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RESEARCH ARTICLE

Sero Prevalence of Virus-neutralizing Antibodies for Rabies in Street Dogs of Kathmandu Valley, Nepal

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Abstract:

Introduction:

Rabies is a vaccine-preventable viral zoonotic disease that remains a serious global public health concern. Rabies vaccination with adequate coverage of the canine population has been shown to control rabies outbreaks among canines and to prevent the transmission of rabies from dogs to humans. As vaccination is the primary control measure for rabies, it is important to determine the level of anti-rabies antibodies in animals in order to determine the effectiveness of the control measures being implemented.

Materials & Methods:

Blood samples were collected from 50 street dogs (August 2016 to December 2016) in Kathmandu, Bhaktapur and Lalitpur districts. Rabies sero-conversion on the separated serum was quantified using PlateliaTM Rabies II Kit (Bio-Rad, China) according to the manufacturer's recommendations.

Results:

Eighty percent (40/50) of the serum samples surpassed the requested level of rabies antibodies, suggesting good coverage of vaccination among street dogs.

Conclusion:

However, an active dog surveillance system with a dog registration process before and after vaccination campaigns, and a multi-dimensional approach including all stakeholders, are necessary to eradicate rabies from the canine population in Nepal.

Keywords: Rabies, Dogs, Vaccination, Antibody titer, Multi-dimensional approach, Stakeholders.

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1. INTRODUCTION

Rabies is a vaccine-preventable zoonosis caused by the virus, genus *Lyssavirus* family *Rhabdoviridae* [1]. It is one of the most feared zoonotic diseases worldwide. Although it remains a serious global public health concern, more than 95% of the world's fatal cases of rabies occur in Africa and Asia [2].

Canine rabies is the most common means of transmission of rabies to the human population [3]. Whereas developed countries have reduced or eliminated human rabies through the control and vaccination of their dog populations, it has remained a challenge for many developing countries [4]. Due to the lack of accurate data on the disease burden, weak or non-existent rabies surveillance systems, under-reporting of cases by local and central authorities, erratic identification of cases based only on clinical diagnosis rather than laboratory confirmation, and inadequate legislation for compulsory notification of cases, rabies remains a neglected disease [5] and its elimination continues to be a challenge.

Rabies is a high priority, endemic, zoonotic disease in Nepal [6]. It occurs in two epidemiological cycles: The urban

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cycle involves domesticated dogs, and the sylvatic cycle involves wild animals [6]. The urban cycle is maintained by the street and community dogs and is the main source of human rabies globally. Frequent transfer from street dogs to owned dogs adds to the already high rabies burden. Recent surveys carried out by animal welfare organizations estimated that the dog population within the ring road area of the valley to be 31000 in 2006, 22500 in 2010 and 22300 in 2012 [7]. However, there is little information on the exact population of street dogs.

In countries where dogs are the primary source of infection to humans, vaccination of dogs can help reduce or eliminate the human rabies burden. Vaccines help to establish pre-exposure immunity and to protect individual animals from contracting rabies, hereby preventing further spread to humans or other domestic animals [8]. Rabies vaccination with 70% coverage of the canine population has demonstrated a reduction in the transmission of the disease, which is enough to control its outbreak in the canine population [9] leading to the prevention of transmission from dogs to humans [10]. As vaccination is the primary control measure, it is important to determine the level of anti-rabies antibodies in animals to know the efficacy of the control measures [11].

No study to determine the efficacy of the rabies vaccine as a control measure in Nepal, has been conducted. Therefore, this study was conducted to determine the level of antibody titers of rabies in street dogs of the Kathmandu valley in order to ascertain the immune status in the street dog population.

2. MATERIALS AND METHODS

2.1. Study Area and Sample Size

This cross-sectional study was conducted from August 2016 to December 2016 in three districts of Kathmandu Valley *viz*. Kathmandu, Bhaktapur and Lalitpur. A total of 50 dogs were selected randomly from different animal shelters. The sample size was calculated using Unknown Population Size, 10% Error, 15% Prevalence at 95% Level of Confidence with the statistical program at winepi.net. Eighteen samples from Kathmandu, 16 samples from Bhaktapur and 16 samples from Lalitpur were randomly collected when the dogs were brought to these shelters for sterilization purpose.

