weak PEP access and animal bite reporting despite the efforts of the GAVI-project to increase PEP and animal rabies diagnosis availability.

Not surprisingly, affordability of human rabies vaccine is among the main issue determining PEP access. People and providers are unanimous on this point: PEP is extremely expensive. During the GAVI project human vaccine for PEP was provided free of charge in health centers and hospitals participating in the bite survey, provided they could insure cold chain. The cost of human RIG could not be covered by the project and equine RIG, which is less expensive is not licensed for use in Chad. Outside of the project activities, the price of human vaccine was estimated by the respondents at 12,000 CFA francs (18 EUR) per dose and the overall cost of PEP at 50,000 CFA francs (76 EUR), excluding the cost of consultations and wound treatment. This amount is out of reach for poor households in the country. Obstacles to treatment in health facilities resulting from the high cost are a consequence of poverty as a study in Cameroon has highlighted (26). Chad, although an oil country, is a poor country with about 47% of the population living on <US$2 per day (http://data.worldbank.org/pays/Tchad). Affordability of veterinary services was not investigated by this study. For both PEP access

and animal investigation, long distances to facilities, transport difficulties related to poor road infrastructure and adverse climatic conditions during some time of the year add to the cost of the actual service. The same factors also explain another major obstacle, which is geographical accessibility of human and animal health facilities. For access to PEP this problem is exacerbated by the low availability of human vaccine in local health facilities. Our results illustrate previous quantitative findings on affordability and geographical accessibility of PEP (18, 24). The average distance patients travel to a health center, for example, is 16 km and to a hospital is 62 km (information from Ministry of Health health statistics, https://reliefweb.int/report/chad/tchad-annuaire-des-statistiques-sanitaires-tome-31-me-dition-ann-e-2017). Geographical distance was also found to be a major determinant of access to PEP in other countries such as India (27) and Tanzania (28). Unable to access primary health facilities or secondary facilities they are referred to, poor communities turn to other therapeutic methods such as traditional treatment. Health providers therefore stated quite clearly: If nothing is done about the cost and availability of the vaccine, people will continue to die of rabies. The solution to this problem would be government investment in human vaccines for PEP to ensure availability and accessibility to prevent deaths. The new GAVI investment strategy could support governments of eligible countries in the free provision of human rabies vaccine for PEP, provided that a country has adopted a national rabies action plan (6). The implementation of this strategy was unfortunately delayed due to the COVID-19 pandemic. Moreover, Chad currently has no rabies action plan validated on the ministerial level.

Depending on the schedule, a course of PEP includes between three and five visits to a health center. Therefore, affordability, accessibility and availability are all impacting on patience adherence as our qualitative data clearly illustrate. In Tanzania, too, the risk of non-adherence PEP was high for people living far from health facilities (28). On the other hand geographical accessibility of communities is mentioned by service providers as an obstacle to carrying out their work, such as sample collection for rabies diagnosis. It also hampers the outreach and sensitization strategy of the human health sector to cover very remote populations and therefore adequacy.

Another constraint to accessibility of rabies care identified in this study is the decision-making process. The results of the interviews highlighted the parental influences and power differentials that condition health seeking behavior (Q18). Due to the high costs of PEP or the distance to a health facility, decision-making constraints often have negative implications for health care, especially when the victim is a child or a woman. In general, when it comes to health in a household or family, the head of the household decides and his or her agreement is a prerequisite in this process. Similar parental dynamics have been observed in Tanzania (29). However, to investigate on the dynamics of gender and age dependent access barriers in Chad a further in depth study would be needed.

The interviews reveal that traditional care is strongly used by Chadian communities. In the quantitative baseline study, 24% of bite victims consulted traditional healers (18), and the qualitative results also confirm this trend. The follow-up of the therapeutic itinerary of bite victims in the study conducted by Mindekem et al. (24) identified the same problem. In some cases, traditional care providers even seem to discourage patients from seeking PEP (Q9). According to health service providers, this attitude of local healers has caused the death of several victims during the GAVI-project, which shows that even if solutions to geographical accessibility, availability and affordability can be found, issues concerning acceptability of PEP by some people will remain. The importance attached to traditional care gives providers a mere parallel role in the provision of care for a disease as severe and fatal as rabies. However, it seems clear that difficulties related to cost, availability or geographical inaccessibility is the main driver for bite victims to resort to other therapeutic alternatives at the expense of proper post-bite medical care.

Health seeking after a bite is also strongly influenced by risk perception and in consequence by the level of knowledge a community has about the severity of rabies to adequately realize the importance of accessing PEP. Although most participants from the community knew that rabies is a disease transmitted from dog to dog or from dog to humans by bite, knowledge of other vector animals or of the paralytic form or rabies seems to be lacking. Moreover, many victims seem to neglect minor wounds or scratches (Q19) and think that the risk is mitigated when the wound has healed (Q4). Since all participants in our study had sought help at health facilities, we were unable to assess the impact on knowledge on health seeking in more detail. However, the sad story of the death of a child that a nurse shared during one of the interviews (Q8) indicates that the lack of awareness of the lethal nature of rabies might have an influence on the efforts undertaken by victims or parents to access PEP. Interestingly knowledge level was not significantly linked to preference for either medical or traditional care in Cameroon (26), but results from Kenya show that knowledge of rabies was the main driver in seeking medical attention after a bite (30).

Another factor influencing the motivation to access a service is a client's perception of the quality of this respective service. Quality of services is besides availability also impacted by adequacy and compliance. The FGDs and individual interviews denounce especially that the veterinary services that would be responsible to provide animal vaccination, observation of biting animals and rabies diagnosis are not readily available and accessible. Participants reported a lack or inadequacy of technical and logistical facilities and trained rabies personnel, which limits the performance of this sector. Similarly to PEP, animal observation includes several visits and therefore geographical accessibility is also impacting on adherence (if the dog owner needs to bring his animal to the veterinary facility) or compliance (if the animal health worker needs to visit a dog owner). The challenges for rabies control related to availability and poor quality of veterinary services was also encountered during the extended surveillance study of the GAVI-project (21) and are also reported from other rabies endemic countries, for example Ghana (31). The qualitative findings of this study confirm results from a knowledge, attitudes and practices survey done on the occasion of the training workshops at the beginning of the GAVI-project. This study revealed deficits in knowledge and practice (KAP) for rabies service delivery in both human and animal health personnel (23). Even if there are facilities and trained personnel, rabies control tasks seem to be neglected in favor of other, more lucrative activities. Adding to this are religious barriers to work with dogs, as our qualitative data clearly illustrate. Especially animal health workers with a Muslim religious background express their reluctance to collect and diagnose samples from dogs. They feel that their families and close relatives would not eat off the same plate with them if people were to learn that they had handled a dog carcass. This could be an explanation for the very few samples received from the northern study area during the GAVI project, indeed one of the decentralized diagnostic units established in the Ouaddaï province only sent a donkey sample to the central rabies laboratory in N'Djaména (21). This lack of compliance of animal health workers further undermines veterinary services and on the community level the same hesitance to handle dogs constitutes a barrier to dog vaccination (23). On the other hand, in the southern regions of the country, dominated by a Christian religious background, dog meat is a source of protein for some communities. This practice results in the abusive slaughter of dogs after a bite case, which seriously compromises dog observation and sample taking. Again, our qualitative data confirms quantitative findings on this practice published in Naïssengar et al. (21). Finally, our study shows a lack of reinforcement of the law on compulsory reporting of rabies suspicious animals. The community and the animal health services seem to blame themselves vice versa for this shortcoming. However, it should be a government's responsibility to adequately inform the public about laws and provide the means to the respective services to reinforce these laws. Because of this negligence on governance level, animal health services are not really contacted by dog owners after a bite, because they are not aware that they are obliged by law to do so and they do not trust this service to be able to respond to their demand.

A similar neglect is observed on the human health side. Providers deplore that nothing has been done about rabies since the adoption of the law mentioned above: there is no national health action plan on rabies and the disease is not included in the list of diseases under surveillance in humans. The monthly activity reports of health facilities mentioned by participants (Q24 and Q25) exemplify the lack of data. For some providers, this lack of data explains why rabies remains neglected. A study in Côte d'Ivoire documented the very positive effect that increased efforts on human rabies surveillance can have on the epidemiological data situation (32). Another possible explanation is that human health professionals think it is the responsibility of the animal health sector to control this disease since rabies is of animal origin. However, it would be clearly the responsibility of the human health services to provide PEP to potentially rabies exposed people. Theoretically human rabies vaccine should be included in the MPA of health facilities, but in practice, availability and affordability of this life saving product constitute a huge barrier to access. Human health officials explained that in the past few years, the free human rabies vaccine supply through the ministry of public health has been suspended for reasons that are unknown. This suspension is having a very detrimental impact on PEP coverage with only not even 10% of bite victims accessing PEP in Chad as quantitative results from the GAVI-project household survey show (18). Compliance of human health workers to WHO recommended PEP schemes could not be assessed by this study, but the afor mentioned KAP study (23) and the PEP follow-up data on the health facility level (18) during the GAVI-project found deficits in provider knowledge and compliance.

On the positive side, providers show committement to the strategy of collaboration between the human and animal health sectors. This confirms findings of the KAP survey back in 2016, where both human and veterinary workers well identified the need and value of better collaboration (23). Nevertheless, the interviews also revealed that practice lags behind the positive intentions to collaborate. A similar lack of practical implementation of intersectoral collaboration has been reported from Gomma in the South West District of Ethiopia (33). Challenges for this collaboration result mostly from lack of capacities on the animal health side. This is illustrated by the fact that the number of veterinary structures and staff is numerically much lower than on the human health side. At the regional level, the ratio is about four health centers for one veterinary post. This means that animal health facilities are even harder to access than human health facilities and on the other hand animal health workers often have to travel long distances for animal investigation. Besides the obvious need for capacity strengthening on facility level and training of personnel, ways to make rabies control activities more profitable for the animal health sector would need to be found to provide sufficient incentive for OH collaboration. For example, samples collected in the study areas by designated animal health personnel were rewarded with 5,000 FCFA (about 9 USD), which was certainly one of the main drivers for reporting and testing suspect cases (21). Another option would be to quantify the economic losses due to rabies in the livestock sector as recently done by a study in Ethiopia (34). This would show the benefit of investment into rabies control for this important economic sector in Chad.

