



# International Journal of Current Research in Medical Sciences

ISSN: 2454-5716

[www.ijcrims.com](http://www.ijcrims.com)

Volume: 1- Issue: 1 July 2015



## Research Article

### Epidemiological study of Leptospirosis in humans associated with canine population in and around Namakkal

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#### Abstract

The aim of the present study was to examine the prevalence of leptospiral antibodies against serovars of *L.interrogans*, *L.icterohaemorrhagiae*, *L.grippityphosa*, *L.canicola*, *L.pomona* and *L.autumnalis* among domestic animals and Humans in and around Namakkal. The serovars used in this study were chosen because of their clinical importance in human disease. Blood samples were received from clinically ailing human patients attending various hospitals, with the history of fever, myalgia, headache, nausea, conjunctival effusion, oliguria and disturbed sleep. Data relating to the illness were collected from the patients or their relatives through questionnaires. Blood samples were also collected from apparently healthy individuals who had associated with dogs. A sero-prevalence of 47.4% to 51.6% patients was found to be positive for Leptospirosis in Namakkal, Tamilnadu. The main serogroups identified in human cases are also found in animals (*icterohaemorrhagiae* and *autumnalis*). The maximum (MAT) titres observed were 1:640 (*autumnalis*) and 1:320 (*icterohaemorrhagiae*). Notably, the dominant serovar *icterohaemorrhagiae* is associated with most cases of weil's disease in humans and dogs.

**Keywords:** Human, Leptospirosis, Microscopic Agglutination test (MAT) & Weil's disease.

#### Introduction

Leptospirosis is a disease that is under-reported and lack of knowledge are multi-factorial which include lack of awareness, changes in environmental factors and changing clinical spectrum. In India, since 1980s the outbreaks are being increasingly reported especially from the states of Tamil Nadu, Kerala, Karnataka and Maharastra indicating its re-emergence in recent years. Unless you seek you shall not find, unless you know, you will not look and unless you look,

you will not see (1). The global burden of disease is unknown because of the lake of data, but incidence estimates range from 0.1 to 1/100,000/year in temperate areas, to over 100/100,000/year during epidemics in the tropics. Estimated 300,000-500,000 severe cases occur each year, with case-fatality reports of up to 50 percent (2,3). Humans are usually infected through indirect exposure with a freshwater or humid environment contaminated with the urine

of reservoir animals. Many of the serovars circulating in animals reservoirs have been shown to cause disease in humans.

## Materials and Methods

Blood samples were received from clinically ailing human patients attending various hospitals in and around Namakkal (Salem, Erode and Karur) with a history of fever, myalgia, severe headache, nausea, conjunctival effusion, oliguria and disturbed sleep. Data relating to the illness were collected from the patients and their relatives referred by Human physician. Blood samples were also collected from apparently healthy individuals who had association with dogs. After centrifugation, sera were stored at -20 C before sending to the Leptospirosis laboratory, for microscopic agglutination tests (MAT), Department of Veterinary Epidemiology and Preventive Medicine, Veterinary College and Research Station, Namakkal.

### Leptospira Antigen

Five to seven days old live leptospiral cultures of *L.interrogans* serovars viz., Autumnalis (strain Akiyami A), Canicola (strain Hond IV), Icterohaemorrhagiae (strain RGA), Pomona (strain Pomona) and Grippityphosa (strain Maskova V) supplied by Dr. L.D.Smythe, WHO/FAO Collaborating Centre on Leptospirosis, Australia, were used in this study.

### Leptospiral antibodies

Hyperimmune sera prepared as per the WHO, Manual on Control of Leptospirosis (1982) was used as positive control in MAT.

### Maintenance of Leptospire

All the five leptospiral serovars were grown separately in duplicate 10 ml culture tubes. The leptospire were subcultured in semi-solid EMJH and Fletcher's media once in 30 days. Sub-culturing of leptospiral serovars was carried out in EMJH liquid media twice in a week. The cultures

were screened by DFM on the 5<sup>th</sup> day after sub-culturing to observe the viability and growth of leptospire. Cultures showing 100-200 leptospire per field were used for conducting MAT as per the standard laboratory procedure.

### Microscopic Agglutination Test (MAT)

MAT was performed as per the method of (4). In MAT test 5-7 day-old live culture of serovars Icterohaemorrhagiae, Pomona, Canicola, Autumnalis and Grippityphosa were used separately. A homologous, high-titred, antiserum was included in each testing session. Serum dilutions were prepared in 8 well "U" bottomed disposable microtitre plates (Tarson). A serial, two fold dilution of each serum was made in phosphate buffered saline solution (pH7.2) starting with an initial dilution of 1:10. An equal volume (i.e. 50µl) of live culture antigen was added to each well, mixed by gentle rocking and incubated at 37 C for two hour after sealing with polyethylene sheet. The MAT titre was the reciprocal of the highest dilution of the serum in which >50 % of the antigen was agglutinated. A minimum cut off of 1:40 and above was taken as the positive agglutination reaction in endemic areas.