2.2. Sample Collection

Dogs were restrained physically and muzzled using either a commercial muzzle, or a single loop in a length of gauze bandage that was looped over the dog's muzzle, tied under the mandible, and the ends brought behind the dog's ear and tied to prevent the dog from removing it. The dog was then placed either in lateral or sternal recumbency on the examination table and a tourniquet was applied above the site of blood collection. Blood was drawn aseptically from either the cephalic or saphenous veins into vacutainer tubes, then stored in an ice-box for transport to the laboratory of National Zoonoses and Food Hygiene Research Center (NZFHRC), Kathmandu, Nepal. There, serum was separated from the blood and stored in a freezer at 18 to 20 ^{o} centigrade for further processing.

2.3. Antibody Titer Detection Using *ELISA – Platelia TM Rabies II kit ad usum Veterinarium*

For the detection of rabies virus, anti-glycoprotein antibodies from the dog serum samples, an indirect immune-enzymatic assay (*PlateliaTM Rabies II kit ad usum Veterinarium,* Biorad) was used according to the manufacturer's recommendations and instructions. This test was chosen for rapidity in obtaining results (<3 hours), simplicity in comparison to virus neutralization, the quantitative and qualitative results, and safety for the laboratory personnel. This test has 98.6% specificity and 88.8% sensitivity.

Diluted serum samples, positive and negative controls, and the quantification standard were distributed into the microplate and incubated at 37ºC for one hour. Three washing steps 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 37ºC for one hour, and an additional 5 washing steps to remove unbound conjugate. The microplate was then incubated at room temperature for 30 minutes and 100µl solution of H2SO-1N was added to stop the enzymatic reaction. The microplate was read bichromatically at 450 and 620 nm.

To quantitatively determine anti-rabies antibodies, a standard curve was constructed using the quantification standards (S1 to S6) obtained by serial dilutions of the R4b calibrated positive controls. The optical density values for the unknown samples were compared with the positive sera titers in quantification tests obtained after a direct reading on the standard curve, and expressed as Equivalent Units per ml (EU/ml) unit equivalent to the international units defined by seroneutralization (Fig. **1**). The results of our determinations were included in the next categories: high seroconversion level (>4 EU/ ml), sufficient seroconversion level (0.5-4 EU/ml), insufficient seroconversion level (0.125-0.5 EU/ ml) and undetectable seroconversion (<0.125 EU/ml).

2.4. Data Analysis

Microsoft Excel was used for calculating the descriptive statistics, and plotting standard curve.

3. RESULTS

3.1. Qualitative and Quantitative Interpretation of Results

A total of 50 serum samples from street dogs were screened for virus-neutralizing antibodies, 40 (80%) samples overcame the requested level of seroconversion (≥ 0.5 IU/ml) and 10 (20%) samples were under the range (< 0.5 EU/ml) according to PLATELIA™ RABIES II test. The highest seroconversion rate was found to be 16 (88.88%) in Kathmandu followed by Bhaktapur 14 (87.50%) and Lalitpur 10 (62.50%). The mean titer and SD of positive seroconversion were found to be 1.79;1.11 in Kathmandu, 1.72;1.02 in Bhaktapur and 1.55;1.45 in Lalitpur districts (Table **1**). The quantitative interpretation was made with the help of a standard curve. This curve was plotted in Microsoft excel sheet provided by PLATELIA™ RABIES II test. The antibodies titer of each sample was evaluated and compared with the optical density of

the standard positive control and blank as described in the manufacturer's protocol (Fig. **1**).

