

## Seroprevalence of Leptospirosis in Human beings and Animals in Central and North Kerala

<sup>1</sup>Manju Soman\*, <sup>2</sup>V.Jayaprakasan and <sup>3</sup>M.Mini

<sup>1,2,3</sup> Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, , India

---

**Abstract:** A study was undertaken to assess the prevalence of leptospirosis in animals and man in Central and North Kerala. Five hundred and one sera samples collected from dogs, cattle, pigs, rodents (bandicoots and rats) and human beings were serologically tested for leptospiral antibodies by Microscopic Agglutination Test (MAT) and Passive Haemagglutination Assay (PHA). The MAT detected a prevalence of 36.36 per cent in dogs, 47 per cent in cattle, 23.80 per cent in pigs, 21.42 per cent in rodents and 54.54 per cent in human beings. Passive Haemagglutination Assay detected a prevalence of 50.41 per cent in canine, 23 per cent in bovine, 19.04 per cent in porcine, 26.19 per cent in murine and 42.85 per cent in human sera. The most predominant serovar infecting dogs, pigs and rodents was identified as *Leptospira interrogans* serovar Pomona. Humans showed maximum prevalence to serovar Australis followed by Pomona while cattle showed the highest prevalence to serovar Hardjo of serogroup Sejroe. High prevalence of leptospiral antibodies in apparently healthy animals like cattle proves the major role played by animals in the dissemination of infection in animal and human population.

**Keywords:** Leptospirosis, MAT, PHA, Prevalence, Serology

---

### I. Introduction

Leptospirosis continues to be a major health problem in India, with Kerala witnessing yearly spurts in human and animal leptospirosis in the post monsoon periods causing heavy mortality in human and animal population in rural and urban settings. Though rapid and sensitive diagnostic tests have helped in optimal treatment and patient management, lack of knowledge of the identity of circulating serovars has greatly impaired the epidemiological and public health surveillance programmes. Immunization strategies in animals fall short due to the emergence of new predominant serovars infecting man and animals in the region. Taking this into deliberation a study was undertaken to detect the prevalence of leptospiral antibodies in animals and man by MAT and PHA and to determine the predominant leptospiral serovars circulating in the region.

### II. Materials and Methods

Sera samples collected from suspected cases of human and canine leptospirosis and apparently healthy bovine, porcine and murine (bandicoot and rats), in Central and Northern Kerala, were subjected to MAT and PHA.

#### 2.1. MAT

One hundred and twenty one canine, 100 bovine, 84 porcine, 42 murine and 154 human sera samples were subjected to MAT as per [1] with minor modification. A battery of nine pathogenic reference serovars viz., *Leptospira interrogans* serovars Australis, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pomona, Pyrogenes, Hardjo (serogroup Sejroe), Tarassovi and nonpathogenic Patoc I strain of *L. biflexa* serovar Patoc (serogroup Semaranga) procured from National Reference Centre, Port Blair were used as antigen in MAT. Five to ten day old cultures of the reference strains grown in EMJH liquid media with a density of approximately  $2 \times 10^8$  leptospores/ml of media was used as the antigen.

#### 2.2. PHA

Passive Haemagglutination Assay was performed as per [2] with minor modification. The ethanol precipitable erythrocyte sensitizing substance (ESS) was prepared as per [3] with minor modifications. Fresh unmodified sheep erythrocytes were used for antigen preparation.

### III. Statistical Analysis

The results obtained from the two serological tests were analyzed for percentage agreement and relative sensitivity and specificity by Kappa ( $\kappa$ ) statistics.

IV. Results

4.1. MAT

A titre of 1: 80 and above was considered as positive in dog, cattle, pig & human sera where as a titre of 1: 20 [4] was taken as positive in rodent . Microscopic Agglutination Test detected a prevalence of 36.36 per cent in dogs, 47 per cent in cattle, 23.80 per cent in pigs, 21.42 per cent in rodents and 54.54 per cent in human beings (fig 2).The MAT detected highest titres of 1:5120 to serovar Pomona in dog, 1:20480 to serovar Hardjo (serogroup Sejroe) in cattle, 1:640 to Pomona in pig, 1:20480 to Pomona in rodent and 1:20480 to Australis in human beings.