Regarding the community level, we identified limitations of awareness to inform and influence individual and community habits about rabies that might hamper success of OH interventions. Although the knowledge of the main transmission pathway from dogs to human is known, there are deficits in risk perception and help seeking in human health facilities after a bite. This alone constitutes a limiting factor to IBCM, but the biggest challenge are cultural practices regarding the reluctance of dog handling on the one hand side and consumption of dog meat on the other hand. Both practices limit access to dog investigation and hence efficient IBCM. Lack of community awareness that could negatively affect appropriate rabies prevention measures are also reported from other African countries, for example the Democratic Republic of Congo (35). Interpersonal communication (with rabies images) should help reduce prejudice and could have positive influence on attitudes and practices toward the use of medical and veterinary services in case of a bite. To this end, it would be interesting to develop integrated communication systems using community relays, community-based associations and religious channels. Since traditional healers are frequently contacted after a bite, they would need to be included in a sensitization and awareness campaign.

Conclusion

Rabies prevention and control faces enormous challenges in Chad. These challenges are multilayered, meaning encountered on community, human health and animal health service level. They are closely interrelated and of various nature including economic, socio-cultural and organizational. Looking at all these obstacles one could become easily discouraged, but the interrelatedness of the access elements and factors affecting them is also a chance: minimizing or eliminating one barrier will have positive effects on several others. For the conclusion of our study, we would like to highlight two leverages by level of actors that we feel would be the most urgent to be addressed and also highly impactful to improve IBCM access. Firstly, on the community level public awareness campaigns will be an activity with the strong potential to overcome barriers related to lack of knowledge and erroneous attitudes and practices.

On the human health level, the most urgent task would be to decrease the price of human vaccine for PEP or provide it entirely free of charge and to start reporting human rabies deaths. On the animal health side, it would be crucial to provide a financial stimulus for rabies control activities and increase government investment in the veterinary sector. Doing so would decrease cost in the human health sector and potentially has economic benefits for the livestock sector. Finally, from the experience of our work we think that joint training workshops with human health and animal health workers fuel intersectoral collaboration besides the positive effect on provider knowledge and compliance.

As we write this publication an interministerial OH platform is being established in Chad. We strongly hope that one of the first actions of this platform will be the adoption of a national rabies action plan similar to those existing already in other African countries (36). Besides securing free human rabies vaccine through the GAVI-investment strategy this would be a strong sign for commitment to the global agenda toward zero human deaths from dog mediated rabies by 2030 (7).

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of North Western and Central Switzerland (EKNZ). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

AM: health geographer, study design, data collection, analysis, and manuscript writing. ML: veterinarian and rabies expert, study coordination, and manuscript writing support. KH-T: sociologist and manuscript proofreading and revision. JZ: veterinarian, One Health expert, and study PI. MD: One Health expert, support for analysis approach, and manuscript revision. NS: sociologist, methodological, and manuscript writing support. NM: sociologist, study co-design, data collection, and analysis support. 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.

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Supplementary material

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General insights on obstacles to dog vaccination in Chad on community and institutional level

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Domestic dogs are responsible for 95% of all human rabies cases worldwide and continue to be the main reservoir for this fatal virus in African and Asian countries. Interrupting the spread of the disease in the domestic dog population is therefore necessary for long-term, sustainable rabies control. Chad has been recognized as a rabies-endemic country since 1961, but no national control strategy is in place to date and dog vaccination coverage is very low. This qualitative, descriptive study aims to describe the main barriers to dog vaccination on both the community and the institutional level from a socio-anthropological point of view in Chad. The study was embedded in an overall project conducted from 2016 to 2018, to determine rabies burden and vaccine demand in West and Central Africa, funded by GAVI, the vaccine alliance. Data collection was conducted on the occasion of the project's closing workshops with stakeholders organized between August to September 2018 in the four (4) project areas: Logone Occidental, Ouaddaï, Hadjer Lamis and Chari Baguirmi. We conducted interviews and focus group discussions (FGD) among veterinary officers and dog owners. Participants were selected purposively based on their place of residence (dog owners) or work place (veterinary officers) and their previous contact with the project through reporting (dog owner) or management (veterinary officers) of a suspect dog rabies case. In each region, one FGD was organized with dog owners, and one FGD with heads of veterinary posts. At the end of the FGDs, a few participants were randomly selected for interviews. In addition, in each region an interview was conducted with the head of the livestock sector, the chief district medical officers and the head of a civil society association. The identified barriers to dog vaccination access are grouped into three main aspects: the economic, the socio-cultural and the institutional level. Economic constraints encountered relate to the cost of the vaccine itself and the expenses for transporting the dogs to the vaccination site. The cultural belief that the vaccine will have an impact on the therapeutic properties of dog meat for consumers (observed in Southern Chad), and the fact that dogs are considered impure animals in Muslim faith, which prohibits handling of dogs, are obstacles identified on the sociocultural level. At the institutional level, the unavailability of vaccines in veterinary services, the lack of communication about the law on dog vaccination, the absence of rabies in the training curricula of veterinary agents, and the lack of intersectoral collaboration limit vaccination coverage. In order to improve vaccination coverage and rabies surveillance with a view to eradicate rabies by 2030, communication strategies that are adapted to the context and that take cultural obstacles into account must be put in place in a synergy of interdisciplinary action. In addition, factors such as affordability, geographical access and availability of dog rabies vaccines needs to be addressed throughout the country. Although our study design did not allow a detailed analysis of obstacles related to socio-economic level, gender and age the broad insights gained can provide general guidance for future interventions in Chad and similar countries.

KEYWORDS

rabies, cultural context, dog vaccination, veterinary services, Chad

Introduction

Rabies, a lethal zoonotic disease, is mostly transmitted to humans by infected dogs and mainly affects marginalized communities in Africa and Asia (1). In many low-income countries, the classical rabies virus (RABV) is endemically present in dog populations (2, 3). At the same time most of the dogs are free roaming leading to frequent human to dog contact (4). Humans usually get infected accidentally through a bite, scratch, or lick of excoriated skin by a rabid animal and tragically, children are disproportionally affected (1). The disease is almost invariably fatal once clinical signs appear (5). Timely administration of rabies vaccine to bite victims can prevent the onset of rabies (6), but this measure called Post-Exposure-Prophylaxis (PEP) is very costly, hard to access for marginalized communities and most importantly does not reduce overall exposure risk for those communities. With 25,000 deaths per year, Africa is one of the continents most affected by rabies (7). The recognized most cost-effective control measure that also holds the potential for elimination of dog-mediated human rabies is large-scale vaccination of dogs (8–10). Rabies control became a flagship of the One Health approach because it illustrates very well the added value of intervention in animals to improve health not only in animals but for humans and the ecosystem overall (11), which is the core idea behind this concept. The feasibility of dog vaccination to reduce the burden of human rabies is proven by the success of this intervention to eliminate canine rabies from large parts of Latin America (12) and by many successful local interventions in Africa and Asia (13–15). However, the same studies point out the need for sustained control measures, the necessity for locally adapted methods and the importance of accurate sensitization through local awareness campaigns. For example in the case of N'Djamena, the capital city of Chad, two consecutive mass vaccination campaigns were sufficient to temporarily eliminate rabies from the city, but after the interventions, rabies was reintroduced from the rural area (16). During the same vaccination intervention, considerable differences were noted between the achieved vaccination coverage in different quarters of the town depending on the socioeconomic and cultural context (13). In a similar intervention in Bamako, Mali, vaccination coverage did not achieve the level needed to interrupt rabies transmission in the dog population (17). This indicates that sociocultural factors determine the accessibility of dogs to vaccination because they determine adequacy and acceptance of the intervention measures, as described by Obrist et al. (18). Local economic and socio-cultural context also determines the most effective tools and strategies for awareness raising. Therefore, it is important to study human attitudes and practices toward dogs and rabies in order to better plan and undertake dog vaccination campaigns.

To achieve sustainability, dog vaccination campaigns need to be institutionalized and taken over by the governmental veterinary services. Therefore, institutional factors also influence the long-term success of vaccination interventions. Finally, accessibility of dog vaccination is heavily impacted by affordability, especially in the context of livelihood insecurity faced by many dog owners in endemic areas (19).

In Chad, as in many other low-income countries, the accessibility of health facilities is often challenging which has a large impact on access to PEP (20). At the same time incidence of rabies in the dog population is observed to be high in areas where surveillance is implemented (21). Therefore, it would be all the more important to decrease exposure risk through dog vaccination. However, veterinary services are even more neglected than human health services and are mostly limited to livestock vaccination. Although vaccination of dogs in Chad is compulsory by law, the estimated vaccination coverage of 0.5% in the dog population is extremely low (22). There exists no governmental led national dog vaccination strategy. Past interventions, mainly in the capital N'Djamena have been based on intervention research projects implementing dog mass-vaccination for a short period of time and in a limited geographical setting.

Dog keeping households are widespread in Chad, and dogs are used for many purposes, ranging from protection to hunting and breeding (22). Dog ownership is most common in the predominantly Christian southern regions of the country, but is also observed in Muslim areas (22). According to Anyiam et al. (23), Chad's dog population is estimated at 1,205,361 dogs. Only around 10% of the overall dog population in Chad are stray (ownerless) dogs (16), but most of the owned dogs roam freely due to lack of means to adequately feed the animals. Dogs supplement their diet from food scraps in the environment such as human feces, wildlife, slaughter offal, and other waste. This creates a lot of contact with people, especially children, who are often unsupervised. Challenges are noted on many levels and show that rabies is a topic that deserves special attention. This paper aims to contribute to the knowledge basis for future planning of control programs in Chad through identification of the socio-economic, socio-cultural and institutional barriers that limit access to dog vaccination in the various cultural contexts of the country. These are very diverse and range from regions dominated by sedentary farmers with a Christian background to regions with a predominantly pastoralist population and a mainly Muslim rooted culture.

FIGURE 1
Map of Chad showing the provinces in which the GAVI-project was implemented.

Materials and methods

Study background

The qualitative study presented in this paper is part of a large-scale research project implemented in Chad, Côte d'Ivoire and Mali, to estimate the burden of rabies and vaccine demand in West and Central Africa, funded by GAVI, the vaccine alliance (GAVI-project) (24). In Chad, this research project lasted from January 2016 to September 2018 and was implemented in three study areas belonging to four administrative provinces (Figure 1). The provinces were selected based on their various demographic and socio-cultural characteristics to cover the range of backgrounds in the country. The quantitative studies undertaken during the project are already published in different papers. They included a household survey on bite occurrence (20), a health facility-based study on reporting of bite cases, PEP access and follow-up (20) and extension of animal surveillance to the project areas (21).