4. DISCUSSION

We analyzed 50 street dogs as a part of our study, of which 40 (80%) had a protective level of anti-rabies antibodies (≥ 0.5 IU/mL). This result was consistent with a study conducted in Bangkok, Thailand, where the antibody prevalence was between 49-86% [12]. We found a higher anti-rabies antibody prevalence in street dogs in three districts of Kathmandu Valley, Nepal, than in Japan, where one study showed that only 27.7% of dogs had protective immune status [13], or in Chandigarh, India where only 1% of the street dogs had a protective level of anti-rabies antibodies [14]. The variation between our results and studies in other countries may be due to overall small sample size (n=50) from animals captured by animal welfare organizations, which may not be indicative of the street dog population in the Kathmandu valley as a whole. Our findings were lower compared to the study conducted in Sweden, where 91.9% of pet dogs had an approved test result of ≥ 0.5 IU/mL [8]. Prevalence of rabies antibody titers was higher in our study compared to the study in Uganda where seroprevalence was 20% [15] and Kenya where prevalence was 21% [7] in owned dogs. In Nigeria, 43% of pet dogs had antibody titers exceeding the positive threshold [16].

Nepal has experienced rapid urbanization in the last two decades, with the highest in Kathmandu valley and in South Asia throughout the 1990s. In 2011, the urban population growth rate in Nepal was 3.38 percent whereas; rural and total

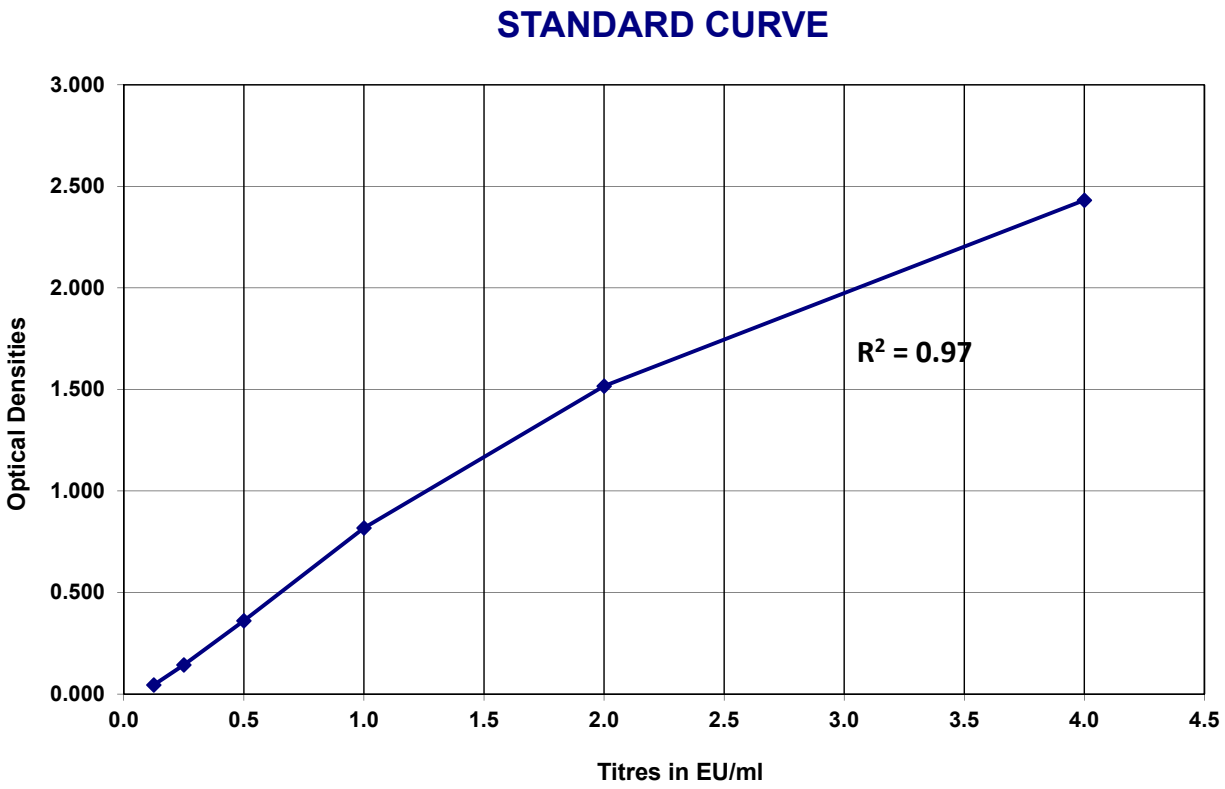

Fig. (1). Standard curve for the quantitative determination of anti-rabies antibodies in Equivalent units per ml obtained with standards and controls supplied by Platelia™ Rabies II kit ad usum Veterinarium, Biorad, France (edited with Rabies QT-ELISA BIORAD-Vers.201610.K.XLS).

