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Seroprevalence of the Cattle Leptospirosis in South Gujarat Region of India

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Abstract:*To evaluate the serological findings of cattle Leptospirosis which is a zoonotic disease with worldwide distribution caused byLeptospirainterrogans. It is usually mild and often subclinical in cattle but it may lead to higher incidence of abortion, stillbirth, infertility, mastitis, weak progeny and decreased milk production in cattle. 676 cattle serum samples were collected during 2008-2011 and stored at -20 °C. All the collected serum samples were subjected to Micro-agglutination test (MAT) and Real time polymerase chain reaction (PCR) tests for leptospirosis.A total 676 cattles were included in present study from which 195 (29%) samples were positive by MAT and 75 (12%) positive by Real time PCR. 21(3%) samples were positive by MAT and PCR both tests.40% cattle detected as carrier of Leptospiraorganism in present study. So cattle act as a maintenance host for leptospirosis in this geographical area. The combined laboratory methods for Leptospiradiagnosis enable the Veterinarian officers to assure their decisions. Further investigation on physiological adaptation of cows as a host of this organism must be supported for studying and creating new preventive strategies.*

Keywords: *Leptospirosis, Micro-agglutination test (MAT), Real time polymerase chain reaction (PCR*).

I. Introduction

Leptospirosis is common bacterial zoonosis worldwide, caused by spirochetes of genus Leptospira. There are more than 20 species of leptospires, consist of more than 200 serovars circulating in a wide range of animal reservoir host like rats, other rodents, livestock and domestic pets [1] .Wild rodents serve as a natural reservoirs of infection, human and few others domesticated animals are accidental hosts in the transmission cycle of leptospirosis [2, 3]which can lead to abortion, stillbirth, infertility, mastitis, weak progeny and decreased milk production in them [4, 5] .The key feature in the transmission of Leptospirosis between animals, and between animals and man, is infection of renal tubules and excretion of infectious leptospires in the urine of carrier animals. Urine shed from carrier animal can result in direct transmission of the infection via contamination of mucous membranes of another animal, or in indirect transmission via contamination of the environment. The scenario of leptospiral infection is different in developed and developing countries. In developed countries, infection is increasingly being associated with outdoor recreational exposure and international travel. In rural areas of developing countries, transmission is usually associated with farming and livestock. In urban areas, infection is associated with overcrowding, poor hygiene standards, inadequate sanitation and poverty, all of which typically takes place in urban slums of developing countries [6].

Suitability of environmental condition for survival of leptospires appears to be critical factor in maintaining the infection. Leptospires have good affinity to areas where heavy rainfall resulted in water logging of land. Human population residing in such areas are at higher risk of acquiring infection [7]. A basic knowledge of serovars and their maintenance hosts is required to understand the epidemiology of leptospirosis in a region. Though there is distinct variation in maintenance hosts and the serovars they carry can occur throughout the world [8].Generally dairy cattle have a role as a natural host of serovarsHardjo, Pomona and Gripphotyphosa, while pigs may harbour Pomona, Tarassovi, and Bratislava. Sheep may harbour serovarsHardjo and Pomona, and dogs may harbour serovarCanicola[9].

Diagnosis of leptospirosis in animals is done by three different methods which include the isolation from samples, detection of leptospiral DNA by real time polymerase chain reaction and detection of anti-leptospiral antibodies. Isolation by culture is very time consuming, laborious and depends upon the presence of live leptospira in sample, so PCR and serology are the only method used for diagnosis. The detection of antileptospiral antibodies can be done with MAT (Microscopic Agglutination Test) and ELISA (enzyme linked immunosorbent assay)[10]. MAT test can be used qualitatively and quantitatively to detect infecting serovars as well as give the titre of individual serovars. Furthermore the sensitivity and specificity of MAT in reported study were 91.94% and 73.77% respectively[11].It is therefore important to have knowledge of the serovars present and their reservoir host. So this study will determined the prevalence of leptospirosis among cattle by MAT, using 12 different serovars in order to assess the risk of infection to humans and to apply the control measures.

II. Material & Methods

In this retrospective study, total 676 cattle serum samples were collected during the year 2012-2013 and stored at -20 °C. The animals included in 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 from randomly selected animals with their owner's consent and not on the basis of the Leptospirosis- like symptoms or any other indication of the disease. The animals included in the study were not vaccinated against Leptospirosis. All the collected serum samples were subjected to MAT and Real time PCR tests for leptospirosis. The study was approved by Human Research Ethics Committee, Government Medical College, Surat, Gujarat for research purpose.