The quantitative studies combined revealed a high occurrence of dog rabies in Chad and in consequence, a high dog to human transmission risk that calls for urgent control measures such as dog vaccination. At the same time, the study team members encountered differences in attitudes toward dogs and practices for rabies prevention across the study areas that might hamper future control interventions, in particular dog vaccination. Therefore, we identified the need to investigate on these differences through a qualitative study, which for the first time, would provide some broad insights on the influence of socio-cultural factors on rabies control in Chad. Data collection for the study was conducted during the closing workshops of the project conducted for each study area. The study's aim is to gain a general overview of obstacles to dog vaccination against rabies in Chad combining the perspectives of different cultural and socio-economic contexts on the population/client side with institutional perspectives of the dog vaccination service provider side (in our case governmental veterinary services). It is a socio-anthropological study, based on a qualitative approach with focus group discussions (FGD) and individual semi-structured interviews (25). Such a research design allows gaining real life examples of how dogs are perceived and treated, the practices the population and animal health professionals adopt regarding dogs in the context of rabies prevention as well as the underlying institutional factors influencing these practices. A detailed analysis of different socio-demographic characteristics, gendered analysis at household level or a deeper investigation into the dynamics at play in government institutions were beyond the scope of this study.

Description of study areas

The first study area includes the province Logone Occidentale, with the city of Moundou, the economic capital and second largest town of Chad (Figure 1). Logone Occidentale is the smallest province in southwestern Chad and is populated mostly by Christians and animist communities. Outside of the regional capital in the rural areas, people live mostly on small-scale farming. The population of the Logone Occidentale province is estimated at 790,694 inhabitants with a human density of 88.68 inhabitants per square kilometer (26). The second zone covers the sparsely populated Ouaddaï region in the northeast, with a predominantly Muslim population. Its regional capital is Abéché situated very close to the Sudanese border in the northeast of Chad (Figure 1). In 2009, Ouaddaï had a population of over 700,000 with a density of 24 inhabitants per square kilometer (26). In the vast rural areas of this province, people are mainly pastoralists. The third study area is defined by a 100-kilometer radius around the capital city of N'Djamena situated in the central-west of the country. It includes parts of the province Chari Baguirmi to the south of the capital, and parts of the province Hadjer Lamis to the north of the capital (Figure 1). These two administrative provinces have a culturally mixed population of sedentary farmers and pastoralists. The population density is about 18 inhabitants per square kilometer (26).

The study areas were broadly divided into urban and rural strata: The provincial capitals (Moundou and Abéché) are considered urban and the rest of the respective province is considered rural. The study area covering parts of Chari Baguirmi and Hadjer Lamis was considered rural.

Target population, recruitment, and participants' background

Based on the experience form the various quantitative studies of the GAVI project, we identified the following stakeholder groups to be interviewed:

Community level:
- Dog owners
- Heads of the regional civil society association

Institutional level
- veterinary officers (heads of veterinary posts)
- administrative authorities of the study regions (heads of district veterinary delegations, heads of regional livestock sectors and chief medical district officers).

For the purpose of the study a dog owner is defined as a person that keeps one or more dogs at his household for which he provides care to some extent (food, shelter etc.) and to which he attributes a role (watchdog, guard dog, etc.). Dog owners were selected in a purposive manner with the help of veterinary officers. Most of them had no formal employment and were farmers, herders, informal traders, housewives or students. The only selection criteria for the institutional representatives was the prior participation in the extended animal surveillance and bite reporting activities of the overall GAVI-project. Public veterinary officers in Chad most often do not have a university degree in veterinary science, but are graduated livestock technicians. Veterinary officers and dog owners came from various districts in rural and urban settings of the respective regions. In total, the study gathered 42 participants in 6 different FGDs and conducted 21 individual interviews. The discussion points of the FGDs and the interview guides are provided in the Supplementary Document (S1). Two thirds of the institutional representatives were Muslims (18) and the other third Christians (9). Study participants representing the population and dog owners included 13 Christians and 11 Muslim. It is important to note that local belief systems based on a rich ethnic background persist in the population, even if people go to church or pray at the mosque. Furthermore, formal education with a Francophone heritage add to the cultural complexity. As mentioned above the aim of the study was not to analyze the different socio-cultural profiles in detail, but to identify major trends about beliefs and practices concerning dogs, their vaccination, rabies and institutional shortcomings. Considering the rather broad level of analysis that we aimed at (rather than depth), we did not pay particular attention to gender, neither age (see Table 1 below). However, both demographic characteristics are mentioned to provide some information to the reader about the positionality of the different quotes.

Three (3) languages were used depending on the region and characteristics of the interviewees to allow everyone to participate in the discussion. French was used to talk to service providers in all regions. To talk to dog owners in the Logone Occidental region in southern Chad, Ngambay was used, whereas in rural N'Djamena and the Ouaddaï region, Arabic was used in interviews and FGDs.

In a society that values seniority, the constitutions of groups in relation to age always has an influence on how participants of FGD express themselves. Therefore, individual interviews were added to complement the data

TABLE 1 Socio-demographic characteristics of research participants.

Data collection tool	Site	Sex	Age range (years)	Religious affiliation	Dog owners	Heads of civil society associations	Heads of veterinary posts and livestock delegation	Heads of livestock sectors	Chief district medical officers
FDG	Logone occidental	Men: 5 Women: 2	22–67	Christian: 6 Muslim: 1	7	-	-	-	-
		Men: 6 Women: 1	33–59	Christian: 5 Muslim: 2	-	-	7		
FDG	Ouaddaï	Men: 6 Women: 1	25–58	Muslim: 7	7	-	-	-	-
		Men : 6 Women: 1	34–50	Muslim: 6 Christian: 1	-	-	7	-	-
FDG	Hadjer Lamis and Chari Baguirmi	Men: 5 Women: 2	30–66	Christian: 3 Muslim: 4	7	-	-	-	-
		Men: 4 Women: 3	44–52	Christian: 4 Muslim: 3	-	-	7	-	-
Interview	Logone Occidental	Men: 5 Women: 2	43–65	Christian: 6 Muslim: 1	3	1	1	1	1
Interview	Ouaddai	Men: 6 Women: 1	40–56	Muslim: 7	3	1	1	1	1
Interview	Hadjer Lamis and Chari Baguirmi	Men: 5 Women: 2	45–57	Christian: 2 Muslim: 5	3	1	1	1	1

Source: field data.

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sources (triangulation). Men and women were mixed in FGDs of dog owners. This choice was taken based on the focus on economic, religio-social and institutional factors (rather than gender). For the client vs. provider perspective adopted in this article, it was crucial not to mix service providers with the client (dog owner) side, as this may have limited dog owners from expressing their views on the service received out of fear of disadvantages in case of future consultations. Before the discussions and interviews, each participant signed an informed written consent.

Table 1 describes the number of FGD and interviews by study site and the study participants' backgrounds.

Data collection techniques and tools

This qualitative data collection was conducted on the occasion of the above mentioned stakeholder workshop, during which the results of the quantitative studies undertaken during the GAVI-project were shared. The workshops were held between August to September 2018 distributed as follows: from August 22 to 23, 2018 in Abéché, from August 27 to 28, 2018 in Moundou and on September 10, 2018 in N'Djamena.

Data were collected by FGDs and by direct semi-structured interviews using an interview guide (S1). With dog owners, veterinary officers and heads of livestock delegations both in-depth interviews and FGDs were conducted. With heads of civil society associations, district medical officers and heads of livestock sectors only individual interviews were conducted. The FGDs were held separately for community representatives and the institutional representatives (see Table 1). This allows to juxtapose perspectives on dog vaccination from the client's point of view (the dog owners) with the institutional point of view, as well as to compare formal rules with actual vaccination practices. There were seven participants per FGD. In average, the FGD lasted about 45 min.

The interview guide was structured along the following thematic aspects: Economic and socio-cultural factors affecting access to dog vaccination and institutional factors that hinder access to dog vaccination. The interviews lasted about 30 minutes each.

Data processing and analysis

With the participants from the institutional level, interviews were conducted in French. With the dog owners, Arabic and Sara was used, in addition to French. The lead author who did the field research speaks all three languages fluently. The data collected through the FGDs and individual interviews were translated into French if needed and transcribed and entered into Word version 2010 and then processed using MAXQDA version 2018 software. The data was analyzed using content analysis. Coding was done inductively. The text broken down into thematic paragraphs and coded with the help of the software.

The first and second author of this paper were members of the local GAVI-project team and as such involved on all levels of the quantitative data collection: Household survey, bite case reporting and animal rabies case surveillance. In addition, the main author was responsible for the free hotline established during the project to facilitate communication between the animal health and human health workers and reporting of bites or animal rabies incidents by the public (27). Together with the main author's participation in prior rabies research studies in Chad (22), the presence in the field and close contact with both the community and the institutions during the GAVI-project period has allowed him to gain the needed experience to appropriately interpret the FGDs and interviews.

Ethical considerations

Ethical approval for the overall study, covering research activities in the three GAVI-project countries (Mali, Côte d'Ivoire and Chad), was granted by the Ethics Committee of Northern and Central Switzerland (EKNZ). The specific research activities of the GAVI-project in Chad received research approval from the Chadian Ministry of Public Health (MSP) (No. 1569/MSP/SE/SG/DGAS/2016) and the National Bioethics Committee of the Chadian Ministry of Higher Education (No. 298/PR/MESRS/SG/CNBT/2016). Written consent was obtained from each participant before data collection.

Results

The data presented here address the obstacles to dog vaccination in Logone Occidentale (LO) province, in Ouaddai (O) province and rural Ndjamena (Chari Baguirmi (CB) and Hadjer-Lamis (HL) provinces) of Chad. It is structured in two sections: The first section presents the economic and sociocultural factors that constitute obstacles to dog vaccination on the population side, and the second point deals with the institutional factors that prevent dogs from being vaccinated on the provider/veterinary side. At the end of the result section, we summarize the topics discussed in a table to give a general overview (Table 2).

Economic and socio-cultural factors affecting access to dog vaccination

Economic and sociocultural factors that hinder dog vaccination include: (1) the high cost of the vaccine; (2) the

TABLE 2 Summary of factors influencing access to dog vaccination in Chad.

