

Seroprevalence of Rabies Virus in Dogs in the Mekong Delta, Viet Nam

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ABSTRACT

Rabies in dogs continues to be a serious public health threat in many regions worldwide. Viet Nam is one of endemic countries of the disease and many fatal cases in dogs and human have been frequently reported over years. This study aimed to determine the seroprevalence and factors associated with the seroprevalence of captured dogs in slaughterhouses in Can Tho and Ben Tre provinces, the two epidemiologically representative locations of rabies in the Mekong delta (MD), Viet Nam. During 2020–2021, a total of 470 canine serum samples were collected from slaughterhouses in six districts for each of Ben Tre and Can Tho provinces and these collected sera were tested for detection of anti-rabies antibodies using indirect enzyme-linked immunosorbent assay (ELISA). Seroprevalence of rabies virus in Ben Tre was 5.94% (17 positive samples out of 286 collected samples) which was lower than the seroprevalence in Can Tho at 14.13% (26/184), respectively. Similarly, the mean value of antibody titer against rabies virus of captured dogs in Ben Tre (1.19 ± 0.45 IU/mL) was lower than that of in Can Tho (5.98 ± 4.84 IU/mL). Effects of several demographic factors such as breed, region and sex were not found for canine rabies seroprevalence. This study provides important information about seroprevalence of rabies for dogs in the MD and highlights necessity for vaccination program and surveillance for control and prevention of rabies in the area.

Key words: Rabies Virus, Antibodies, Seroprevalence, Mekong Delta, Viet Nam.

INTRODUCTION

Rabies is an infectious viral disease that affects the central nervous system in humans and warm-blooded animals. The viral infection almost results in fatal outcome following the onset of clinical symptoms and absence of postexposure prophylaxis (Tarantola et al. 2019). Rabies is caused by rabies virus which is the prototype species of the *Lyssavirus* genus order *Mononegavirales* and family *Rhabdoviridae* (Madhusudana et al. 2012; Walker et al. 2022; Javed et al. 2023). The predominant mode of rabies transmission, responsible for 90% of cases, is through the bite of infected animals, notably dogs and cats, due to their close proximity and interaction with humans (Baer et al. 1971; Chhabra and Ichhpujani 2003; Blanton et al. 2009). Based on the severity of mortality it induces in humans worldwide, rabies occupies the seventh position among infectious diseases (Coleman et al. 2004). Despite the

implementation of advanced therapeutic measures, rabies continues to manifest as a fatal disease in humans (Jackson 2013). According to the World Health Organization (WHO), it is estimated that approximately 59,000 fatal cases in human were reported annually due to rabies in over 150 countries, with 95% of cases occurring in Africa and Asia (Organization). However, it is noteworthy that this is just an estimate and the actual number of cases may be higher or lower due to underreporting and limited access to healthcare in some areas (Organization).

Viet Nam is considered as a high-risk country for rabies in which there are approximately 76 deaths due to rabies in humans and over 400,000 people receive postexposure prophylaxis every year (Thuy and Dang 2016). For example, in 2011, there were 110 fatal cases of rabies in Viet Nam, while the number of deaths increased to 266 cases during the period of 2012-2014. Most recently in 2022, number of deaths due to rabies were 41

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cases in which Ben Tre (12 cases) and Kien Giang (five cases) in the Mekong delta (MD) reported the highest fatal human cases of rabies (Viet Nam Ministry of Health 2022). Several epidemiological studies have identified risk factors for rabies infection in dogs and humans such as dog population demography, bite risk factors and population knowledge (Hampson et al. 2008; Chevalier et al. 2021). The genetic characterization of rabies recovery from Burkino-Faso and Viet Nam demonstrated that the isolates exhibited similarities with those obtained from Mauritania and China, respectively (De Benedictis et al. 2010; Nguyen et al. 2011). Another example, dog slaughterhouses are considered as hotspot of rabies and dog butchers become at a high risk of rabies virus infection (Nguyen et al. 2021). This suggests that rabies has the potential to spread across borders, underscoring the importance of dog trading and slaughtering in the epidemiology of rabies and warranting further concern.