2.1 Microscopic Agglutination tests (MAT): The MAT test was performed using standard procedure [12]. 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. hebdomadis (Hebdomadis), L. pomona (Pomona), L. semeranga (patoc), L. pyrogen (Pyrogen), L. icterohaemorrhagiea (Icterohaemorrhagiea) 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 28-30°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 presence of viable leptospires. Incubation was done at 30°C for five to seven days. Densities of approximately 2-3x 108leptospira/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. 50ul of the specific serovar was added to all the wells. One of the wells included only the antigen without addition of antibody 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 end point titre. A titre of 1 in 40 or more was considered positive.

2.2 Real Time PCR assay: Total DNA from cattle serum (200 µl) was prepared using QIAamp DNA Mini Kits (QIAGEN, USA) according to the manufacturer's instructions. The primers and probes were designed from alignments of available Leptospira spp. LipL41 sequences obtained from the GenBank nucleotide sequence database. The program used was Primer Express™ (Applied Biosystems, USA). For real time PCR, 5 µl of DNA was added to the 45 µlTaqMan Universal PCR Mastermix Mix (Applied Biosystems, USA) in final concentrations of 3 pmol/µl of each primer and 2 pmol/µl of the FAM-TAMRA labelled probe. A negative control without added template in the above reaction mixture was used as a control to detect the presence of contaminating DNA. Amplification and fluorescence detection was conducted in an ABI Prism 7700 sequence detector (Applied Biosystems, USA) with a program of 40 cycles, each cycle consisting of 95°C for 15 seconds and 60°C for one minute as per the manufacturer's instructions.

III. Results

A total 676 cattles were included in present study from which 195 (29%) samples were positive by MAT and 75 (11%) positive by Real time PCR. 21(3%) samples were positive by MAT and PCR both tests. Figure-1 illustrated the seropositivity among the cattle in South Gujarat region. The annual cattle leptospirosis cases averaged over the study period showed a distinct seosanal pattern with an increased percentage of cases diagnosed from July to December. With respect to the MAT titres of 676 samples, L. autumnalis and L. ballum were the most strongly reacting serovars (shown in Table-1) and also almost 80% cases of human leptospirosis cases were occured in rural area associated with agricultural work.

195 of the 676 (29%) suspected cases showed anti-leptospiral antibodies by MAT (Table 1) with the L.Ballum (19%) as a predominant serogroup followed by L.Autumnalis (18%), L.Icterohaemorrhagea and L.Hardjo (8%). The other serovars observed were L.Pomona (7%), L.Hebdomadis (7%), L.Canicola (7%), L.Australis (5%), L.Pyrogen (4%) and L.Batavia (4%). SerovarL.Gripphotyphosa did not show any significant titer in MAT test. 106 (54%) samples showed antibody titer between 1 in 80 to 1in 160. It is noteworthy that the highest antibody titer was recorded against serovarL.Autumnalis and L.Ballum.

IV. Figures And Tables

Figure-1: MAT and PCR results for diagnosis of Leptospirosis.

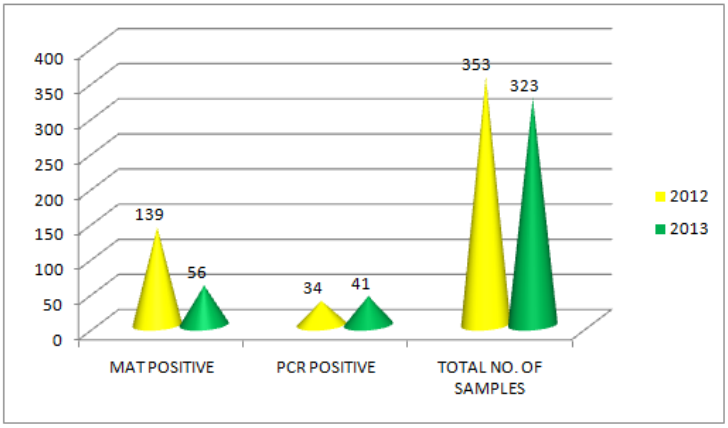

Table-1: Details of MAT titres against predominant serovars.

