

Seroprevalence of the Rodent Leptospirosis in South Gujarat Region of India

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Abstract: To evaluate the serological findings of rodent Leptospirosis which is a zoonotic disease with worldwide distribution caused by *Leptospira interrogans*. Wild rodents serve as natural reservoirs of infection, human and few others domesticated animals are accidental hosts in the transmission cycle of leptospirosis. 459 rodents serum samples were collected during the year 2012-2013 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 459 samples were included in present study from which 65 (14%) samples were positive by MAT and 122 (27%) positive by Real time PCR. 11 (2%) samples were positive by MAT and PCR tests. The present study surveyed that 40% rodents *Leptospira* carriage were observed in South Gujarat region where human leptospirosis is hyperendemic. If rodent control measures are properly planned and implemented, this contribution may in turn to reducing the burden of human leptospirosis.

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

I. Introduction

Leptospirosis is a global public health burden occurring primarily in tropical zones. It is caused by spirochetes belonging to the genus *Leptospira*. The annual incidence rates of leptospirosis range from approximately 0.1–1 per 100,000 persons in temperate climates; and from 10–100 per 100,000 persons in humid tropical climates^[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 leptospire 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. Rodents are very efficient maintenance hosts because they remain healthy during lifelong renal carriage. 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 leptospire appears to be critical factor in maintaining the infection. Leptospire has 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 serovars Hardjo, Pomona and Grippityphosa, while pigs may harbour Pomona, Tarassovi, and Bratislava. Sheep may harbour serovars Hardjo and Pomona, and dogs may harbour serovar Canicola^[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 anti-leptospiral 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 determine the prevalence of leptospirosis among rodents 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 459 rodents serum samples were collected during the year 2012-2013 and stored at -20 °C. The rodents 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 randomly and not on the basis of the Leptospirosis- like symptoms or any other indication of the disease. The rodents 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. icterohaemorrhagica* (Icterohaemorrhagica) *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 (Difco, 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 leptospire. Incubation was done at 30°C for five to seven days. Densities of approximately 2-3x 10⁸ 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. 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 leptospire 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 µl TaqMan 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 459 rodents were included in present study from which 65 (14%) samples were positive by MAT and 122 (27%) positive by Real time PCR. 11 (2%) samples were positive by MAT and PCR tests (Figure-1). Figure-1 illustrated the diagnosis of rodent leptospirosis carriage rate. With respect to the MAT titres of 459 samples, *L. Pyrogen* is the most strongly reacting serovars shown in Table-1. 65 of the 459 (14%) suspected cases showed anti-leptospiral antibodies by MAT (Table 1) with the *L. Pyrogen* (36%) as a predominant serogroup followed by *L. Hebdomadis* (10%), *L. Autumnalis* (9%), *L. Pomona* (4%) and *L. Icterohaemorrhagica* (4%). The other serovars observed were *L. Hardjo* (3%), *L. Australis* (3%), and *L. Grippotyphosa* (2%). Serovar *L. Batavia* did not show any significant titer in MAT test. 22 (33%) samples showed antibody titer of 1 in 40 and 15 (23%) samples showed titer of 1 in 160. It is noteworthy that the highest antibody titer was recorded against serovar *L. Autumnalis*. This data suggests that rodents play a role in epidemiology of leptospirosis in the South Gujarat region. Also almost 80% cases of human leptospirosis cases were occurred in rural area associated with agricultural field work.