Dimension	Variables	Obstacles	Propositions
Economic	Cost of the vaccine	- Low purchasing power - Transportation costs	- Provide vaccine free of charge - Increase local availability of vaccine
	Geographic accessibility	- Distance to vaccination site	- Conduct door-to-door vaccination
	Means for transport	- Transportation difficulties - Aggression by other dogs	- Conduct door-to-door vaccination
Sociocultural	Social function of the dog	- Belief in the negative effects of the vaccine on dogs	- Conduct awareness campaigns
	Perception of the dog	- Negative attitude toward the dog as an impure animal - Lack of awareness	- Conduct awareness campaigns
Institutional	Vaccine availability in veterinary posts	- Lack of electricity - Supply difficulties	- Use of solar panels for conservation - Improve supply management
	Reinforcement of the law on dog animal vaccination	- Lack of data on rabies - Low involvement of authorities	- Increase surveillance - Conduct participatory workshops on rabies - Conduct awareness campaigns
	Knowledge of the animal and human health workers about rabies	- Absence of the rabies topic in the curricula	- Strengthen rabies in the training of medical and veterinary students - Conduct joint training workshops
	Intersectoral collaboration	- Lack of communication - Lack of notification of rabies cases - Neglect of rabies on the national level	- Improve intersectoral communication on rabies - Increase reporting of rabies - Allocate means for One Health collaboration

Source: field data.

distance between home and veterinary posts; (3) the lacking means of transporting the dog to the vaccination post and (4) the use of dog meat for therapeutic purposes in some socio-cultural milieus.

Cost of the vaccine

The cost of the animal rabies vaccine varies between 10,000 and 15,000 CFA francs (15 to 23 Euros) depending on the locality in Chad. The majority of respondents found the price of the vaccine very expensive. With a Gross Domestic Product (GDP) of less than one (1) Euro (<500 CFA francs) per day (https://www.data.worldbank.org/country/chad), most people were unable to pay for vaccines. Respondents from all focus groups in the different regions said that the high cost and local unavailability of the vaccine prevent them from vaccinating their dogs.

> *"Dog vaccines are too expensive, and it is difficult to find them even in Beinamar. I went but I could not find it."* (Dog owner, Christian, M, 31, LO)
>
> *"When I wanted to vaccinate my dog, I looked for the vaccine until Koumra [town about 275 km from owner's place of residence] and they charged me 80.000 FCFA [122 Euro], I was unable to buy it."* (Dog owner, Christian, M, 46, LO)
>
> *"The vaccine is too expensive, that's why we don't vaccinate the dogs. The state must make the vaccine free for us, otherwise we cannot vaccinate our dogs."* (Dog owner, Muslim, M, 57, CB)
>
> *"The state must subsidize the price of vaccines to allow the population to get them."* (Dog owner, Muslim, M, 53, HL)

Some study participants even say:

> *"We don't have money to pay for bread and you ask us to pay for vaccine that is expensive for dog only."* (Dog owner, Christian, W, 41, CB)
>
> *"The vaccine costs are too much, the state must give it for free. We want to vaccinate our dogs well, but with this price, we can't."* (Dog owner, Christian, M, 32, LO)
>
> *"In our community, dogs are not vaccinated, because of the cost of vaccines. A dose of dog vaccine costs up to 20,000FCFA [30 Euro]. And our purchasing power does not allow us to buy them."* (Dog owner, Muslim, M, 48, O)

These comments show that there is a willingness to vaccinate dogs in the different communities, but the cost of the vaccine is a common barrier to access on the national scale. Furthermore, dog owners complain about the unavailability of vaccines at institutional level and lack of information where to receive it:

> *"One day, I took my dog to the veterinary station in Beinamar. But the agents told me that they do not have a refrigerator to store the vaccines.* (Dog owner, Christian, W, 39, LO)
>
> *"We are not informed about where dog vaccines are sold."* (Dog owner, Christian, M, 27, LO)

Geographical accessibility of the vaccine

The distance between homes and veterinary facilities that might provide vaccine is a major concern for our study participants. In all four regions of the study, rabies vaccines are stored in the regional capitals (Moundou, Abéché, Massakory, and Massenya), because of the lack of electricity for storage at veterinary district levels. In Logone Occidental, the distance between the district veterinary office and the provincial livestock delegation that manages the vaccines is between 60 and 80 km. In Ouaddaï, it is between 150 and 200 km. The one of Chari-Baguirmi is in the range of 80–100 km away and in Hadjer Lamis, the provincial delegation in Massakory is located between 100 and 150 km from the district offices of the region. Thus, when in need, people are obliged to travel at least 60 km to have their animals vaccinated and some up to 200 km. The average cost of a single trip for transportation between the districts and the regional livestock delegation is 3,000 to 6,000 FCFA *[4.5 to 9.- Euro]*. Given the poor condition of the road, people and animal have to calculate about 5–8 h (in the rainy season even up to 10–12 h) to get the vaccine. Those who do not have a means of transportation find it impossible to have their dogs vaccinated. Interview respondents said:

> *"It must be recognized that the rabies vaccines are only available in Moundou. And we who are in the other localities, do not have the possibility to keep them because we do not have the electricity to keep them. When someone wants to vaccinate their dog, they are directed to Moundou and because of the distance, they refuse to go."* (Veterinary officer, Christian, M, 58, LO)
>
> "*We have problems with vaccine supply. When it rains, the road is cut by rainwater and we are isolated. It takes 3 to 4 months for our roads to be cleared. Under these conditions, access to vaccine is difficult"* (Veterinary officer, Muslim, M, 46, HL)
>
> *Our district is very far from the regional hospital in Abéché and sometimes in Abéché itself, we cannot find vaccine for the dogs. If needed, we are sent all the way to Ndjamena, more than 1,000 kilometers to get the vaccines."* (Dog owner, Muslim, M, 39, O)

During the focus groups, respondents expressed themselves in these terms:

> *"There are no vaccines in the veterinary station at our home in Abdi. If someone wants to vaccinate their dog, we send them to Abeche. But as the journey is long, the owners of the dogs refuse to go. We have no way to store the vaccines here."* (Dog owner, Muslim, M, 53, O)
>
> *"We can't transport the dogs more than 150 kilometers for vaccination. One has to send the vaccines to the districts."* (Dog owner, Muslim, M 60, O)
>
> *"During the rainy season, with the rising waters, we are cut off from other towns for at least 3 months. It is difficult for us to get supplies of vaccine."* (Veterinary Officer, Christian, M, 47, O)
>
> *"Everything is centralized in Abéché. We need to supply the other districts with vaccine to reduce the distance and facilitate accessibility."* (Veterinary Officer, Muslim, M, 50, O)
>
> *"In the rainy season, we have difficulties accessing products such as vaccine because the conservation conditions are not met. To go to Ndjamena, the roads are impassable because of the rainwater."* (Veterinary Officer, Muslim, 36, O)
>
> *"To get the vaccine, we have to travel several kilometers because the vaccine does not exist in our district. So this distance does not allow us to access the product."* (Dog owner, Christian, 42, CB).
>
> "*To get your dog vaccinated, you have to go all the way to Ndjamena, because here in Dourbali (106 km de Ndjamena), there is no rabies vaccine."* (Dog owner, Christian, M, 52, CB)

Means of transporting dogs to the vaccination post

One of the difficulties raised by our respondents is the lack of means to transport the dog to the vaccination site. In this regard, about three quarters of the respondents stated that it is impossible to drag the dog on foot for miles to vaccinate it. Since most of the dogs are free roaming taking the dog on foot for vaccination entail the risks for dog-to-dog aggression. In addition, there is the risk that the dog might aggress other people in the street. The cost of transporting dogs in a public vehicle (between 2,000 and 3,000 CFA francs, or 3 to 5 Euros) makes it difficult to travel longer distances to access the vaccine. Furthermore, in the case of public transport, some truck owners refuse to transport dogs, because according to their belief the dog is an animal of misfortune whose presence in the truck can cause accidents.

Some of the comments made in the interview were:

> *"When you bring your dog somewhere, the other dogs chase you and the dogs fight."* (Dog owner, Christian, M, 54, HL)
>
> *"The dog is a complicated animal. If you take it somewhere, the other dogs will follow you and fight, or even bite you. If you take him in the truck, he attacks the other passengers. That's why we can't get our dogs vaccinated."* (Dog owner and head of social civil society association, Christian, M, 31, LO)
>
> *"I would like to vaccinate my dog, but I do not know how to transport it to the vaccination site. If the vaccine is in our district, we will get our dogs vaccinated. But we have to go all the way to Moundou. We don't know how to transport our dogs."* (Dog owner, Muslim, W, 39, HL)
>
> *"Transporting dogs to the vaccination site is always a problem in our communities. We need to organize door-to-door vaccination. We don't know how to transport the dogs to the vaccination [site]."* (Dog owner, Muslim, M, 46, O)
>
> *"We have no means of transporting the dogs to Ndjamena for vaccination. And the vehicle owners refuse to take the dogs, because they will cause an accident."* (Dog owner, Muslim, M, 60, CB)

Social value and perception of the dog

In addition to its value as a guardian and pet, many male respondents of the Logone Occidental, region of southern Chad mentioned that they eat dogs due to its ascribed therapeutic properties. This cultural practice of dog meat consumption persists in Christian communities in southern Chad and is not practiced among Muslims. Several research participants mentioned that the vaccination of dogs could destroy the virtues contained in the dog. The statements about this specific function and perception of the dog are as follows:

> *"The meat of the dog is a medicine for us. It preserves against bad spells, evil spirits and rejuvenates the cells of the body."* (Dog owner, Christian, M, 59, LO)
>
> *"We raise the dog to eat. When you hear the dog howling at night like a wolf, it means it has seen a spirit. So we eat the dog's meat to protect ourselves from evil spirits. But when you vaccinate the dog, it loses all these virtues and it has no longer any effect."* (Dog owner, youth Association Leader, Christian, M, 43, LO).
>
> *"The dog is used for hunting in our country. But when it dies, we make medicine with its flesh against disease. That's why we don't want to vaccinate it."* (Dog owner, Christian, M, 39, CB)

To the contrary, research participants with a predominantly Muslim background in the Ouaddaï region, consider the dog as an impure animal that should not be approached according to the religious and cultural practices. According to our study participants, even if a dog owner is willing to handle the dog and bring it to a vaccination location, the community's negative perception of someone handling a dog is an obstacle for owners to bring their dogs to vaccination.

> *"The dog is not appreciated in our community. Those who approach the dog are also hated. I can't take my dog to vaccination because of the community's view."* (Dog owner, Muslim, M, 41, O).
>
> *"At home, if you are seen handling the dog or touching the dog, people do not greet you, and do not eat with you in the same dish. You are considered dirty, unclean. We don't accept the dog near us. Even if we raise it for our own safety, it stays in the front yard and we give it food, but it does not come near the family because it is too dirty."* (Veterinary officer, Muslim, M, 54, O)

Both the positive perception of dog meat on the one side and the negative perception of the dog as a dirty animal on the other side constitute important cultural barriers to dog vaccination.

Institutional factors that hinder access to dog vaccination

This section focuses on institutional factors that limit access to vaccination. These include the absence of vaccines at veterinary posts, lack of communication about the Animal Health Law, lack of awareness of rabies among veterinary officers, and lack of intersectoral collaboration.