4.2. PHA

A titre of 1:32 and above was considered as positive in dog, cattle, pig and human sera while the cut off titre for rodents was taken as 1:16. In canines, 50.41 per cent tested positive with the highest titre of 1:512. Twenty three per cent of bovine sera samples were positive with a maximum titre of 1:128. In pigs, 19.04 per cent of sera samples were positive, while in rodents, 26.19 per cent were found to be positive for antibodies to *Leptospira*. The maximum PHA titres recorded in pigs and rodents were 1:32. In humans, 42.85 per cent of the sera samples tested positive with highest recorded titre of 1:512 (fig 2).

Fig 1. Prevalence of leptospiral antibodies to various serovars in the five species

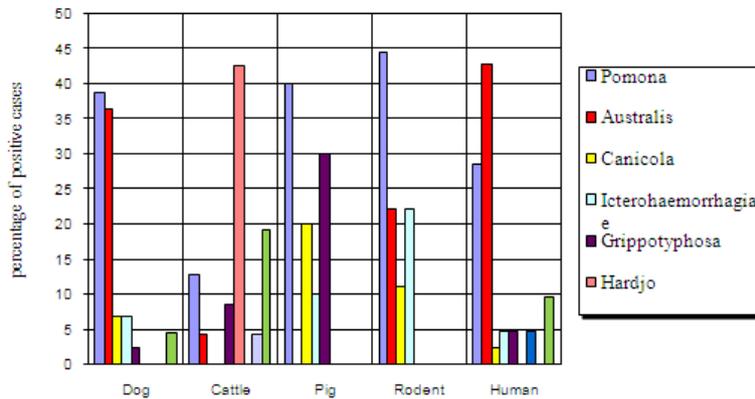
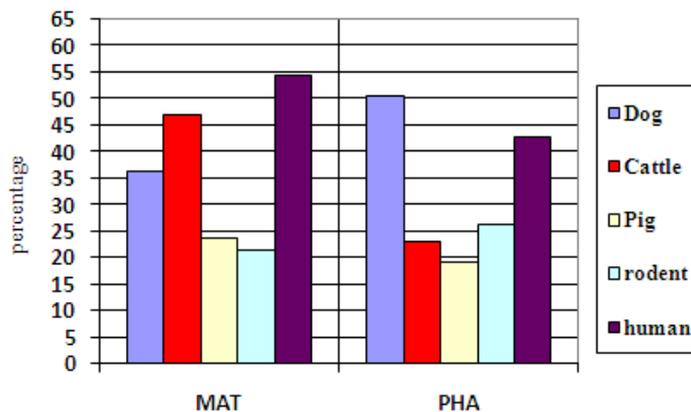


Fig 2. Prevalence of leptospirosis in the five species by MAT and PHA



4.3. Statistical Analysis

Percentage of agreement between the two tests showed kappa values above 0.9 ( $\kappa > 0.9$ ) for each species tested. As per [5],  $\kappa$  values above 0.81 indicated perfect agreement.