In contemporary times, the efficient management of animals is commonly regarded as the primary approach for preventing and controlling the dissemination of rabies (Meltzer and Rupprecht 1998; Larghi 2004; Blancou 2008). On a fundamental level, vaccination is one of the most effective control measures for rabies. It leads to significantly reduce the number of dogs susceptible to infection and therefore it contributes to reduce dog-mediated human rabies cases (Thuy and Dang 2016; Nguyen et al. 2019; Acharya et al. 2020). However, routine surveillance, especially sero-surveillance to estimate immunity coverage and levels against rabies virus remains largely limited (Pham et al. 2021). In this study, we carried out serological surveillance of rabies in dogs in Ben Tre province (hotspot of rabies) and Can Tho (central administration unit) in the MD, Viet Nam.

MATERIALS AND METHODS

Ethical Approval

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Can Tho University, Viet Nam. Capture and blood collection of dogs were ethically performed following the guideline in accordance with the Regulation on Animal Experimentation of Can Tho University.

Study Areas and Period

This cross-sectional study was conducted between August 2020 to October 2021. The sampling procedure was performed in slaughterhouses in six districts of each Ben Tre and Can Tho provinces. These are two (out of ten) provincial administration units of the MD, Viet Nam (Fig. 1).

Sample Collection

A total of 470 serum samples of dogs at slaughterhouses were collected in Ben Tre and Can Tho provinces in which 286 serums samples were collected in six districts in Ben Tre province, meanwhile remaining 184 samples were collected at six districts in Can Tho province. General information about the sampled dogs such as age estimates, sex, breed and location were recorded by sampling investigators.

Before blood collection, dogs were restrained and muzzled properly. About 3-5mL of blood was drawn aseptically from each dog at jugular or cephalic veins using sterile needles and syringes (22-gauge, 5mL syringes). Blood was kept in the syringes without anticoagulant and allowed to clot for 4-5 hours at 4°C. The sera were then gently decanted into 1.5mL Eppendorf tubes and stored at -20°C until being laboratory tested.

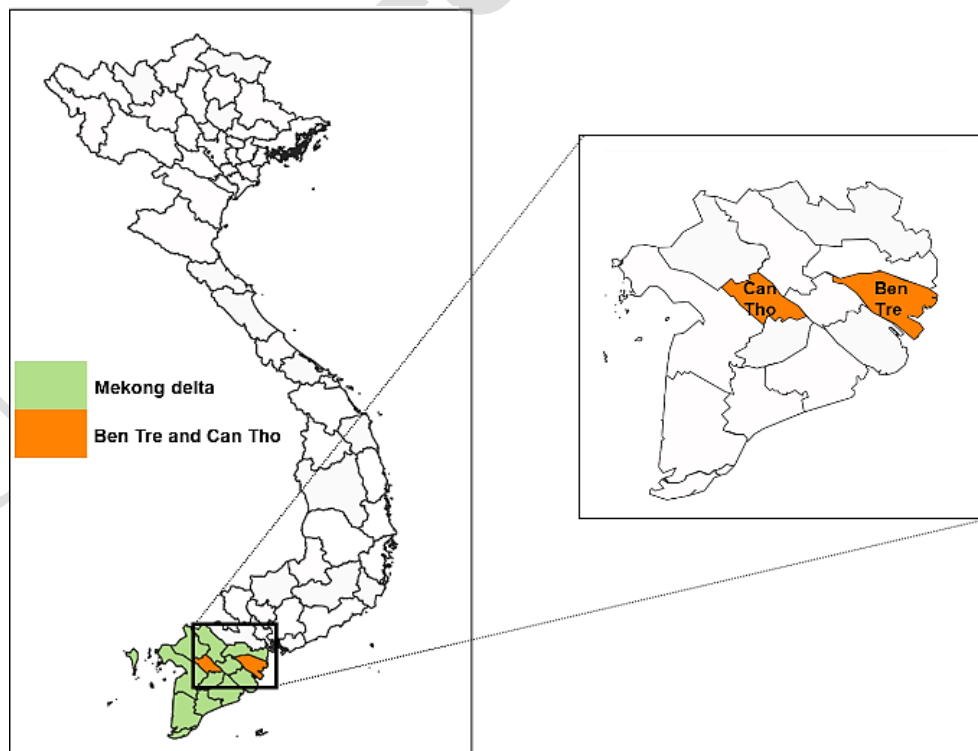


Fig. 1: Map of Viet Nam and the MD showing locations where sampling was performed in this study. The MD is colored green and two Ben Tre and Can Tho provinces of the MD are colored orange.

