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Research Article

Sero-Detection of *Leptospira hardjo* in Cattle of Bhaktapur District of Nepal

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Abstract

Leptospira hardjo is the most commonly reported cause of leptospirosis among cattle globally. The objective of this study was to determine sero-detection of *Leptospira hardjo* in cattle of Bhaktapur district of Nepal. A cross-sectional study was conducted in cattle pockets located at 4 different village development committees (VDCs) in the Bhaktapur district of Nepal. The sample collection was done in cattle to determine the sero-detection of *Leptospira hardjo* from February 2014 to June 2014. A total of 176 serum samples were collected from four VDCs of Bhaktapur district namely Sipadol, Dhadikot, Duwakot and Nangkhel, selected purposively. Forty samples from Sipadol, 46 Dhadikot, 42 Duwakot and 48 from Nangkhel were collected. 5 ml of blood was collected aseptically from jugular vein using 5 ml sterile disposable syringe. After that blood was transferred to the plain vacutainer. The harvested sera were transferred to serum vials and stored at in -20˚c deep freeze of Central Veterinary Laboratory until used for ELISA test. For screening of *Leptospira hardjo*, the *Leptospira hardjo* antibody test kit, ELISA (Prionics, Netherlands) was used. ANOVA along with multiple comparison test Tukey was used to compare frequency of detection across different locations in Bhaktapur district using SAS 9.4. MS-Excel was used to manage ELISA data from four different VDCs and to extract information regarding frequency of detection. There was 5.11% sero-detection in cattle of Bhaktapur district. Location wise sero-detection was 5% in Sipadol, 4.3% in Dhadikot, 4.76% in Duwakot and 6.25% in Nangkhel. The study showed that the detection of *Leptospira hardjo* in cattle. There was no statistical difference (P>0.05) between location suggesting that cattle in all areas are equally at risk of this pathogen. Further study is suggested on isolation and identification of disease in Nepal.

Keywords: *Leptospira hardjo*; Bhaktapur; sero-detection; cross-sectional study; cattle

Introduction

The cattle population of Nepal was about 7.2 million as per data from Ministry of Agriculture and Development (MOAD, 2012/2013). Leptospirosis is a systemic disease of humans and domestic animals including cattle, swine, and canine. This disease is highly prevalent globally and has proved endemic potential mainly in countries with humid subtropical or tropical climates (Bharti *et al.*, 2003). The most common cause of leptospirosis among cattle is infection with *Leptospira* belonging to *serovar hardjo* and appeared to be the primary maintenance host of this serovar.

In cattle, clinical signs including reproductive failures, abortions, mummified fetus, still births and weak calves are found although in most cases showed asymptomatic infection with host adapted serovars such as *Leptospira hardjo* (Grooms, 2006). There has been detection of *Leptospira hardjo* in infertile cattle and buffaloes in the western hills of Nepal (Joshi and Shrestha, 2011).

Many cases of abortion and reproductive inefficiency are reported in the district, but their actual cause is remaining undiagnosed. The geographic distribution of leptospirosis is widespread, but no national surveillance program exists in Nepal to establish the incidence of leptospirosis or the burden of disease in livestock of the country. In case of Bhaktapur district 65% of people are engaged in agriculture and the population of cattle is 21,568, buffalo 8,941, sheep 2,690, goat 25,500 (MOAD, 2012/2013). Up to now very few researches have been done in finding out the detection of leptospirosis in cattle of Nepal. The reliable estimates of the detection of serovar hardjo infections are lacking (Grooms and Bolin, 2005). Hence, the objective of this study was to understand the sero-detection of *Leptospira hardjo* in cattle of Bhaktapur district of Nepal using indirect Enzyme Linked Immunosorbent Assay (ELISA).

Materials and Methods

Study Design

A cross-sectional study was carried out in the Bhaktapur district of Kathmandu valley from February 2014 to June 2014. Dairy farms from four VDCs of Bhaktapur district including Sipadol, Dhadikot, Duwakot, Nangkhel were selected purposively and sampled using serum samples.