Unavailability of vaccines at veterinary posts

When asked if the vaccine is available at the veterinary posts, more than half of our respondents said that there is no vaccine at the posts, which confirms the experiences shared by the dog owners. In the district of Moundou, which is the vaccine storage center, cases of vaccine shortages have been noted. In this regard, the shortages are noted during certain periods as a result of lacking supply chain management, as the respondents stated during the interviews:

> *"When we order the vaccine, it takes time to reach us and this can create the shortages."* (Veterinary officer, Muslim, M, 34, O)
>
> *"We looked for the vaccine in all the districts of the region, but we did not find it. We are told that only the district of Moundou has the vaccine. I went there but they tell me there is a break"* (Dog owner, Christian, M, 51, LO)

The absence of rabies vaccines at local veterinary posts results in the barriers to accessing dog vaccination described above such as geographical distance coupled with transport difficulties.

Lack of communication about the dog vaccination law

Regarding knowledge of the law that requires owners to vaccinate their pets and report cases of mandatory rabies, almost all were unaware of it.

The interviews yielded the following statements:

> *"There is no law for dog vaccination in this country."* (Dog owner, Muslim, W, 43, O)
>
> *"Nobody told us that there is a law that obliges dog owners to vaccinate them. But when your dog bites someone and if it dies, you are arrested in prison."* (Head of civil society association, Christian, M, 55, LO)
>
> *"Although this law on the vaccination of dogs exists but it needs to be popularized to the public. We don't have the means to raise awareness."* (Head of district veterinary delegation, Christian, M, 39,CB)

We note from the opinion of the respondents that the majority is not informed of the law on dog vaccination and mandatory reporting of suspected animal bites. This situation is due to the lack of communication and awareness of the population by the authorities in charge of animal health and rabies.

Absence of rabies in the training curricula of veterinary officers

The data collected from our respondents regarding their knowledge and practice of rabies reveal that animal health professionals do not have a good understanding of rabies. The majority of them stated that rabies was not part of their training, as some of them testified:

> *"In our training, we have never heard of rabies. We do not know the manifestations or clinical symptoms of this disease in dogs."* (Veterinary officer, Christian, M, 48, LO)
>
> *"Rabies is considered a neglected disease by our authorities and no one cares about this disease. Yet, it kills many children. The state must develop a plan to fight this disease."* (Head of livestock sector, Muslim, M, 57, HL)

According to them, this lack of training in rabies control is due to the negligence of the authorities in charge of animal health. The lack of knowledge of rabies by veterinary officers does not allow them to organize an awareness or communication campaign about rabies. In order to effectively communicate the danger of rabies to the population, agents must be well-trained about the subject. The absence of this training result in an access barrier related to lacking knowledge about the disease in the community as a whole.

Lack of intersectoral collaboration

Another important factor for access to dog vaccination is collaboration between human and animal health services. According to the data collected, the majority of our respondents stated that there is no collaboration between these two sectors:

> *"We don't have collaboration as such with the human health sector. Sometimes, we meet at workshops but afterwards, each one goes its own way."* (Veterinary officer, Muslim, M, 49, LO)
>
> *"Since we have been practicing, we have no relationship with human health workers. Everyone evolves on their own and in case of rabies, it is difficult for us to communicate."* (Head of livestock sector, Christian, M, 38, CB)
>
> *"Collaboration with human health was non-existent. It is from the GAVI study that we had collaboration in the monitoring and diagnosis of the biting animal."* (Veterinary officer, Christian, M, 43, LO)

Indeed, when there is a case of rabies, the two services must work together to adequately treat the victim, but also to follow up on the bitten animal to stop the spread of rabies. The lack of collaboration also results in inadequate notification of cases and in consequence, the burden of rabies is underestimated and authorities do not perceive the need for a national action plan.

Discussion

The studies objective was to combine perspectives on the community and institutional level to gain a first overview of where some of the main obstacles to dog vaccination in Chad might lie. The results reveal several economic, socio-cultural and institutional factors that prevent access to dog vaccination in Chad. Some of them have been observed in other studies (8, 28), but to our knowledge this is the first qualitative study that brings together insights from both the population and the service provider level. These insights will be valuable to overcome barriers to vaccination in future planning of rabies control measures. Like for other goods, access to dog vaccination is subject to the dynamics between availability and demand. Both positive and negative factors associated to dog vaccination on the community level thus influence the factors on the institutional level and vice versa, forming an access cycle. If for example animal health professionals are not well-trained on rabies prevention and control, they are not able to provide adequate sensitization to the public to increase awareness among the community and hence demand by dog owners will remain low. On the other hand low purchasing power and limited geographical access on the community level results in low demand by dog owners and in turn, availability will remain low on the institutional level due to the unprofitability of providing dog vaccination. These examples show that the combined perspective allows us to better understand drivers or barriers influencing this access cycle.

In our study we have only looked at the provision of vaccine in public veterinary facilities, but we assume that the interplay between availability and demand will be even more negatively affect in the private veterinary sector due to the higher need for profitability. In Senegal for example the public sector funded through the government provides livestock vaccine free of charge and farmers pay only a service fee, whereas in the private distribution system, herders bear the full cost of the vaccine (29).

The study took place in four provinces of Chad that together represent the two major religio-cultural backgrounds. Moreover, the study covered the rural and urban context. The results of this study can thus provide guidance to implementation of a rabies control program at the national level. In Chadian communities, dogs are kept for a variety of reasons, ranging from protection of premises and livestock, to hunting and even consumption (22). Accordingly, the perception and role of the dog varies from one community to another based on the ethno-cultural and religious context. Despite the general picture, we are able to provide by looking at the two main religio-cultural contexts, our study has limitations. The breadth (sample size) and depth (detail) of data did not allow us to deepen the analysis of perceptions and practices that would enable us to distinguish between different ethno-linguistic groups, socio-demographic and socio-economic characteristics. Such a study could be a next step helping to design locally tailored measures to increase acceptability of interventions (18). Nonetheless, the main access barriers identified here can already be useful to prepare a general national action plan for rabies control.

A first issue to address would be the cost of the vaccine. Chad remains one of the poorest countries in the world with a Human Development Index (HDI) of 0.401 (187th out of 189 countries). The majority of study participants representing the population have low monthly incomes (<60,000 CFA francs or 90 Euros) and have no formal employment, with low purchasing power. In N'Djamena, a free mass dog vaccination campaign achieved the required level of 70% coverage (13), whereas a previous campaign in the same setting that charged around 4 USD for vaccinating a dog achieved only a coverage of 24% (30).

Studies from Uganda and Peru also show that poverty influences not only vaccination coverage, but also dog keeping practices (31, 32). Another previous study in Chad shows that lack of financial means for vaccination or to pay for PEP in a case of a bite even influences the decision to raise a dog (33). Indeed, when a rabid dog bites individuals, it is the dog owner's responsibility to take care of these victims regardless of the number of victims. Given the unavailability and cost of human rabies vaccine (20), some dog owners end up in prison, or social ties with their neighbors or the community are weakened after a dog bite incident, because they are unable to pay for PEP [main authors' own experience gained during his service as a rabies hotline agent during the GAVI-project (27)]. Risks and cost related to transport of a dog to distant veterinary facilities with vaccine in stock highlight the need for localized approaches to improve availability of services. Distance and transport were also identified to be major barriers for dog vaccination in Ethiopia (34) and Tanzania (35).

Free public rabies vaccine and bringing veterinary posts closer to communities could improve motivation of dog owners to vaccinate their animal, but our results also show that such measures alone might not be sufficient due to lack of disease awareness and socio-cultural barriers observed. A study in Ethiopia found that a dog owner's knowledge of rabies (34) is a significant predictor of the level of intention to vaccinate a dog. A finding that is supported by a study in Côte d'Ivoire reporting that low vaccine access appears to be influenced by ignorance and negligence (35). Overall these findings are not new since already back in 2010 a study conducted in several developing countries, where canine rabies is endemic, the most common reasons for dog owners not to vaccinate their dogs are lack of knowledge about the disease burden and prevention, vaccination costs, and ease of catching dogs (8). In fact, the neglect of rabies in sub-Saharan Africa is largely attributed to a lack of recognition of the infection as a significant public health threat (36).

Our qualitative data confirm the hypothesis derived from quantitative data on vaccine coverage (13) and dog population estimates (23) in Chad, that ethnic beliefs influence dog breeding/keeping in some localities. Some respondents believe that the consumption of dog meat protects them against evil spirits and helps to cure certain diseases. This power is according to them destroyed by the vaccine and therefore this aspect constitutes an obstacle to rabies control. The Christian religion considers the dog as a companion that deserves care and affection. According to Akakpo (37), a Christian priest points out that "on the day of the last judgment, all the animals rescued by your care will come to testify in your favor."

In the Muslim communities, dogs are less appreciated. This conception is also reported in Senegal. Leye [1989, cited in Migan (38)] had stated that in Senegal, "it is common to hear that the Muslim should not raise a dog, because it is an impure animal." Indeed, some Hadiths of Islam cited by Migan (38) teach the following: "The angel does not enter the room where there is a dog"; "The black dog is a Satan (demon)"; "If the dog licks the dish, it must be washed 6 times in natural water and the 7th time with soap." It should be noted that in the Muslim community in Chad, the dog is called "khèleb" which means (impure, dirty, hated, smells bad) to the point where someone who is hated by society is called "wadam khèleb" which translated means "damned dog." This concept leads the community to chase dogs off their premises and even keeps a dog, it is held in the front yard to ensure their safety, but the dogs are not allowed in the house. Such dogs that are not close to their owners and not used to be handled are difficult to reach during vaccination campaigns. In Bamako, Mali, for example, the inability to handle aggressive dogs was an important reason for non-participation in a centralized vaccination campaign (39). However, the situation is paradoxical in Pikine (a suburb of Dakar in Senegal) where the Muslim community is the one that owns most dogs (37). At a closer look the topic is more complex that commonly thought. According to cited hadiths in Migan (38), the Prophet Mohammed also said: "the best dog is the one who guards the herd and the house. He also promised paradise to the believer who quenched a dog's thirst by giving it a drink from his shoe." In any case, in spite of religious or ethnic prohibitions, confronted with the problem of insecurity, in the cities and the countryside alike, people with different beliefs decide to keep a dog to ensure their safety.

