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Analysis of Prevalent Leptospira Serovar in Different Animals of South Gujarat Region during the Year of 2020

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Abstract

Aim of Study: Leptospirosis is a bacterial zoonotic disease transmitted through contact with animals that are harbouring leptospira. Knowledge of prevalent leptospira in a particular animal of a particular geographical area is essential to understand the epizootiology of disease, to understand the linkage between circulating serovars in animals and in humans, and to apply appropriate control measures, etc. **Material and Methods:** Animal samples from different districts of the south Gujarat region received in the Microbiology department during the year of 2020 for the Microscopic agglutination test (MAT) of leptospirosis were included in the study. Results of MAT which was already performed using 12 different serovars were analysed to prevent serovars in a particular animal. Quantitative data were analysed using frequency and percentage. **Result:** Out of 1406 animal samples, 151 (11 percent) were positive from animals like cows, buffalos, bullocks and goats. More prevalent serovars in cows were *L. ictrohemorrahiae* (22%), *L. hardjo* (19%), *L. patoc* (17%) and *L. pyrogen* (16%). In buffalo, *L. patoc* (58%) and *L. hardjo* (27%) were found. *L. hardjo* (50%) in bullock and *L. automonalis* (50%), *L. australis* (22%) and *L. patoc* (14%) in goat were found as prevent serovars. **Conclusion:** Different prevent serovars has been observed in different animals from the different district south Gujarat region which will be helpful to trace the source of infection in human, to apply control measures, to know the epizootiology of disease, for developing strategies in the future during vaccine development with emphasizing more on the prevalent serovars.

Keywords

Leptospirosis, Animal Samples, Serovars, Prevalence

1. Introduction

Leptospirosis is a globally widespread bacterial zoonosis caused by spirochetes belonging to the genus *Leptospira* [1]. An estimated 500,000 cases occur annually, with the fatality range rising up to 70% as mentioned in different cohort studies [2]. It has been classified as an emerging or re-emerging infectious disease by the Centers for Disease Control and Prevention and World Health Organization (WHO) [3] [4]. Leptospirosis disease mainly affects the agricultural farmers and urban slum dwellers as in resources poor developing countries are unable to provide basic medical services in these areas, so the WHO considers leptospirosis to be a neglected zoonotic disease [5] [6]. It circulates in a wide range of animals like rodents, cattle, buffalo, sheep, goats, pigs, deer, dogs, camels, horses, raccoons, etc. Infection to humans is usually transmitted by direct or indirect exposure to the skin (cuts/abrasions), mucous membranes (intact) to contaminated urine, placental fluids, etc. of these animals. The disease is maintained in nature by chronic renal infection of carrier mammals which excrete the organism in their urine or through indirect contact via contaminated water or soil [3]. Identifying leptospirosis is a diagnostic challenge, because of its protean manifestations which vary from asymptomatic or mild flu-like cases to a severe fulminant disease presenting with jaundice, renal failure, pneumonia or haemorrhage and shock [7].

Knowledge of prevalent leptospira serovars in particular animals of a particular geographical area is essential to understanding the epizootiology of disease. It would be helpful to understand the linkage between circulating serovars in animals and humans. So, this study was designed with the aim to detect the prevalent leptospira serovar of animals and humans circulating in the South Gujarat region and to decide the strategies to control the burden of leptospirosis disease [3] [4].

2. Material and Methods

Study Samples: In this retrospective study, a total of 1406 animal serum samples were collected during the year 2020 and stored at −20˚C. The animals included in the present study were from various sources representing the diverse livestock production system e.g. rural subsistence, periurban, semi-commercial and organized commercial dairy farms, where human leptospirosis cases were known to occur. The samples were collected randomly and not on the basis of Leptospirosis, like symptoms or any other indication of the disease. All the collected serum samples were subjected to MAT test for leptospirosis. The study was approved by Human Research Ethics Committee, Government Medical College, Surat, Gujarat for research purposes.

Microscopic Agglutination Tests (MAT): The MAT test was performed using standard procedure [8]. Serogroups included in the antigen panel are *L. australis* (Australis), *L. autumnalis* (Bangkinang), *L. ballum* (Ballum), *L. sejroe* (Hardjo), *L. grippotyphosa* (Grippotyphosa), *L. canicola* (Canicola), *L. hebdo-*

madis (Hebdomadis), *L. pomona* (Pomona), *L. semeranga* (patoc), *L. pyrogen* (Pyrogen), *L. icterohaemorrhagiea* (Icterohaemorrhagiea), and *L. bataviae* (Batavia). All the strains were obtained from the National Leptospirosis Reference Centre, Regional Medical Research Centre (World Health Organization collaborating centre for diagnosis in leptospirosis, ICMR) in Port Blair, Andaman and Nicobar islands. The cultures used as antigens should be checked by MAT against homologous antisera frequently for quality control. These serovars were maintained in 0.1% semisolid EMJH agar by using Leptospira medium base supplemented with 10% enrichment (Diffco, USA) at 28˚C - 30˚C in screw-capped test tubes.

Preparation of Antigens: A 0.5 ml of each representative strain from the panel of 12 serovars was inoculated into 10 ml of liquid EMJH medium. A loopful of culture was checked under dark field microscopy to confirm the absence of contamination or clumps and the presence of viable leptospires. Incubation was done at 30˚C for five to seven days. Densities of approximately 2 - 3 × 10^8 leptospira/ml of media were used as an antigen.