IV. Figures And Tables

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

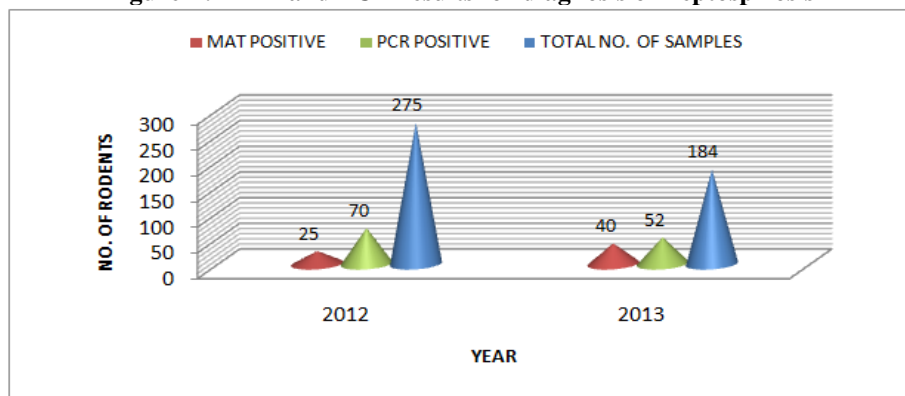


Table-1 Details of MAT titers against predominant serovars

MAT titre	Pyrogen	Australis	Autumnalis	Grippityphosa	Patoc	Pomona	Icterohaemorrhagea	Hebdomadis	Canicola	Hardjo	Ballum	Batavia	Total
1:20	--	--	--	--	3	--	--	--	--	--	--	--	3
1:40	8	1	1	--	--	1	1	7	1	1	1	--	22
1:80	5	--	3	--	--	1	1	--	--	--	--	--	10
1:160	13	1	--	--	--	--	--	--	--	1	--	--	15
1:320	--	--	2	1	8	1	1	--	--	--	--	--	13
1:640	--	--	--	--	4	--	--	--	--	--	--	--	4
Total	24	2	6	1	15	3	3	7	1	2	1	--	65

V. Discussion

Leptospirosis is common bacterial zoonosis worldwide. 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 (13). Although the incidence of the disease seems to have decreased in developed countries, it is apparently emerging rapidly as a significant public health problem in developing countries. Some of the countries where leptospirosis under surveillance have recorded this increase in incidence. (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.

Exposure to *Leptospira* depends on chances of contacts between humans and infected animals or a contaminated environment. The names for some forms of leptospirosis (e.g. rice field fever, Weil's disease, sewer's disease, cane cutter's disease, swineherd's disease, dairy farm fever, mud fever) reflect transmission conditions. 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. Sources of non-occupational leptospirosis in temperate climates are mainly rodents, dogs, leisure activities and travel (e.g. hobby farming, rafting, camping, boating, swimming) affecting city and rural dwellers. The magnitude and severity of illness and case fatality is determined by, geographical and related factors which govern the distribution and types of carrier animals, e.g. cattle in temperate, rodent-controlled environments, compared with rodents in tropical uncontrolled areas, and occupational-cultural factors governing the proximity to animal sources of leptospires. (15)

Outbreaks of leptospirosis are recognized through occupational exposure, such as rice farming and other agricultural activities in rural areas of the tropics. (16) *Leptospirosis* has also become a health problem in urban slums in developing countries. (17, 18) In 'developed countries', recreational activities have recently been identified as a significant risk factor for leptospirosis. (19,20) The present study suggests that humans could

contract leptospirosis through occupational exposure or exposure during activities of daily life in environments contaminated with rat urine containing leptospires in endemic geographical areas in India. Physicians and public health authorities should, therefore, be aware of the severe risk of contracting leptospirosis associated with rats in urban areas.

The rapid diagnosis of Leptospirosis in rodent samples by PCR method was more sensitive than MAT. [21] The combination techniques for *Leptospira* diagnosis enable to calculate the actual seroprevalence in rodents of these geographical area. Further investigation on physiological adaptation of rodents as a host of leptospirosis must be supported for studying and creating new preventive strategies. Different geographical areas showing seropositivity but there is difference in prevalent circulating serovars. *L.pyrogen*, *L.patoc* and *L.hebdomadis* are the most strongly reacting serovars in rodents in present study. Balakrishnan, G et al [22] studied antibody to serogroups *L.australis*, *L.autumnalis*, *L.canicola*, *L.grippotyphosa*, *L.icterohaemorrhagiae*, *L.javanica* and *L.pomona* in rat serum. Demers et al (1985) studied the risk of Leptospiral exposure to rodent control workers in Detroit by a comparative cross-sectional study among the workers and two control groups. A statistically significant higher risk was found in rodent control workers (OR: 4.37; 95% CI: 3.0, 6.3 and OR: 11.08, 95% CI: 5.6, 22). [23]

VI. Conclusion

To conclude, the present study surveyed rodents and their *Leptospira* carriagee in South Gujarat region where human leptospirosis is hyperendemic. Overall, the observed *Leptospira* prevalence was 40%. However, significant variations were observed both in the abundance of rodents and their *Leptospira* carriage. The climatic conditions could lead to increased leptospires dispersal by the rodent reservoir and increased exposure of humans to risk situations (e.g. flood, mud). Because rodent control measures were demonstrated elsewhere to be cost effective if correctly planned and implemented, this contribution to a better knowledge of rodent and leptospires dynamics provides useful information and may in turn allow developing relevant rodent control actions aimed at reducing the burden of human leptospirosis.

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