Description of The Research Area

Bhaktapur district has got 21,568 cattle, 8,941 buffalo, 2,690 sheep and 25,500 goats (MoAD, 2013). Intensive cattle farming is increasing in the Bhaktapur district the number of commercial dairy farms is rising. Infertility, abortion, repeat breeding, abortion, milk loss and birth of weak calves with a reduced survival rate is one of the common problems faced by farmers in this area.

Sample Collection and Processing

One hundred and seventy-six serum samples were collected using 5 ml sterile disposable syringe aseptically from the jugular vein. The blood was transferred to vacutainer and they were transported to laboratory in ice-packed containers within three hours of collection. The blood samples were centrifuged at 1500 rpm for 10 minutes and serum was separated, and the harvested sera were transferred to serum vial. Sera samples were stored in -80˚C freezer until testing.

Leptospira hardjo ELISA

ELISA was done by using PrioCHECK® *Leptospira hardjo* Antibody Test Kit provided by Prionics, Netherland. It has 99% sensitivity and 85% specificity. The procedure followed was as per the directions mentioned below.

Incubation of test samples:

100 µl of ELISA buffer was dispensed to wells A1 and B1 of the test plate (= blanks) and 90 µl of ELISA buffer to wells C1 to H1. Similarly, dispense 10 µl of 1:20 diluted reference serum was dispensed (= positive control) to wells E1 and F1. So, the final serum dilution was 1:200. Again 10 µl of 1:20 diluted reference serum was dispensed to (= weak positive control) wells G1 and H1 so that final serum dilution is 1:200. When testing serum samples, 90 µl of ELISA buffer was dispensed into the remaining wells along with 10 µl of 1:20 diluted sera in each of these wells so that final serum dilution is 1:200. Serum samples can be titrated by making two-fold serial dilutions in dilution buffer. Seal and shake the test plate gently and incubate for 60±5 minutes at 37±1˚C.

Incubation with conjugate and chromogen (TMB) substrate:

Wash the test plate 6 times with washing solution. Dispense 100 µl of diluted conjugate to all wells. Cover the test plate and incubate for 60±5 minutes at 37±1˚C. Wash the test plate 6 times with washing solution. Dispense 100 µl of the chromogen (TMB) substrate (Component 10) to all wells. Incubate the test plate for 15 minutes at 22±3˚C. Add 100 µl of stop solution (Component 11). Agitate the test plate to mix the content of the wells prior to measuring.

All the laboratory work was carried out at Central Veterinary Laboratory (CVL), Tripureshwor, Kathmandu.

Statistical Analysis

Statistical analysis was conducted with SAS 9.4 software (SAS Institute Inc., Cary, North Carolina) with a level of significance of 0.05. Analysis of variance (ANOVA) along with multiple comparison test Tukey was used to compare frequency of detection across different locations in Bhaktapur district. MS-Excel was used to manage ELISA data from four different VDCs and to extract information regarding frequency of detection. A total of n= 176 samples was used for the final analysis.

Results and Discussion

The overall sero-detection of *Leptospira hardjo* was found to be 5.11% (9 of 176) in the cattle sampled across the Bhaktapur district as shown in Fig. 1.

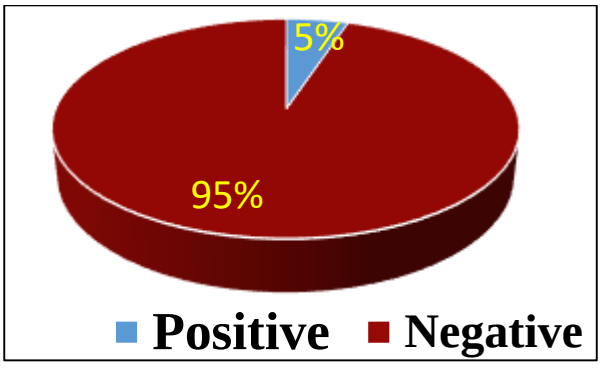

Fig. 1: Overall sero-detection of Leptospira hardjo

The frequency of detection of seropositive cattle in Sipadol was 5% (2 of 40), Dhadikot was 4.3% (2 of 46), Duwakot was 4.76% (2 of 42) and in Nangkhel was 6.25% (3 of 48) cattle sampled as shown in Fig. 2. No statistically significant difference was found in the frequency of detection across location (P<0.05) which indicated the equal potential of risk of this pathogen in all the sampled VDCs of Bhaktapur district. The higher frequency of detection of *Leptospira hardjo* in Nangkhel VDC could be due to presence of higher number of paddy fields in that region which increased the incidence of rat that has a potential to be a carrier of leptospirosis.

