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Research Article

Detection of Antibodies Against *Leptospira hardjo* in Large Ruminants of Nepal

DR Khanal[1]*, N Paudyal[1], S Khanal[1], M Prajapati[1], P Shrestha[1], R Bowen[2], MP Acharya[1], SP Shrestha[1], UM Singh3, RP Thakur[1], I Naletoski[4] and BR Joshi[3]

[1]*Animal Health Research Division, NARC, Khumaltar, Lalitpur, Nepal*

[2]*College of Veterinary Medicine and Biomedical Sciences, CSU, Fort Collins, Colorado, USA*

[3]*National Animal Science Research Institute, NARC, Khumaltar, Lalitpur, Nepal*

[4]*International Atomic Energy Agency, Vienna, Austria*

***Corresponding Author:** D R Khanal, Animal Health Research Division, NARC, Khumaltar, Lalitpur, Nepal.

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Abstract

Leptospirosis is a life-threatening zoonotic disease caused by pathogenic spirochete bacteria of the genus Leptospira. A cross sectional investigation using dot-ELISA technique was carried out to detect the antibodies against *Leptospira hardjo* in sera of 640 domestic ruminants inhabiting at three different ecological zones in two seasons of the year: pre and post-monsoon. Dot-ELISA of pre-monsoon samples (n = 320) revealed 12 (3.75%) samples positive for antibodies against *Leptospira hardjo* while that of 320 post-monsoon sera, 22 (6.88%) were positive. Species wise prevalence in cattle, buffalo and chauries (cross of yak and cattle found in high mountain) was 6% (10/160), 0.64% (1/156) and 25% (1/4), respectively in pre-monsoon and 4.51% (7/155), 0.69% (1/145) and 70% (14/20), respectively in post-monsoon samples. Incidence was highest in chauries followed by cattle and buffaloes; post-monsoon sera demonstrated higher prevalence than the pre-monsoon samples. Furthermore, seasonality had shown clear effect on higher incidence of leptospirosis as evidenced by the detection of leptospiral antibodies in post-monsoon sera. Present data indicated the circulation of *Leptospira hardjo* antibodies in absence of vaccination program and thus, attributing the on-going leptospiral infection in large ruminants, requiring urgent attention to safeguard the public and animal health.

Keywords: Zoonotic Disease; Leptospirosis; Eco-Zone; Dot-ELISA; *Leptospira hardjo*

Introduction

Leptospirosis, a zoonotic disease caused by pathogenic spirochete of several serovars including that of *Leptospira hardjo* can result in life threatening infection in human and heavy economic losses in the diseased animals. Generally, infection spreads from urine of the infected animal or food or water contaminated by urine or the entry of pathogen through skin penetration. Leptospirosis has been distributed worldwide and classified as an emerging infectious disease [1]. Chronic infection of cattle with *Leptospira borgpetersenii* serovar *hardjo* is attributed to reproductive failure and poses a persistent health threat to workers in the animal industry [2]. In a Belgian study, pooled milk samples collected from 2000 Belgian dairy herds in the autumn of 1989 revealed antibodies against *Leptospira interrogans* serovar *hardjo* in 9.2% of the herds, with the incidence being higher in the southern part of the country [3]. *Leptospira hardjo* has been reported most prevalent in the urogenital tract in cattle. The genital tract (57%) appeared to be as important a site of hardjo localization as the urinary tract (62%) [4]. Of the several serovars, *L. interrogans* serovar *hardjo* is the most prevalent leptospiral agent in Brazil [5] and Australia [6] and is related to clinical signs of leptospirosis leading to economic losses in production animals. Likewise, serovar hardjo was shown to be present in 18% of 338 Dutch cattle herds [7]. Microscopic agglutination test (MAT) using live leptospiral antigen is a gold standard test for diagnosing leptospirosis. Recently, it has been reported that enzyme linked immunosorbent assay (ELISA) is also showing 90% correlation with MAT which is an advantageous alternative to the latter for diagnosing leptospirosis. Due to unknown serostatus of leptospiral antibodies in large ruminants in Nepal, the present study was aimed to investigate the seroprevalence of Leptospira spp. infection in large ruminants and the possible relationships between seroprevalence and season. Due to unavailability of live cultures of different serovars of Leptospira for MAT, an alternative ELISA test was chosen.