An element that we were unfortunately not able to address with our study, is the effect of gender-dynamics on dog vaccination access. We did not particularly pay attention to gender balance during recruitment of participants and the fact that FGD were mixed certainly had an influence on the way women participated. Two new studies highlight the importance of adopting a gender sensitive approach when identifying obstacles to vaccination in the field of livestock vaccines (40). The role of managing and controlling livestock diseases in these communities was culturally ascribed to men. This is also the case for dog keeping in Chad and the decision power on whether to vaccinate or not lies almost inclusively in the hands of the male representative of the household. Moreover, dog meat consumption is limited to men (main author's experience). Even in cases where women are the primary caretakers, for example in the case of poultry farming in Senegal, there are cultural and social barriers to their ability to access vaccination services (29). Similarly, children are most often those that can handle their household dogs very well to bring to vaccination posts, but they depend on the decision of their parent. Further, more fine-grained studies of the social dynamics related to age and gender at household level would certainly provide valuable insight into gender or age related differences to access.

Obstacles also result from the low involvement of the authorities in dog vaccination campaigns. The majority of our respondents cited ignorance of the law on health police organizing dog vaccination (Law No. 04-009 2004-05-19 PR of 19 May 2004). According to this law, dog vaccination is mandatory in Chad, and sanctions are provided for all those who do not respect this law. The lack of knowledge of the law by the majority of owners is explained by the lack of awareness in the community (22, 41), lack of knowledge of animal and human health providers (42, 43) and low priorization of rabies control by concerned authorities resulting from a virtually absent rabies surveillance system (21). Results from a global study on rabies surveillance shows that Chad is not an exception in this regard (44). In fact, a very recent article reflecting on the factors hampering advances on the road to zero dog mediated human rabies cases by 2030 identified these very obstacles described here on the institutional and socio-cultural level (28). Intersectoral collaboration between human health and veterinary workers is a key factor in rabies control (11). Lack of collaboration and lack of expertise in rabies control is a challenge for the organization of vaccination campaigns. A Knowledge Attitude and Practices (KAP) study in human and veterinary health workers conducted during the GAVI-project revealed a considerable lack of knowledge about rabies and recommended treatment among the participants and confirms the negligence of the rabies topic by the training curricula of both sectors (43). The need for cross-sectoral collaboration of actors in a synergistic transdisciplinary way highlights the usefulness of a "One Health" approach for rabies control (11, 45). This approach gives an important role to animal health professionals and animal owners as well as to people in regular contact with domestic and wild fauna and the environment. To be effective, control actions must address all levels of society, all political, religious or associative groups. Although it primarily concerns the Ministries of Health and Agriculture or Livestock, rabies control also requires the involvement of the Ministries of the Interior, Education and Communication, and Research (36). All stakeholders, including universities, learned societies and associations of physicians, pharmacists and animal health professionals, all schools, the media, local authorities and religious groups, and even neighborhood communities, must be sensitized and involved in the fight against rabies if the disease is to be defeated in the near future.

Conclusion

Socio-cultural and institutional factors influence a dogs' access to vaccination. Therefore, these factors, which may constitute barriers to rabies control, must be addressed and eliminated through context-specific communication strategies to improve vaccination and surveillance coverage. In Chad, this study demonstrated three major problems that impede dog vaccination. These obstacles are economic (cost of vaccines), sociocultural (belief of loss of valued characteristics of dog meat in case of vaccination on the one hand and stigmatization of dogs on the other), and institutional (unavailability of vaccine and lack of knowledge and communication of the law on dog vaccination).

To achieve efficient control and ultimately elimination of rabies the collaboration of several actors intervening in the field of human and animal health, safety, education, communication, research, environment, etc. is crucial. These actors need to have sufficient resources to respond to the existing demand for dog vaccine but also to engage in awareness raising to increase demand by dog owners.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Northern and Central Switzerland (EKNZ). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

NM: sociologist, study design, data collection and analysis, and manuscript writing. AM: health geographer, study co-design, data collection, and analysis support. DA: sociologist, methodological, and analysis support. PN: veterinarian and manuscript writing support. JZ: veterinarian, one health expert, and overall study PI. KH-T: sociologist, methodological support, and manuscript revision. ML: veterinarian and rabies expert, study coordination, data collection, supervision, and manuscript revision. 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.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.866755/full#supplementary-material

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United Against Rabies Forum: The first 2 years

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Rabies continues to kill an estimated 59,000 people annually, with up to 99% of human cases transmitted by domestic dogs. The elimination of human deaths from dog-mediated rabies is achievable by applying a One Health approach, and the framework to do this is outlined in Zero by 30: the Global Strategic Plan to end human deaths from dog-mediated rabies by 2030. To build on this global goal, and implement the approaches set out in Zero by 30, the United Against Rabies Forum was launched in 2020. This paper gives a review of the objectives, governance, activities and achievements of the United Against Rabies Forum to date. It also outlines ongoing work, and next steps as the United Against Rabies Forum reviews its first 2 years of activities and identifies priority areas for the coming 12 months.

KEYWORDS

rabies, United Against Rabies Forum, rabies elimination, Zero by 30, One Health, neglected tropical diseases, zoonosis

Introduction

Rabies, one of the world's most ancient infectious diseases and most lethal viral zoonosis of mammals, kills an estimated 59,000 people annually across large regions of the globe (1). Transmitted by domestic dogs in up to 99% of human cases, effective control methods to tackle the disease have been long proven but often do not reach the population in need—poor and marginalized communities (2). The elimination of human deaths from dog-mediated rabies is achievable by applying a One Health approach, and the framework to do this is outlined in Zero by 30: the Global Strategic Plan to end human deaths from dog-mediated rabies by 2030 (*Zero by 30*) (3). Developed by the Food and Agriculture Organisation of the United Nations (FAO), World Organisation for Animal Health (WOAH), World Health Organisation (WHO) (the Tripartite) and the Global Alliance for Rabies Control (GARC) in 2018, this country-centric strategy prioritizes the changes needed to reach zero human deaths from dog-mediated rabies with three core objectives: to effectively use vaccines, medicines, tools and technologies; to generate, innovate, and measure impact; and to sustain commitment and resources (3).

To use the momentum created by *Zero by 30*, the Tripartite launched the United Against Rabies Forum (UAR Forum) in 2020 to create a broad and inclusive network of stakeholders who share a vision for the elimination of dog-mediated rabies, and wish to work collectively to achieve it (4). Three working groups, encompassing more than 30 institutions, were established to progress activities that contributed to each of the three *Zero by 30* objectives (4). In line with a One Health approach, these working groups actively engage a wide range of stakeholders including participants from national and regional entities, human and animal health sectors, NGOs, academic and research institutions, and private sectors (4). This paper gives a review of the objectives, governance, activities and achievements of the UAR Forum to date. It also outlines ongoing work, and next steps as the UAR Forum reviews the first 2 years of activities and identifies priority areas for the coming 12 months.

UAR Forum governance and mode of operation

Zero by 30 established an achievable goal and a common plan for the international community to work toward. However, further work was needed to actively bring stakeholders together. To address this, the Tripartite set out to establish a network, the UAR Forum, that could bring partners together to collectively implement the objectives set out in the global strategy, and accelerate progress toward their shared vision of ending human deaths from dog-mediated rabies.

The UAR Forum is hosted by WOAH, on behalf of the Tripartite organizations, with the Terms of Reference undergoing review by focal points and legal teams of each organization until these were agreed upon. The Tripartite maintains oversight of the UAR Forum, with strategic direction provided by the Steering Group, which is comprised of representatives of the three Tripartite organizations and chairs of each working group (5). The Steering Group meets quarterly to review progress, ensure that working group activities are coordinated and synergistic, and provide strategic direction in planning next steps.

The wider UAR Forum consists of member organizations that are committed to implementing the approaches set out in *Zero by 30*, while sharing knowledge, experience, ideas and information. UAR Forum members include intergovernmental and non-governmental organizations, national governmental institutions, private sector entities, philanthropic foundations and academic and research institutions. At the time of publication, more than 50 organizations have become members of the UAR Forum. Organizations can view the UAR Forum Terms of Reference and apply for membership through the UAR Forum website (www.UnitedAgainstRabies.org).

To ensure that practical outputs were developed to support countries in progressing with control and elimination of dog-mediated rabies, results-focused working groups were established to address priority activities that contributed to the three objectives outlined in *Zero by 30*. Three working groups were established in 2021: Working Group 1—Effective use of vaccines, medicines, tools and technologies; Working Group 2—Strategic and operational support to countries; Working Group 3—Advocacy and resource mobilization. The workplans of each working group initially focused on priority activities that were realistic and could be developed over a 12–18 month period, with the intention of expanding the workplan to include further activities at a later date.

Participation in the working groups is not mandatory for UAR members. Instead working groups consist of participants that represent a small a number of UAR member organizations, and have expressed their interest and availability in actively contributing to focused activities (5). Working group chairs provide coordination and leadership for working group activities and lead working group discussions to prioritize topics and review progress. Workstreams are developed within each working group, with a workstream lead nominated to coordinate the work. These workstreams meet virtually on a regular basis to progress activities, reporting back to the larger working group every 2–4 weeks. To ensure the working groups remain inclusive and productive, participation is flexible, with workstream participants contributing to multiple workstreams over time, or providing short-term contributions to specific activities. Contribution in the working groups is unpaid and voluntary, with each working group participant dedicating time and expertise according to their individual availability and capacity. Stakeholders interested in finding out more or contributing to specific activities can contact the UAR Forum through the contact page of www.UnitedAgainstRabies.org.

Key achievements

Facilitating knowledge transfer—The United Against Rabies Forum webinars and website

The inaugural stakeholder event, entitled "United Against Rabies: One Health in Action—Partnering for Success" was held virtually in September 2020 (6). This event not only acted to officially launch the UAR Forum, but provided an opportunity for stakeholders to define and recommend the priority areas that should be progressed by UAR Forum working groups. In 2021, a series of three webinars were held in September and October to update stakeholders on the progress and outputs of the UAR Forum, and post-webinar evaluation forms were also disseminated to allow stakeholders to provide feedback on the priority areas that should be addressed, and areas that needed to be reviewed (7). Between 200 and 400 participants representing both human and animal health sectors from 92 countries attended each webinar (7). A further webinar was held in 2022, focusing this time on a single priority area that stakeholders had identified—"Tackling Rabies and Dog Population Management: the Role of Local Authorities" (8).

UAR Forum members also presented on the UAR Forum activities and outputs in more than 20 virtual and in-person events over the course of 2021 and 2022, advocating for *Zero by 30*, and inviting stakeholders to contribute to the collective effort. However, as much of the engagement was virtual over the first 12 months of the UAR Forum due to the COVID-19 pandemic, and there was no central platform to direct stakeholders to, there were challenges in achieving an inclusive network and disseminating Forum outputs. The UAR Forum website (www.UnitedAgainstRabies.org) was launched in early 2022 and helped overcome these challenges by providing a central platform where stakeholders could access technical resources, sign up for news and events and express interest in UAR Forum membership.