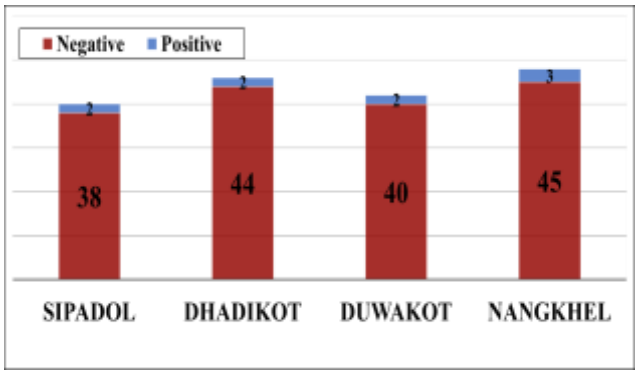

Fig. 2: Sero-detection of Leptospira hardjo by location of sample collection. Same letter indicated no statistically significant difference at P<0.05.

Joshi and Joshi, 2000 in western region of Nepal, reported 11% sero-detection in cattle which was higher to the finding in our study. Likewise, in the previous study done by (Joshi and Joshi, 2000) in the hills of Nepal the prevalence rate was found to be 8.5 % in cattle which was higher to the finding in our study. Both of these studies conducted in Nepal, revealed higher rate of detection than our finding which could be due to difference in topography, types of methods used and other associated risk factors of *Leptospira hardjo.*

Anwar *et al.*, 2013 in Peshawar district of Pakistan found a sero-prevalence of 5.26% in cattle which was similar to what we found in our study. In the previous research done by Vakili *et al.*, 2013 in Mianeh-Iran, 7.14% sero-prevalence was found in cattle and similarly by Ajaj and Farwachi (2013 in Nineveh Province, Iraq found sero-detection of 6.3% for *Leptospira hardjo* which was higher to the finding in our study. However, the previous study done by Sharma *et al.*, 2006 in Andhra Pradesh, India and Ebrahimi *et al.* (2004) in Shahrekord district, Central Iran found sero-detection of 39% and 17.33% in cattle which was higher to the finding in our study. India and other neighboring countries reporting this disease further increased the risk of *Leptospira hardjo* in Nepal. Similarly, higher prevalence of 29.35% was reported by Atherstone *et al.* (2014) in Southwest Uganda in the cattle. These differences might be due to geography, differences in test procedure and time and method of sample collection.

Conclusion

Leptospirosis is a cardinal example of a zoonotic disease. Recent information clearly shows that leptospirosis is the leading zoonotic disease in Nepal and is of major concern in view of its impact on both animal production system and public health. In this study, sero-detection of leptospirosis in Bhaktapur district was found to be 5.11% in cattle. This seems to be distressing situation in the district or we can generalize this condition in a country as a whole. There was no statistical difference between location suggesting that cattle in all areas are equally at risk of this pathogen. Proper and timely formulation and implementation of strategy for prevention, control and treatment of disease is important. The incidence of leptospirosis has been reported to vary significantly in different seasons, and to be higher in monsoon. In the current study, the samples were collected between February and March. Hence, the result obtained may not represent the actual prevalence of leptospirosis. People are found to be very much unaware about the zoonotic diseases. They even do not know that the diseases can be transmitted from animals to man.

Ethical Aspects

We received consent from the cattle owners for the collection of blood with the help of District Livestock Service Office of Bhaktapur

Author's Contribution

Both authors contributed equally in all works related to the present publication.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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