## V. Discussion

The present study was carried out to detect the presence of leptospiral infection in animals and human beings in Central and North Kerala by MAT and PHA and identify the common serovars infecting animals and man by MAT. Serovars Pomona and Australis were detected as the most prevalent ones infecting dogs in the region (Fig.1) where as in cattle, serovar Hardjo of serogroup Sejroe was identified as the predominant one (fig 1). The results substantiated the previous serological studies on bovine and canine leptospirosis [6],[7],[8],[9],[10]. The cattle screened in this study were apparently healthy and hence those detected as positive could have been subclinical carriers. Subclinical leptospirosis in apparently healthy cattle caused by serovars Hardjo and Andamana has been reported by [11]. The high prevalence of leptospiral antibodies in cattle, in this study, indicates the high possibility of disease transmission by these animals. Porcine and rodent sera, in this study, showed high prevalence to serovar Pomona. In India, porcine leptospirosis caused by serovar Pomona has been reported by several authors [13], [14]. Antibodies to serovar Grippotyphosa were also seen at a higher level in pigs in this study (fig 1). Reproductive abnormalities in pigs caused by serovars Pomona and Grippotyphosa have been reported earlier [15], [16]. Pigs have been reported to have a special significance in the epidemiology of leptospirosis because of the high intensity and long duration of leptospiuria [17]. Serovar Pomona infection in rodents in Tamil Nadu has been reported by [12]. Rodents are susceptible to acute infection only in the early days of their life. Later the immune system develops and surviving ones become resistant to further infection [1]. This could be a possible reason for the low levels of antibodies detected in rodents. As the leptospires get lodged in the renal tubules of the rodents, rodent urine becomes a source of leptospiral infection in grazing animals like cattle, which in turn contribute to infection in human beings [12]. In this study, Australis and Pomona were identified as the predominant serovars infecting humans (fig 1). Human leptospirosis in Kerala has been reported by several authors [18],[19] and Australis has been detected as the most prevalent serovar infecting humans in Diglipur district of North Andaman by [20].

The PHA detected a maximum prevalence of leptospiral antibodies in man and dogs and a minimum in pigs and cattle (fig 2). The PHA titre of 1:16 or below indicated an infection which might have occurred two months earlier while titres of 1:32 and 1:64 indicated recent infection within two months [21]. It was suggested by [22] that in PHA, the substance used for sensitization of unmodified red cells (ESS) contained large amount of carbohydrate (17.18 per cent w/w) and much less of protein (4 per cent w/w) and that the carbohydrate moiety was largely responsible for the genus specificity of the test. It was found that most of the antibodies active in IHA using ethanol extracted antigen were in the IgM fraction [2]. Hence it could be assumed that sera samples showing high PHA titres were from acute cases of leptospirosis and this could be the probable reason for the low levels of PHA antibodies in cattle which are usually subclinical carriers.

The results of the two serological tests were analysed by Kappa statistics. The percentage agreement between MAT and PHA showed Kappa values above 0.9 for all the species tested. This indicates perfect agreement as per [5].

## VI. Conclusion

The present study could successfully detect leptospiral antibodies in the sera of human beings, the end host, animals, the propagative hosts and rodents, the reservoir hosts of *Leptospira*. The common serovars prevalent in man, animals and rodents were identified as Pomona and Australis. High prevalence of leptospiral antibodies in apparently healthy animals like cattle proves the major role played by these animals in the dissemination of infection in animal and human population. As the currently employed canine whole cell inactivated vaccines incorporates none of these emerging serovars, and as it confers only serovar specific immunity, vaccinated animals are becoming increasingly susceptible to leptospiral infection. The changing predominance of different leptospiral serovars in canines and humans stresses the need for a modified genus specific vaccine for both the species in this region. Based on the limited data obtained from this study, a more elaborate study on the epidemiology of leptospirosis in Kerala has to be carried out to identify the important reservoirs and serovars of *Leptospira* responsible for the endemicity of the disease and suggest a foolproof prevention strategy for leptospirosis in the state.

## Acknowledgement

The authors are highly thankful to the Dean, College of Veterinary and Animal Sciences, Mannuthy, for providing all kind of facilities.

## References

- [1]. S. Faine. Guidelines for the control of leptospirosis. World Health Organisation, offset publication No.67, Geneva, Switzerland. 1982. P.171