Procedure: Doubling dilutions from 1 in 10 to 1 in 640 were prepared by using phosphate buffer saline as a diluent. 50 ul of the specific serovar was added to all the wells. One of the wells included only the antigen without the addition of antibodies and served as the antigen control. The final dilutions after adding the antigen were from 1 in 20 to 1 in 1280. The plates were closed with aluminium foil and incubated at 37˚C for 2 h. The highest serum dilution showing approximately 50% agglutinated leptospires or a reduction in the number of leptospiral cells as compared to the antigen control was taken as endpoint titre. A titre of 1 in 40 or more was considered positive.

3. Results

District wise analysis is shown in Table 1. A total of 1406 samples that were received were from 4 major districts of the south Gujarat region that were Navsari, Surat, Tapi and Valsad. These are the area from where the majority of cases of leptospirosis in humans during monsoon season were observed and the majority harbours animals in their homes or they come into contact with animals. Moreover, animals with whom they frequently come in contact are Cow, buffalo, bullock and Goat, so the majority of samples which were received were from these animals only. Out of 1406 samples, 11% (151) samples were positive by MAT, out of which 7% (64) samples were from cow, 9% (24) were from buffalo, 15% (4) were from bullock and 25% (59) were from goat.

As shown in Table 2, in cows, the more prevalent serovars which were observed by MAT test were *L. ictrohemorrahiae* (22%) followed by *L. hardjo* (19%), *L. patoc* (16%) and *L. pyrogen* (16%). In buffalo, *L. patoc* (58%) is more prevalent followed by L.*hardjo* (27%). In bullock *L. hardjo* serovar is observed with a 50% prevalence. In goat *L. automonalis* (50%) followed by *L. australis* (22%) and *L. patoc* (14%) were observed.

Table 1. District wise analysis of total samples received and positive samples.

District	Total No of samples	Positive by MAT Number (percentage)	Positive samples/total samples in different animals			
			Cow	Buffalo	Bullock	Goat
Navsari	546	76 (14%)	18 (7%) 270	2 (3%) 68	0 2	56 (27%) 206
Surat	105	14 (4%)	8 (11%) 70	5 (45%) 11	0 0	1 (4%) 24
Tapi	330	13 (12%)	2 (1%) 213	5 (5%) 107	2 (20%) 10	2 (100%) 2
Valsad	425	50 (12%)	36 (11%) 325	12 (14%) 85	2 (13%) 15	0 0
Total samples	**1406**	**151 (11%)**	**64/878 (7%)**	**24/271 (9%)**	**4/27 (15%)**	**59/232 (25%)**

Table 2. Percentage of different serovars found in different animals.

Animal	Total No of samples	Positive by MAT No (percentage)	Different Leptospira Serovars positive by MAT. No (percentage)									
			L.australis	*L.automonalis*	*L.canicola*	*L.hardjo*	*L.hebdomadis*	*L.ictrohemorrahiae*	*L.patoc*	*L.pyrogen*	*L.pomona*	*L.griphotyphosa*
Cow	878	64 (7%)	3 (5%)	6 (9%)	3 (5%)	12 (19%)	5 (8%)	14 (22%)	11 (17%)	10 (16%)	0	0
Buffalo	271	26 (10%)	0	1 (4%)	1 (4%)	7 (27%)	0	0	15 (58%)	0	0	2 (8%)
Bullock	27	4 (15%)	0	1 (25%)	0	2 (50%)	0	0	1 (25%)	0	0	0
Goat	232	59 (26%)	13 (22%)	29 (50%)	0	0	0	1 (2%)	8 (14%)	0	3 (5%)	5 (8%)

4. Discussion

During the year 2020, 1406 animal samples were tested for MAT from the villages of the district of Surat, Navsari, Tapi and Valsad, which had shown seropositivity for MAT test is 11%, 14%, 4% and 12%, respectively. This suggests that Navsari and Valsad areas have higher seropositivity in animals for Leptospirosis. The animal wise analysis had shown that Goat in Navsari, Bullock and buffalo in Valsad, buffalo in Surat and bullock in Tapi are more potential animals in that area for Leptospirosis transmission in between animals and also in humans.

Serovars wise analysis showed that *Ictrohemorrahiae*, *hardjo*, *patoc and pyrogen* in cow, *patoc and hardjo* in byffalow, *hardjo* in bullock and *automonalis* and *australis* in goat were predominant serovars. The predominant serovars pattern is similar to the studies conducted earlier by Balakrishnan *et al.* [9], Prameela *et al.* [10], Anusha *et al.* [11] and Gaurav *et al.* [12].

5. Limitations

Still, there is a scope of test in other domestic and wild animals like pigs, dogs, etc., which may be harbouring the leptospirosis and causing zoonosis. Although there have been studies conducted in different parts of India, it is difficult to as-

sess the true nature of the disease from an epizootiological point, due to inadequate sample size. Continuous observation every year from the same area was not analysed, which can be the future scope of more observation and improvement.

6. Conclusion

The seroprevalence of leptospirosis among different animals in different districts of South Gujarat was significant. These prevent serovars analyses will be helpful to know the epizootiology of leptospirosis. It is also helpful to trace the source of infection in leptospirosis infected human subjects. Moreover need for control measures can be strategies based on these data. Development of a vaccine against leptospirosis which is a challenging thing can be developed or prioritized for these areas on these prevalent serovars first as compared to focusing on all 20 serogroup or 200 serovars.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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