Materials and Methods

Site selection

A total of 11 sites were selected representing three distinct ecological zones (Terai, Hills and Mountains) from south to north altitudinal variation across east-west length of the country as shown in Figure 1. Terai region with elevation less than 500 metres comprised of four sites (Sunsari, Bara, Bardiya and Kanchanpur); hills with altitudes ranging from 500-1800 metres comprised of three site districts (Surkhet, Lamjung and Dhankuta) and four sites (Jumla, Mustang, Gatlang of Rasuwa and Murtidhunga of Dhankuta) were selected from the mountain region with elevation ranging from 1800-2300 metres.

Sample collection

Field stockpersons were mobilized in each site district for serum collection before and after monsoon season. A total of 640 sera samples collected without the knowledge of vaccination history, parity and herd health status from cattle, buffalo and chauries, of which 320 sera were collected before monsoon and 320 sera after monsoon (Table 1). Sera thus collected were stored in the deep freezer (-80°C) until the time of dot-ELISA test at Animal Health Research Laboratory.

Assay Procedure

The presence of IgG antibodies against *Leptospira hardjo* in blood sera of cattle, buffalo and chauries was determined using Immuno Comb® Bovine Leptospira Antibody Test Kit (Biogal, Galed Labs, Israel) following the instruction manual provided in the kits. Briefly, after thawing of the test kits and sera samples to room temperature, 5 µl each of positive control (C+), negative control (C-) and test serum sample were dispensed into each well of Row A followed by well mixing. The Immuno Comb card was incubated in Row A for 10 minutes with proper mixing at the start of incubation. Excess liquid in the comb was shaken off; the comb was inserted into wells of Row B and allowed to incubate for 2 minutes. Then it was transferred to Row C, incubated for 10 minutes followed by 2 minutes incubation each in Rows D and E. The comb was incubated for 10 minutes in Row F for colour development and then to Row E again for 2 minutes for colour fixation. After 2 minutes of colour development in Row E, the comb was dried completely and aligned with the tone of positive control spot provided in Comb Scale. All spots were observed separately, and the titer read in the yellow windows.

Species	Terai (< 500 m)		Hills (600 - 1800 m)		Mountain (1800-2300 m)		Total	
Season	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Cattle	108	105	22	20	30	30	160 10	155 7
Buffalo	82	105	68	30	6	10	156 1	145 1
Chauries	-	-	-	-	4 1	20 14	4 1	20 14
Total Sera Positive Sera	190	220	90	50	40	60	320 12 (3.75%)	320 22 (6.88%)

Table 1: Showing the number sera collected before monsoon (pre) and after monsoon (post).

Results and Discussion

Out of 640 sera tested for the leptospiral antibodies, 34 samples (5.31%) were positive with a greater number of positive cases in post-monsoon (6.88% vs. 3.755%) compared to pre-monsoon samples. The positivity of the samples was confirmed by comparing with the tone of the positive control spot (C+) in Comb Scale provided in the test kit. On an average, higher prevalence of leptospiral antibodies in post-monsoon sera of chauries might be due to the impact of heavy rains and flood and the poor level of hygiene in animal sheds although such finding was not noticed in cattle and buffaloes. Furthermore, the human development index of chauries rearing areas is lower compared to that of hills and Terai region of the country. Apparently higher incidence of leptospiral antibodies in those areas is giving alarming signs to both human and animal

health and more studies are warranted in the same location in the spirit of one health. Furthermore, isolation and molecular characterization of pathogenic leptospira from the biological samples (urine, blood, renal tissues or genital swabs) would further elucidate the serovars circulating in the animal population.

Figure 1: Showing serum sample collection sites denoted by from all three ecological zones (Mountain, Hills and Terai).

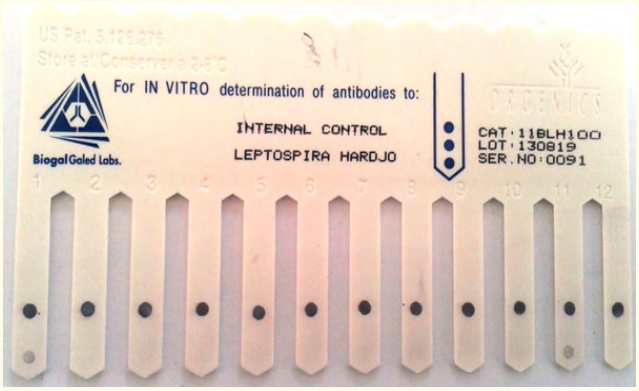

Figure 2: Showing the presence of antibodies against Leptospira hardjo in Comb 11 (Comb Tooth 1: Positive control and Comb Tooth 12: Negative control).

Conclusions

Findings of this study has provided some information on circulating leptospiral antibodies among large ruminants which would be essential in designing intervention measures to reduce the risk of disease transmission.

Acknowledgement

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