## Results and Discussion

### Prevalence of leptospirosis in patients associated with un-vaccinated dogs

From the total number of 197 samples from apparently healthy patients associated with vaccinated, un-vaccinated and no history of contact with dogs. 66 out of 36 (47.4%) patients were found to be positive for leptospire antibody associated with unvaccinated dogs. Of these 36, 11(14.5%), 2(2.6%), 6(7.9%) 6 (7.9%) and 14(18.4%) sera reacted to *icterohaemorrhagiae*, *canicola*, *pomona*, *grippityphosa* and *autumnalis* respectively. Three sera samples showed reaction to both *icterohaemorrhagiae* and *autumnalis*. The highest seroprevalence (18.4%) was to the proposed novel serovars *autumnalis* belonging to the *autumnalis* serogroups. This finding is fully in

concordance with a recent report of Arent *et al.* (5) and Ramakrishnan *et al.* (6) who reported in their study 20-25 percent of residents were exposed directly or indirectly to vaccinated or unvaccinated dogs respectively. The present study indicates that presence of MAT titres in healthy patients associated with un-vaccinated or vaccinated dogs was not abnormally high, but the presence of leptospiral antibodies in human associated with unvaccinated dogs was more. This is in accordance with the findings of Simon *et al.* (7) and Hartskeerl (2) who reported that risk factors associated with leptospirosis included contact with pets, particularly carnivores. The maximum titres observed were 1:640 (*autumnalis*) and 1:320 (*icterohaemorrhagiae*). Notably, the dominant serogroups is *icterohaemorrhagiae* where together with serovars *icterohaemorrhagiae* is associated with most cases of weil's disease in humans and dogs. It agrees with reports of Houwers (8).

#### **Prevalence of leptospirosis in patients associated with vaccinated dogs**

59 out of 9(15.3%) patients were found to be positive for Leptospirosis antibodies associated with vaccinated dogs. Of these 9, 4(6.8%), 0(0%), 2(3.4%), 1(1.7%) and 4(4.8%) sera reacted to *icterohaemorrhagiae*, *canicola*, *Pomona*, *grippotyphosa* and *autumnalis* respectively. Two sera samples showed reaction to both *icterohaemorrhagiae* and *autumnalis*. The highest titre observed was 1:160. This finding is well corroborated with the reported data of Martone and Kaufman (9); Waitkins (10); Ramakrishnan *et al.* (6) and Cedric *et al.* (11) who observed that 25 percent of clinically infected patients owned pets and 3 per cent of cases had contact with dogs and showed seropositivity of Leptospirosis respectively. In the present study it could be opined that the incidence of leptospirosis in humans due to *L.interrogans* serovars *L.icterohaemorrhagiae* and *L.canicola* has reduced by vaccinating dogs with the commonly used inactivated vaccine components containing *icterohaemorrhagiae* and *canicola*. However, dogs are not usually considered as a reservoir for *leptospira*, except for *canicola*. Similar findings

were observed by Ryan *et al.* (12) and Marshall (13). Who reported that sero-prevalence of leptospirosis in dairy cattle in New Zealand, showed that a campaign of leptospirosis vaccination had resulted in a marked decrease in the incidence of the disease in humans.

#### **Prevalence of leptospirosis in patients associated with no history of contact with dogs**

In the present study screening of 62 ailing patients without previous history of contact with dogs revealed that most of them were agriculturist(7), weavers(5), drivers(31), coolies(8), and butchers(1). Among the 62 human patients with no history of contact with dogs, 32(51.6%) were positive to one or more serovars. Of the 32, 12(19%); 2(3.2%); 7(11.2%); 5(8%) and 14(22.5%) were positive to *icterohaemorrhagiae*, *canicola*, *Pomona*, *grippotyphosa* and *autumnalis*, respectively. Some of the sera samples reacted to more than one serovar. Four sera samples reacted to *autumnalis* and *icterohaemorrhagiae*, three to *autumnalis* and *pomona* and one to *icterohaemorrhagiae* and *pomona*. Majority of the patients were frequent travellers, and those in direct contact with nature. This is in agreement with the findings of Feigin *et al.* (14) and Lau *et al.* (3).who reported that agricultural occupations and activities in contact with nature were those most frequently associated with leptospirosis.

Statistical analysis of data by applying non-parametric test namely Scheirer-Ray-Hare Extension of the Kruskal-Wallis test revealed ( $P < 0.01$ ) significant difference between the three groups of ailing humans i.e., those associated with un-vaccinated dogs, those associated with vaccinated dogs and those with no previous history of contact with dogs. But no significant difference between the prevalence of five leptospiral serovars among the three groups was noticed. Statistical analysis revealed no significant difference between the two groups of healthy humans i.e., those associated with vaccinated dogs and those associated with unvaccinated dogs.

Table .1 Sero-prevalence of leptospiral antibodies among patients showing signs suggestive of leptospirosis

Status of the Patient	Number of patients screened	Number of sero positive Humans against each serovar					
		Total positive	Icterohaemorrhagiae	Canicola	Pomona	Grippityphosa	Autumnalis
Patients associated with unvaccinated dogs	76	76(47.4%)	11(14.5%)	2 (2.6%)	6 (7.9%)	6(7.9%)	14 (18.4%)
Patients associated with vaccinated dogs	59	9(15.3%)	4 (6.8%)	0 (-)	2(3.4%)	1(1.7%)	4(6.8%)
Patients with no history of contact with dogs	62	32(51.6%)	12(19%)	2(3.2%)	7(8.0%)	5(8.0%)	14 (22.5%)

In conclusion, that the study shows the biodiversity of the serovars is generally related to the wide range of mammalian reservoir. The disease is endemic in domestic animals and concerns all the species. The diversity of serovars detected is indicative of the possible existence of a variety of animal reservoirs.

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