Enzyme-Linked Immunosorbent Assay (ELISA)

For detection of rabies antibodies in dog serum samples, SERELISA® Rabies Ab Mono Indirect kit was applied and its testing procedure was performed following the manufacturer's recommendations with minor adapted modification (Jin et al. 2019). Briefly, diluted serum samples, positive and negative controls, and the quantification standard were distributed into the microplate and incubated at 37°C for one hour. Three washing steps using PBS were then performed to remove unbound antibodies and other proteins from the samples after incubation, and then 100µL of a conjugate-protein labeled with peroxidase was added to each well. This was followed by a second incubation step at 37°C for one hour, and an additional five washing steps to remove unbound conjugate. The microplate was then incubated at room temperature for 30min and 100µL solution of H₂SO₄ 1N was added to stop the enzymatic reaction. The results by optical density were read using an ELISA reader (Multiskan FC, Thermo Scientific) at wavelength 450nm.

Statistical Analysis

Data collection was undertaken using Microsoft Excel. The prevalence proportion (expressed as a percentage) of seropositive was determined, and its confidence intervals (CI) were calculated as 95% binomial proportions representing Wilson score intervals. Statistical analyses, graphing, and visualization were performed through programming language R software.

RESULTS

Seroprevalence and Antibody Titer of Rabies Virus

Fig. 2 indicates the seroprevalence and the mean values of antibody titers of 470 dogs collected in Ben Tre and Can Tho provinces. Average rabies seroprevalence in dogs in the MD was 9.15% in which seroprevalence in Can Tho (14.13%) was significantly higher than that in Ben Tre province (5.94%). Mean values of antibody titer against rabies virus in the MD, Ben Tre and Can Tho were 3.58±2.64, 1.19±0.45 and 5.98±4.84IU/mL, respectively.

Seroprevalence of Rabies Virus in Dogs (Demographic Factors)

Table 1 provides summary statistics of seroprevalence of dogs characterized by livelihood, breed and gender. It is clear from the Table 1 that there were no significant differences of seroprevalence of dogs among studied demographic factors, meaning that seroprevalence of dogs between urban and suburb livelihood, domestic

and foreign breed, male and female gender were almost equivalent in Ben Tre, Can Tho and MD.

DISCUSSION

Rabies is an endemic and neglected zoonotic disease in many regions in the world where low and middle income countries in Asia and Africa have been particularly affected (Organization). The disease is one of the most important zoonotic diseases since it has posed significant health implications. Domestic dogs and cats are the most common sources of human infections, which is associated with more than 99% human deaths in the world. Viet Nam is the country with rabies epidemic occurring annually and at the high risks of transmission of rabies from animals to human (Thuy and Dang 2016). For example, a total of 378 deaths due to rabies were recorded in 52 out of 63 provinces and cities across the country during the period from 2017 to August 2021 (Viet Nam Ministry of Health 2022). Surveillance and vaccination in dogs and cats are considered as the most effective control measures for the disease in both humans and animals. However, routine surveillance in Viet Nam especially sero-surveillance to estimate immunity coverage and levels against rabies virus remains less investigated (Pham et al. 2021).

(Viet Nam Ministry of Health 2022)

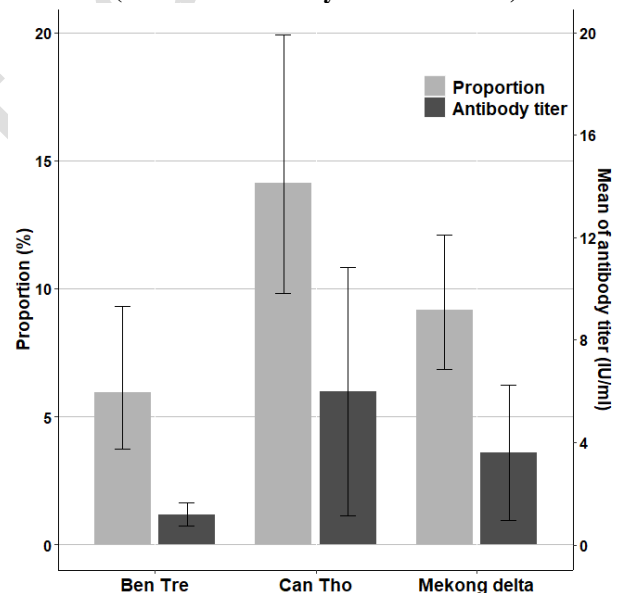


Fig. 2: The seroprevalence (light grey) and antibody titers (dark grey) in dogs in the MD, Ben Tre and Can Tho provinces.