To improve engagement and communication of updates and UAR Forum outputs with stakeholders, the UAR Forum will hold webinars on a quarterly basis, focusing on key priority areas or newly developed resources that can support stakeholders in implementing rabies control programmes. Webinars will be open to all, and provide an opportunity for participants to network and ask questions of experts.

Quarterly newsletters will also be disseminated to the UAR Forum mailing list, providing news, updates on upcoming events, latest outputs and inviting stakeholders to participate in UAR Forum surveys where relevant. Individuals can sign up to the UAR Forum mailing list at www.unitedagainstrabies.org, or contact the United Against Rabies Forum by emailing globalrabiescoordinator@woah.org.

Working group workstreams

Within each of the three working groups, several workstreams were established to focus on specific priority topics. A working group member was identified as the workstream lead, and were responsible for leading the workstream discussions and sharing progress and results with the wider working group on a regular basis. Working group outputs were made available for review by all working groups, before being submitted to the Steering Group for final review. Following approval by the Steering Group, working group outputs were finalized and made publicly available. Several of the completed workstreams, and the respective outputs from these, are discussed below, with Table 1 providing direct links to key outputs of the UAR Forum to date.

Experts within the Forum are available to provide stakeholders with additional information or support to better utilize and implement any of the UAR Forum outputs, and contact can be made through the UAR Forum website to request support, or express interest in contributing to specific areas of work.

National strategic plan template

A critical aspect of *Zero by 30* is that it focuses on country-centric engagement, with countries leading efforts. A key milestone for countries to do this is the development of a national strategic plan—providing a framework outlining targets and measurable deliverables, and demonstrating government commitment to the global goal of *Zero by 30.* However, developing this strategy can often be a challenge for countries. To address this, participants of Working Group 2 (Strategic and operational support) reviewed available documents and existing resources (3, 9–18) to develop a national strategic plan template that follows international guidelines and standards, to help countries develop their own robust, One Health focused canine rabies elimination plan. The template is flexible enough to provide practical guidance for countries in early stages of canine rabies elimination, and countries at more advanced stages, and aligns with the WOAH process for the endorsement of official control programmes for dog-mediated rabies (9). Countries that achieve this endorsement will likely increase chances of obtaining national and international funding (9). The template has been disseminated through regional networks, and used to support the development of several national strategic plans to date, including Burkina Faso, Cambodia, Sierra Leone and Togo. The template will be reviewed and updated regularly according to feedback from national stakeholders, to ensure that this remains useful and fit for purpose. It is available in English and French on the UAR Forum website, and will be linked to the UAR Forum roadmap (discussed below) as a resource to help countries in achieving milestones associated with the development and implementation of a national strategy (19).

Surveillance and minimum data elements

Surveillance is a critical component of national control programmes. Despite rabies being one of the deadliest diseases known to man, in practice it remains a hidden disease due to poor surveillance and underreporting. Rabies-related data span the One Health spectrum, with human deaths and human vaccination data collected by human health authorities (most often Ministries of Health), whereas animal rabies cases and animal vaccination data are often collected by Veterinary Authorities (often Ministries of Agriculture). This has led to constraints in data sharing, and the development of numerous and overlapping guidance documents and data definitions.

Data should be collected for a specific purpose, be that informing interventions (e.g., Post-Exposure Prophylaxis (PEP) or dog vaccination programmes), monitoring trends, or evaluating the effectiveness of disease programmes. Consistent case definitions are a foundational requirement for surveillance programmes, as well as monitoring and evaluation of progress toward *Zero by 30*, and data elements are often informed by standard case definitions. Participants in Working Group 1 (Effective use of vaccines, medicines, tools and technologies) set out to examine commonly used case definitions for rabies programmes and identify the minimum data elements that inform the current status and progress of National rabies control programmes. A landscape analysis of available documents was conducted (2, 9, 20–29), and numerous inconsistencies were identified in national, regional and international data element definitions commonly used by rabies programmes.

To address this, Working Group 1 developed a comprehensive document that provides realistic set of essential data elements that are needed to track progress toward *Zero by 30*, allowing for reliable, comparable estimates in time and between countries (30). Furthermore, the document provides consistent definitions for key case definitions and data elements that align with global standards published by WHO and WOAH (2, 22). Standardizing terminology is essential for communication and comprehension of information between international advisory forums, regional implementation partners and national administrators. National authorities are encouraged to use the minimum data elements guide to adopt globally standardized data definitions, improve in-country rabies data collection and submit data to the WHO Global Health Observatory and the WOAH World Animal Health Information System on an annual basis (24, 25). By providing a common standard for rabies data, the aim is to move toward resolving rabies-related information gaps that would improve health policy decisions globally.

TABLE 1 Links to key outputs of the UAR Forum.

Key outputs	Link
United Against Rabies website	www.UnitedAgainstRabies.org
Webinars	2020: One Health in Action: Partnering for Success
	2021 (series of three webinars): Rabies, One Health and Covid 19; Data gaps, monitoring and tools and technology; National Rabies Control Programmes
	2022: Tackling Rabies and Dog Population Management: the Role of Local Authorities
Template for national strategic plan to control rabies	Access template in English and French
Minimum data elements for surveillance	Access document in English
United Against Rabies toolbox	Visit Toolbox
United Against Rabies roadmap	Visit Roadmap
One Health Joint Plan of action—rabies embedded within "action track 3: neglected zoonotic diseases"	Read the One Health Joint Plan of Action here
Template for landscaping of resource partners for rabies	Access template in English
Case studies	Rabies prevention and control—lessons from Chonqing, China
	How Mexico achieved rabies-free status
	Goa State and Mission Rabies—achieving rabies-free status together
	Namibia's national plan for rabies elimination delivers benefits
Example pitch deck	Access example pitch deck

This document has been disseminated through regional networks, and will be a key topic in an upcoming UAR webinar in 2023, entitled "Rabies surveillance: what gets measured gets done." The UAR Forum includes members that are experts in rabies surveillance, and stakeholders can contact the UAR Forum if support is needed in adopting these standardized data elements.

Tool evaluation

Despite a number of effective tools and applications being available to support national control programmes and canine rabies elimination efforts (e.g., tools for economic modeling, planning and implementation of dog vaccination and IBCM, monitoring and evaluation of national rabies programmes), these are often inaccessible or underutilized by stakeholders. Working Group 1 participants focused on collecting existing tools for canine rabies elimination, evaluating these, and providing guidance to countries on how to adapt and use these to improve their rabies programmes. The identified tools and technical resources have been aligned to the workplan of the Stepwise Approach for Rabies Elimination (SARE) (11), to help stakeholders identify tools that are appropriate and fit for purpose for specific activities. The UAR Forum website provides an interface where users can identify the category of tool they need, the strengths and weaknesses of the tool, then contact developers if required to integrate this into their programme (31).

Roadmap

Although many countries have made progress toward freedom from dog-mediated rabies, countries are at differing stages, and often find it challenging to determine the steps and approaches that should be implemented to scale up efforts, and tools and resources that can be utilized to progress further. The development of the UAR Forum roadmap aims to help countries monitor their progress toward canine rabies elimination using a standardized approach with clear progress milestones, while also introducing countries to the most appropriate tools and resources for their activities. In providing an objective roadmap score for a country, aligned with international criteria and milestones of WHO and WOAH (2), this roadmap will also help monitor progress at a global level. National stakeholders can establish the roadmap score for their country by completing a SARE assessment (11). The SARE assessment helps users to assess the strengths and weaknesses of a national rabies control programme, assists the user with developing a rabies workplan, and provides a SARE score indicating the current rabies situation across a country—a score which provides the basis for the roadmap score. The roadmap builds upon this, by bringing together specific tools and resources, including the aforementioned working group outputs, that are readily available to address pending activities in the country's canine rabies elimination work plan, and thereby provide tangible steps for countries to take to progress to the next milestone. For example, national stakeholders at an early stage of the roadmap with no national strategic plan will be directed to the national strategic plan template, stakeholders identifying a need to improve surveillance will be directed to the minimum data elements document, and specific activities identified within a country workplan will link to tools in the UAR toolbox that can help in implementing these activities. The roadmap is available on the UAR Forum website, and further work on this is anticipated in 2023 to provide direct links to tools and resources (32).

Integration of rabies into the One Health Joint Plan of Action

The COVID-19 pandemic highlighted the need for a One Health approach in overcoming challenges and emerging threats associated with zoonotic diseases. In response to a global call for action, the Food and Agriculture Organisation of the United Nations (FAO), the UN Environment Programme (UNEP), the World Health Organisation (WHO), and the World Organisation for Animal Health (WOAH) (the Quadripartite) launched the One Health Joint Plan of Action (OH JPA) in October 2022 (33). This five-year plan aims to create a framework of actions that contribute to One Health, while aligning and building on existing One Health and coordination initiatives, including *Zero by 30* (33).

The Tripartite and members of the UAR Forum worked to ensure the inclusion of rabies in this wider One Health agenda, resulting in the integration of rabies in Action Track 3 of the OH JPA, "Controlling and eliminating endemic zoonotic, neglected tropical and vector-borne diseases" (33). Having rabies embedded into the OH JPA will provide support for advocacy efforts, demonstrating to investors that their investment is contributing to a plan that is widely and most visibly endorsed by the international community.

Landscaping of national resource partners

Mobilizing domestic and international resources is critical for effectively and sustainably implementing national control programmes. For planning purposes, it is key to identify agencies/organizations that have funded rabies prevention programmes/initiatives in the past, and also to identify resource partners that can provide required resources (including non-financial resources e.g., equipment, consumables, infrastructure, IT or communication services). To assist national stakeholders, Working Group 3 has developed a template to help map and profile potential resource partners that can support the development and implementation of their national control programme. This template can be accessed on the UAR Forum website (34).

Case studies

Four case studies have been developed, and are available on the UAR Forum website (Mexico; Namibia; Goa, India; Chongqing, China) (35). Each case study demonstrates unique strategies and specific partnerships, but these all highlight the catalytic role that resource partners have played in canine rabies elimination. The focus of Working Group 3 was to adapt these case studies in a way that could target investors, advocating for their engagement toward canine rabies elimination, and showing how this could be replicated in other areas with the right support. These case studies have been instrumental in discussions with international investment partners, helping to demonstrate the impact that their investment could have in progressing toward canine rabies elimination.