Table 1. Seroconversion rate and mean titer level in sero-positive dogs of three districts of Kathmandu valley.

District	No. of Samples	Positive/ Négative	Criteria Result Validation	Titer Mean	Standard Deviation (SD)	Prevalence
Kathmandu	2/18	-	Not Sero Converted	1.79	1.11	88.88%
-	16/18	+	**Sero Converted**			
Bhaktapur	2/16	-	Not Sero Converted	1.72	1.02	87.50%
-	14/16	+	**Sero Converted**			
Lalitpur	6/16	-	Not Sero Converted	1.55	1.45	62.50%
-	10/16	+	**Sero Converted**			
Total	50	**40**	-	-	-	80%

population growth rates were measured at 1.03 percent and 1.4 percent, respectively [17]. This unprecedented urbanization has drawn global attention with respect to emerging infectious diseases, especially those on the human-animal interface [18]. According to a dog census study carried out by Kathmandu Animal Treatment Center (KAT) center, 20,000 street dogs were reported in the urban areas of Kathmandu valley. Higher seroprevalence might be a result of mass vaccination campaigns, which have been conducted on a regular basis in the valley by either the government or the private sector.

A study in Sri Lanka showed that there is a strong correlation between interventions and human rabies incidence [19]. These authors have shown that interruption in the natural transmission cycle of rabies in dogs is a logical approach to eliminate dog rabies, which would also result in a decline in human rabies cases. Mass vaccination is currently the most important control measure for the prevention and control of rabies, however, it is difficult to ascertain the protection status of an animal without measuring the humoral and cell-mediated immunogenicity in an animal post-vaccination [20].

Titer testing after vaccination is necessary if we are to know that the expected protection status has been achieved. An active surveillance system helps in monitoring the dog population and its dynamics. Along with it, a dog registration is essential as it will help in the identification of the dogs, their owners, the place where the dogs live and their numbers in that particular area. During mass vaccination campaigns, it is essential to know which dogs have been vaccinated, so that there is a nominal chance of revaccination of the same dogs. Furthermore, it will also help in reducing the titer testing of the same dogs. Control of rabies is only possible through a joint effort of all the concerned authorities and their commitment towards achieving this goal. A broader approach to rabies, with involvement of all related stakeholders including government, public health department, NGOs and INGOs, educational institutions, Kennel Clubs, Private clinics and the community, is necessary for the reduction of rabies in dogs and humans.

CONCLUSION

This study showed that 80% (40/50) of the serum samples overcame the requested level of rabies antibodies. The vaccine efficacy seemed to be effective for controlling canine rabies. However, an active dog surveillance system and dog registration process, both before and after vaccination campaigns, are urgent to control canine/human rabies in Nepal. Furthermore, high-level political commitment and a one health approach are necessary to eradicate rabies from Nepal.

AUTHORS' CONTRIBUTIONS

SR, KCO and DKP conceptualized the study. SR, KCO, DKP and YS designed the study protocol. SR and KCO carried out the sero survey. KCO, DKP and YS carried out the ELISAs and data analysis. SR, KCO, DKP and YS drafted the manuscript. SK, KCO, YS and DKP critically revised the manuscript for intellectual content. All authors reviewed, edited and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval in this study was obtained from Nepal Veterinary Council, Nepal. Standard operating procedures were followed for collection of blood sample (Animal Ethical Clearance Reference No:293-2073/74).

HUMAN AND ANIMAL RIGHTS

No humans were used. All experiements on animals were in accordance with the guidelines of Napal Vetinary Concil.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All relevant data and materials are provided with in manuscript.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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