MAT titre	Pyrogen	Australis	Autumnalis	Grippotyphosa	Patoc	Pomona	Icterohaemorrhagea	Hebdomadis	Canicola	Hardjo	Ballum	Batavia	Total
1:20	--	--	--	--	--	--	--	--	--	--	--	--	--
1:40	4	5	9	2	2	3	5	4	3	4	6	5	52
1:80	--	2	8	2	2	4	7	6	3	4	14	1	53
1:160	2	3	7	--	5	4	3	4	6	3	14	2	53
1:320	--	1	8	--	3	3	1	1	--	5	3	--	25
1:640	2	--	3	--	1	1	1	--	3	1	--	--	12
Total	8	11	35	4	13	15	17	15	15	17	37	8	195

V. Discussion

Leptospirosis is considered as one of the most widespread zoonotic diseases in the world [13]. Althoughthe incidence of the disease seems to have decreased indevelopedcountries; it is apparently emerging rapidlyas a significant public health problem in developing countries. Some of the countries where leptospirosisis under surveillance have recorded this increase inincidence[14].Leptospirosis is frequently under diagnosed, because of the non-specific symptoms in the majority infection cases, what is recorded is the severe form of Leptospirosis with organ involvement. Leptospirosis control and prevention is still not in the list of National health programs so laboratory support is not available at most of the places. Isolation of leptospires from clinical material and identification of isolates is time-consuming and is a task for specialised reference laboratories. Isolation followed by typing from renal carriers is important and very useful in epidemiological studies to determine which serovars are present within a particular group of animals, an animal species, or a geographical region.

Although the basic determinants of leptospirosis transmission, like presence of carrier animals, environment suitability for the survival of leptospires, behavioural and occupational factors of people that predispose them to leptospirosis are common, the magnitude and nature of these factors vary from community to community. Therefore, the specific risk factors for acquiring leptospiral infection could be unique to each community. The organisms die when dry, or in acid conditions (pH < 7.0), so that transmission is confined to wet environments or circumstances. The most frequent sources of infection are urine, kidneys, surface waters, mud and soil.Indirect transmission occurs when an animal or human being acquires Leptospirosis from environmental Leptospires, originating in the urine of excretor animals. Leptospires can survive for long periods of time in the environment and probably multiply when the conditions are favourable. It is considered that the most common portal of entry of Leptospires into the host body is through intact skin. High incidence has been recorded among people who are exposed to wet environments because of occupational or other activities.

Most of the countries in South East Asia areendemic to leptospirosis. A number of outbreaks haveoccurred during the past few years in various placessuch as Nicaragua, [15]Salvador [16] and Rio de Janeiro [17]inBrazil and Orissa[18, 19] Mumbai[20] and Andaman Island [21, 22]in India. Till date there is no published data of

seroprevalence of animal leptospirosis in the South Gujarat area which is an endemic region for leptospirosis. The major public health concerns of leptospirosis are difficulty in diagnosis without laboratory support, high case fatality rate and multiple zoonotic source of infection which are difficult to remove or control. So, this study was planned to determine seroprevalence of cattle leptospirosis in this geographical area.

In study, Govindrajan R. et al [23] from Chennai showed seropositivity of 54.54%, 84.93%, 52.88%, 44.44%, 51.61% and 52.28% in cattle, buffaloes, sheep, goats, pigs and dogs by MAT test. SerovarL.sejroe, L.shermani, L.hebdomadis, L.ranarum and L.mini were found in the cattle. Serologically two leptospiralserovars was revealed from tested cattle sera, i.e. L. ballum (19%) and L. autumnalis (18%) were the most prevalent in present study. L. autumnalis and L. australisare the most predominant serovars in humans leptospirosis cases of South Gujarat region (data was not included in this study).This finding suggests that cattles play a role in epidemiology of leptospirosis in this geographical area. In a study done by Vijayachari P et al [24] from Portblair signifies the transmission cycle of Leptospirosis involves the maintenance hosts, the carrier hosts, the environment and human beings.Sharma S. et al[7]from Regional Medical Research Centre, Port Blair, has done a study on seroprevalence among cattle and goats was 34% and 29% respectively and L.icterohaemorrhagiae (24%), L.hebdomadis (22%) and L.grippotyphosa (20%) were the commonest serovars found in cattle samples. In present study, 39.9% seroprevalence among cattles were detected.

VI. Conclusion

To conclude, 40% cattle detected as carrier of Leptospiraorganism in present study. So cattle act as a maintenance host for leptospirosis in this geographical area. Based on limited data obtained from this study, a more elaborate study on the epidemiology of leptospirosis in South Gujarat should be carried out to identify important reservoir and serovars responsible for endemicity of the disease. The combined laboratory testing methods for Leptospiradiagnosis enable the Veterinarian officers to assure their decisions. Further investigation on physiological adaptation of cows as a host of this organism must be supported for studying and creating new preventive strategies.

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