Pitch deck

Working Group 3 participants worked to develop resources that could support stakeholders in developing their own canine rabies elimination advocacy and investment pitches. Participants reviewed and collated a vast amount of rabies related material, before consolidating this and working with communication experts to transform targeted information into an example pitch deck template to support investment in canine rabies elimination. Stakeholders are able to download an example pdf slide deck, and use this information to develop their own pitch to potential investors. The pitch deck is supported by instructions for helping stakeholders develop the best messaging and targeting for specific categories of investors (36).

Identification of main constraints to rabies control

The objective of this workstream was to identify, analyse and provide information on constraints preventing countries from progressing toward *Zero by 30* and to link to potential solutions. To provide an evidence base, participants carried out a scoping review of the literature, extracting and compiling mentioned constraints and clustering these into overarching categories. These constraint categories were used as a basis for virtual polls (conducted during the 2021 UAR webinar series, 2021 virtual Rabies in the Americas conference, and a 2022 workshop conducted in Côte d'Ivoire), with participants asked to prioritize constraints based on their experiences. This prioritization informed the establishment of a new workstream in Working Group 1, focused on improving dog vaccination coverage (one of the prioritized constraints by poll participants). Investigating such priorities in more depth could help to identify patterns for the most pressing constraints depending on factors such as geography or level of canine rabies elimination progress already achieved, directing support and resources into more focused and meaningful directions.

Ongoing workstreams

Linking tools, the roadmap and the canine rabies blueprint

Further work is underway to integrate the outputs of the tool evaluation and roadmap workstreams, specifically looking at linking specific tools and resources within the roadmap—in this way countries can identify both the key activities they need to undertake to progress to the next roadmap milestone, and the specific tools that can help them achieve these activities. The tool evaluation workstream will also progress with reaching out to tool developers, encouraging them to submit their tools for evaluation by experts, so that these can be included in the UAR Forum toolbox. Tool developers who wish to submit their tools for evaluation can contact the UAR Forum *via* the UAR Forum website.

The canine rabies blueprint is also an online repository of resources developed by a global panel of rabies experts, however the website and much of the content is outdated and in need of review and updating (12). Participants of the UAR Forum working groups aim to review the existing resources and content on the canine rabies blueprint in 2023, update or develop new content where required, and integrate this with the UAR Forum website and toolbox to ensure its ongoing availability and use of the relevant resources.

Partnership map

Despite the increased global commitment toward the elimination of human deaths from dog-mediated rabies, rabies control efforts remain fragmented and uncoordinated in many areas, resulting in duplicated efforts, poor use of resources and ineffective or unsustainable activities. To address these challenges and to improve coordination, transparency and equitable support to canine rabies endemic countries, the UAR Forum is developing a partnership map. This map will provide a regional and global overview of rabies stakeholders and will foster collaboration between stakeholders, helping them to better align efforts, avoid duplication and identify synergies. This workstream is currently collecting data on UAR Forum member activities (types of activity, and where these are being conducted), after which this will inform the development of a publicly available map which will be displayed on the UAR Forum website.

Improving the human-animal bond with dog identification

Despite decades of evidence that dog vaccination is the most cost-effective means to reduce dog-mediated human rabies deaths, most canine rabies endemic countries do not reach needed vaccination threshold coverages to eliminate the virus. Anecdotal evidence from workstream members has suggested that ways to identify vaccinated dogs (e.g., through a collar provided at the time of vaccination) can improve participation in dog vaccination campaigns and have other downstream positive impacts. However, dog identification comprises an additional expense for under-funded vaccination programmes. The objective of this workstream is to use ideas from theory of change literature to develop pilot projects and to collect data to demonstrate that when people can differentiate between vaccinated and unvaccinated dogs, there will be a change in behavior between people and dogs, as well as more responsible dog ownership through community pressure on dog owners. It is hypothesized that this change in human-dog interactions can lead to reductions in dog bites, and therefore reductions in the need for post-exposure prophylaxis. Additionally, improved human-dog bonds may decrease culling of stray dogs and improve utilization of routine veterinary services. These benefits have tangible and intangible benefits that may justify the additional expense of dog identification. The workstream are planning to implement pilot projects in a number of countries in order to collect robust data to help demonstrate the cost-effectiveness of combined dog vaccination and dog identification.

Dog vaccination

Mass dog vaccination interrupts the rabies transmission cycle and is a critical pillar of canine rabies elimination strategies. However, the constraints workstream of Working Group 2 identified improving mass dog vaccination coverage as a main priority for stakeholders. While the improving human-animal bond with dog identification workstream is focused primarily on how dog identification can improve dog vaccination, this workstream is focusing on conducting further stakeholder analysis to determine what constraints or barriers are experienced in planning, implementing or evaluating dog vaccination campaigns, in order to identify practical solutions in overcoming these constraints.

Rapid diagnostic testing

Rapid diagnostic tests (RDTs) are diagnostic assays designed for use at the point-of-care, and can be adapted for use in low-resource settings (37, 38). Accurate, sensitive and affordable diagnostic methods could be a game changer for canine rabies elimination, and RDTs and home-based testing have proven pivotal tools in achieving reductions in other major infectious diseases being targeted for elimination. RDTs for rabies have been previously evaluated by the WOAH Reference Laboratories, and found to have low sensitivity and specificity, as well as inconsistent performance between products and batches. However, recent field-based evidence is showing promising results using a modified protocol (39–42). This workstream aims to review evidence on RDT performance for rabies diagnosis, analyse costs and benefits of diagnostic test modalities, investigate potential for RDT certification, develop guidance to support policy and practice on RDTs and investigate funding opportunities to support wider RDT use.

Oral rabies vaccine recommendations

Oral rabies vaccination (ORV) is conducted in numerous countries for the control of wildlife rabies, but has not been used in any meaningful capacity to control dog-mediated rabies. One constraint to use of ORV in dogs is the lack of international guidance for the selection of safe and effective products and how to incorporate ORV into a large-scale dog vaccination programmes. Guidance for ORV in dogs was last updated in 2007 (43), and does not reflect major advances in the production, safety, efficacy and recent field-use data for ORV in dogs. This workstream is revising the 2007 WHO Recommendations on ORV of dogs, and will focus on developing practical guidance for field implementation of ORV as a complementary measure to parenteral vaccinations. The revised recommendations will be published as a formal Tripartite publication in 2023, and this will be made available on the UAR Forum website.

Cross-cutting opportunities for rabies control

This workstream aims to develop a framework and scientific foundation to support countries in integrating rabies control with other goals, including the World Health Organization Roadmap for Neglected Tropical Diseases 2021–2030 (44). While there have been multiple attempts to combine rabies control with other interventions (e.g., snakebite envenomation or parasitic diseases), there is a need to summarize the nature and findings of these

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approaches to understand their feasibility and added value. This workstream is conducting a scoping review of published and gray literature on cross-cutting approaches to rabies control to compile the evidence. The review will be complemented with a selection of key informant interviews and focus groups, facilitated by the UAR network, that look to capture unpublished experiences.

An additional component of this workstream will be building on the experiences of Sierra Leone and Liberia in conducting integrated rabies and Peste des petits ruminants (PPR) vaccination. Reports from Chief Veterinary Officers in these countries reported improved community participation and awareness resulting from joint campaigns, and noted that partnering with the public health sector had marked logistical and infrastructural benefits that enhanced cost-effectiveness and improved intersectoral collaboration (45). Through support from FAO and WOAH, this project will build on these successes in Liberia and Sierra Leone, and expand this to include Guinea, to support coordinated implementation of joint rabies and PPR vaccination programmes. The evidence generated from this project will help to inform the development of UAR Forum recommendations and potential future frameworks for where adoption of integrated approaches may be beneficial.

Next steps

As the UAR Forum comes to the close of its second year, the Steering Group and Working Groups met during an operational meeting in December 2022 to review the governance, mode of operation and priority areas of the Forum. The aim of this meeting was to review the achievements of the Forum to date, and how to objectively measure outcomes from and maximize future value and impact of these outputs; identify challenges and barriers to progressing ongoing activities, reviewing if these proposed activities are still priorities, and if so, finding solutions in overcoming these barriers; identifying key priority activities for the UAR Forum in the coming 12 months based on reported country needs and developing a concrete work plan to address these; and finally restructuring the Forum to better meet the needs of the global community and support countries in reaching *Zero by 30*.

Key priorities will include focusing on engagement and empowerment of national stakeholders and local authorities, for example *via* the associations of mayors of large cities, while facilitating in-country support, particularly in the development of national strategic plans for rabies control. The UAR Forum will continue to promote the WOAH endorsement (9) of these plans and aim to link these with the agenda of Gavi, The Vaccine Alliance on PEP access (7). Gavi announced in 2019 that it would extend its portfolio to include human PEP, but noted that this would need to be part of an integrated One Health approach (46). While the roll out of this was delayed due to the COVID-19 pandemic, the inclusion of rabies PEP in this portfolio is critical for countries to implement effective One Health rabies control strategies, and the UAR Forum will continue to engage with Gavi to progress this.

In the long term the UAR Forum will continue to provide a platform to strengthen collaboration and coordination among partners, helping to reduce fragmentation, improve cross-sector efforts and facilitate country access to technical expertise. In doing so the UAR Forum will support countries to implement sustained rabies control measures and accelerate progress toward *Zero by 30*, while also building the One Health networks and capacity that are needed to improve the response to other endemic and emerging infectious diseases. In addition, having a central, coordinated network to implement rabies control will provide a more attractive and sustainable option for resource partners to invest in, helping mobilize resources to drive progress toward *Zero by 30*. The UAR Forum is supported by rabies experts that volunteer their time to support the *Zero by 30* agenda; while this approach ensures that any willing volunteer can participate, the sustainability and effectiveness of this approach must be closely monitored. Financial support for organizing the UAR Forum is critical, as key organization requirements such as staffing and website support must be supported to maintain the Forum.

Conclusion

The UAR Forum is committed to actively supporting countries in their efforts to reach the goal of *Zero by 30*. By bringing together a diverse range of stakeholders and experts with a shared vision for the elimination of human deaths from dog-mediated rabies, the UAR Forum is putting One Health into practice and providing concrete outputs to support countries' rabies control efforts. A critical component of this will be ensuring that participants and organizations from canine rabies endemic countries are engaged, and actively participating in the UAR Forum. This will be a priority activity for the coming 12 months, with the UAR Forum exploring ways to better connect country needs with UAR Forum activities, in order to inform the development of outputs that can be most impactful for where they are needed most. In doing so, the UAR Forum will make a significant contribution to health equity and stronger human and animal health systems, and can provide a model for One Health implementation.

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

RT and AF contributed equally to the writing of this manuscript. All authors reviewed and approved the manuscript.

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Working Group 1:

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