Table 1: Sero-prevalence of dogs characterized by livelihood, breed and gender

Variables	Can Tho				Ben Tre				Mekong delta				
	No. of tested	No. of seropositive	Sero-prevalence (%)	P value	No. of tested	No. of seropositive	Sero-prevalence (%)	P value	No. of tested	No. of seropositive	Sero-prevalence (%)	P value	
Livelihood	Urban	108	18	16.67	0.24	136	5	3.68	0.12	244	23	9.43	0.24
	Suburb	76	8	10.53		150	12	8.00		226	20	8.85	
Breed	Domestic	166	22	13.25	0.30	207	14	6.76		373	36	9.65	0.46
	Foreign	18	4	22.22		79	3	3.80		97	7	7.22	
Gender	Male	99	14	14.14	0.99	162	11	6.79	0.49	261	25	9.58	0.72
	Female	85	12	14.12		124	6	4.84		209	18	8.61	

To our knowledge, this is the first study to determine the rabies antibody prevalence of dogs in the MD where rabies cases were frequently reported every few years. The two provinces of the MD, Ben Tre and Can Tho, were selected for the cross-sectional surveillance. Ben Tre is the hotspot of rabies with highest number of human cases reported recently (Viet Nam Ministry of Health 2022). However, rabies human case was not reported over years in Can Tho regardless this is the central administration unit of the MD. Therefore, surveillance in these provinces might provide sufficient knowledge for rabies in the MD. This study demonstrated that both rabies seroprevalence and antibody titers of dogs in Can Tho were significantly higher than that in Ben Tre. Our result suggests that vaccination coverage and its efficacy in Can Tho were more likely better than in Ben Tre, which might explain the fact that the number of human cases of rabies in Ben Tre were routinely higher than in Can Tho over the last recent years. Results of this study are in line with the previous research findings that rabies cases are likely limited to specific areas and new risk areas can be different from the areas with high incidence rate (Lee et al. 2018).

Several previous studies indicated that rabies-infected dogs in slaughterhouses may be a potential source of rabies virus transmission to humans through the trading and butchering activities, slaughtering and processing raw dog meat. In Viet Nam, an across-sectional study revealed that rabies virus was detected in dog brain samples collected in slaughterhouses in the northern area of Viet Nam at detection rate of 0.8%. In addition, 26.4% of the dogs had neutralizing antibodies against rabies virus (Vu et al. 2021). This result reaffirms that there was a potential risk of rabies transmission to humans through dog slaughtering activities in Viet Nam (Nguyen et al. 2021). Therefore, sero or virological surveillance of rabies in dogs can be allocated in slaughterhouses for effective monitoring of the disease.

For better understanding about rabies seroprevalence in dogs in the MD, we further examined influence of several demographic factors of the dogs such as livelihood, breed and gender that are thought to be associated with canine rabies seroprevalence. None of these factors were associated with rabies seroprevalence in Ben Tre, Can Tho and the MD. Results of this study are highly consistent with previous reports in other endemic countries such as Bolivia (Suzuki et al. 2008), Nigeria (Kolawole et al. 2018) and India (Nale et al. 2021).

It is also noteworthy to indicate the several limitations in this study. First, the ELISA assay used in this study could not differentiate between antibodies induced by infection and those induced by vaccination. Secondly, almost all dogs in the slaughterhouses lack background information that impedes further epidemiological investigation. Last but not least, it is also critical to carry out virological surveillance for the detection of rabies virus and its genetic characterizations in the MD.

Conclusion

Rabies is one of the most important zoonotic and fatal disease, but it is fully controllable and preventable via

vaccination in dogs and cats. This study is the first report about the sero-prevalence of rabies in the MD and indicates that rabies immunity in dog population was likely insufficient for complete prevention from disease. Therefore, vaccination for dogs should be strengthened, strictly implemented and frequently monitored to better herd vaccination coverage and immunity.

Acknowledgments

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Competing Interests

The authors declare that there is no conflict of interest regarding the publication of this article.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

Authors' Contributions

This work was conducted with the contribution of all authors. NDH, TPV, TNB and NTL designed the experimental procedures. TPV performed the experiments. NDH, TPV, TNB and NTL interpreted the data and prepared the manuscript. All authors read and approved the final manuscript.

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