- [2]. J.A.Morris, J.E.Gill and S.N. Hussaini An examination of the antibodies active in the indirect hemagglutination test for bovine leptospirosis. *British Veterinary Journal*.133, 1977, 17-24
- [3]. A. Palit, and J. Gulasekharan. Genus specific leptospiral antigen and its possible use in laboratory diagnosis. *Journal of Clinical Pathology*.26,1973,7-16
- [4]. N.B.Vanasco, J. Lotterberger, M.D. Sequeira and H.Tarabla. Development and validation of an ELISA for the detection of leptospire-specific antibodies in rodent..*Veterinary Microbiology*.82,2001,321-330
- [5]. G.D.Raj, V. Jayakumar, A.Thangavelu, A. Koteeswaran and A.T.Venugopalan. Immunoelectrophoresis for the diagnosis of Infectious Bursal Disease. *Avian Disease*.42, 1998, 1087-1095
- [6]. H.V.Batra, N.K. ChandiramaniandU.V.Mandokhot. Prevalence of leptospirosis in farm animals in Haryana. *Indian Journal of Animal Sciences*. 60,1990, 755-760
- [7]. J.Ramakrishna and K.S.Venkataraman . Leptospirosis in dairy cattle- A recent epidemiological study. *Cheiron*. 23,1994,242-245
- [8]. L.Ciceroni, P.D Aniello, N.Russo, D. Nese, F. Lauria, A.Pinto. and B. Cacciapuoti.. Prevalence of leptospire infections in buffalo herds in Italy. *Veterinary Record*. 137, 1995,192-193
- [9]. S. Indu. Prevalence of leptospirosis among dogs in Thrissur. M.V.Sc. thesis submitted to Kerala Agricultural University, Thrissur. 1997. pp.1126
- [10]. R.Ambily, M.Mini,S.Joseph, S.V.KrishnaandG.Abhinay. Canine leptospirosis – a seroprevalence study from Kerala, India..*Veterinary World* 6(1) ,2013, 42-44
- [11]. N. Mrunalini. and P. Ramasastry. A note on seroprevalence of subclinical leptospirosis in organized farms in Andhra Pradesh. *Indian Veterinary Journal*.77,2000,713-714
- [12]. K.Natarajaseenivasan. and S.Ratnam. Seroprevalence of leptospiral infection in an agricultural based village in Tamil Nadu. *Cheiron*. 26, 1997,80-83
- [13]. S.V. Bhagwat.. Serological evidence of *Leptospira pomona* infection in pigs in India (Aurangabad). *Indian Veterinary Journal*. 41, 1964, 792-796
- [14]. M. Rajasekhar and R.D. Nanjiah. Animal leptospirosis in Mysore state-a serological study. *Indian Veterinary journal* 48,1971,1087-1095
- [15]. L.E.Hanson, H.A.Reynolds and L.B.Evans. Leptospirosis in swine caused by serotype gryppotyphosa. *American Journal of Veterinary Research*. 32,1971,855-860
- [16]. R.J. Chappel, R.W. Prime, B.D. Millar, L.J.Mead, R.T. Jones and B. Adler. Comparison of diagnostic procedures for leptospirosis. *Veterinary Microbiology*.30, 1992, 151-163
- [17]. N.D.Sullivan. Leptospirosis in animals and man. *Australian Veterinary journal*.. 50,1974,216-223
- [18]. M. Kuriakose, R.Paul, M.R. Joseph, S.Sugathan and T.N. Sudha. Leptospirosis in a midland rural area of Kerala state.*Indian Journal of Medical Research*,128, 2008, 307-312.
- [19]. J.Antony, T.M. Celine and M. Chacko. Case fatality rate of leptospirosis in a Tertiary Care hospital in Kerala, in India. *Annals of Tropical Medicine and Public Health*.5(3), 2012, 236-239
- [20]. M.V. Murhekar ,A.P.Sugunan, P.Vijayachari, S. Sharma. andS.C.Sehgal. Risk factors in transmission of Leptospiral infection. *Indian Journal of Medical Research*.107, 1998, 218-223
- [21]. S.K.Srivastava, N.C.SrivastavaandP.K.Uppal. Standardisation of an indirect Haemagglutination (IHA) test for detecting antibodies against leptospiralserovars in animals. *Indian Journal of Comparative Microbiology Immunology and Infectious Diseases*. 6,1985,69-75
- [22]. A. Palit, R.C.Hamilton and J.C. Gulasekharan. Further studies on leptospiral genus specific antigen: its ultrastructure and immunochemistry. *Journal of General Microbiology*. 82,1974,223-236