



PREVALENCE OF *Leptospira interrogans* SEROVAR PYROGENES AMONG CLINICALLY ILL DOGS IN WAYANAD DISTRICT

Received: 27.07.2018

Accepted: 03.10.2018

Leptospirosis is an important bacterial zoonosis caused by many pathogenic serovars of *Leptospira interrogans*. Rodents act as reservoir host for the disease and animals like dogs, pigs and cattle act as temporary carriers (Tilahun *et al.*, 2013). The members belonging to the genus *Leptospira* are grouped into 22 antigenically related species and classified into more than 300 serovars (Picardue *et al.*, 2017). South Indian states such as Kerala, Karnataka and Tamil Nadu are endemic for leptospirosis (Himani *et al.*, 2013). Vaccination is an effective method for control of the disease. But, the immunity against leptospirosis is serovar specific. Hence, it is necessary to identify the geographically prevalent serovar and it is necessary to incorporate them in vaccine. So, the present study was undertaken to identify the prevalent serovar of *Leptospira* in Wayanad district of Kerala.

Dogs presented to Teaching Veterinary Clinical Complex, Pookode and Ambulatory Clinic, Meenanagdi with clinical signs such as high rectal temperature, icteric mucous membrane, high leukocyte count, vomiting and diarrhoea were selected for

the study. Thirty five serum samples were collected from these dogs and subjected to Microscopic Agglutination Test (MAT) using a battery of 12 pathogenic serovars viz, *Leptospira interrogans* serovars Australis, Autumnalis, Bataviae, Canicola, Grippityphosa, Hebdomadis, Icterohaemorrhagiae, Pomona, Pyrogenes, Sejrae and Tarasovi. MAT was performed as described by Faine *et al.* (1999).

The test sera were first diluted to 1:200 and then serially diluted two-fold in phosphate buffered saline, to obtain dilutions of 1:100 to 1:800. Fifty microliter of each diluted serum samples were mixed with 50 μ L of five to seven days old live cultures of selected *Leptospira* serovars in a U bottom microtiter plate. Antigen controls were set with 50 μ L PBS and 50 μ L of different live *Leptospira* serovars and the plates were incubated at 37°C for two hours. After incubation, the results were read by examining a drop of serum-antigen mixture from each well under low power of dark field microscope for agglutination of leptospire. Fifty per cent or more agglutination was taken as positive. Samples showing agglutination at 1:200 were further diluted till 1:800 dilutions.

Out of thirty-five serum samples tested, five (14 per cent) were found to be positive for *Leptospira interrogans* serovar Pyrogenes. The Microscopic agglutination test (MAT) titres varied from 1:200 to 1:800 (Fig. 1 and 2). Leptospirosis is one of the most underdiagnosed diseases in our country. Detection of antibodies against leptospira using (MAT) considered as gold standard for the diagnosis of leptospirosis (Galton *et al.*, 1965). MAT is a serovar specific test. Titer of 1:100 and above is considered as positive in low endemic areas and 1:200 and above is considered as positive in high endemic areas (Vijayachari *et al.*, 2001). The prevalence of serovar Pyrogenes observed in this study was 14 per cent which is high compared to studies conducted in other parts of Kerala. Ambily *et al.* (2013) reported 7.5 per cent prevalence of serovar Pyrogenes in samples collected from Thrissur, Kozhikode and Palakkad. Abhinay *et al.* (2012) also reported the prevalence of serovar Pyrogenes as 6.98 per cent at Mannuthy, Kerala.

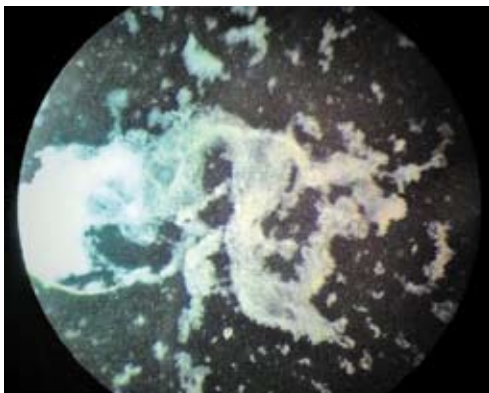


Fig 1: Microscopic Agglutination Test at 1:200 dilution of serum

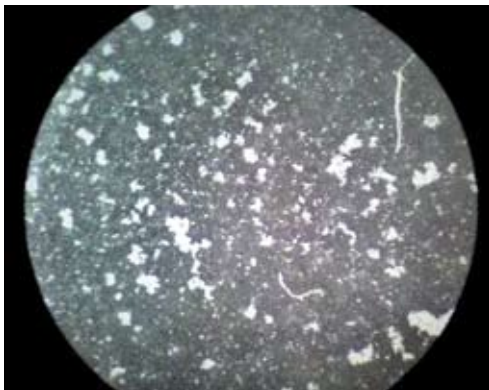


Fig 2: Microscopic Agglutination Test at 1:400 dilution of serum

Sathiyamoorthy *et al.* (2017) conducted a similar study in Tamil Nadu and reported 12.67 per cent of seroprevalence of serovar Pyrogenes among canine population. Balakrishnan *et al.* (2008) reported 16.67 per cent seroprevalence of serovar Pyrogenes in Andhra Pradesh. These studies suggest that Pyrogenes is a prevalent serovar in South India. The current study is the first report showing high prevalence of serovar Pyrogenes in Wayanad as well as from Kerala.

Summary

Immunity against Leptospirosis is serovar specific (Adesiyun *et al.*, 2006). Commercially available vaccines against leptospirosis provide immunity only against serovar Icterohaemorrhagiae, Pomona, Canicola and Grippotyphosa. Current study is the first study indicating high prevalence of serovar pyrogenes among dogs belonging to Wayanad district. So, it is necessary to incorporate geographically prevalent serovars of *Leptospira* for successful immunisation and prevention of the diseases.

References

- Abhinay, G., Joseph, S. and Ambily, R. 2012. Seroprevalence of canine leptospirosis. *Indian Vet. J.* **89**(2): 72-73.
- Adesiyun, A.A., Hull-Jackson, C., Mootoo, N., Halsall, S., Bennett, R., Clarke, N.R., Whittington, C.U. and Seepersadsingh, N. 2006. Sero-epidemiology of canine leptospirosis in Trinidad: serovars, implications for vaccination and public health. *J. Vet. Med.* **53**(2): 91-99.
- Ambily, R., Mini, M., Joseph, S., Krishna, S.V. and Abhinay, G. 2013. Canine leptospirosis-a seroprevalence study from Kerala, India. *Vet. World.* **6**(1): 42-44.
- Balakrishnan, G., Govindarajan, R., Meenambigai, T.V., Jayakumar, V. and Murali Manohar, B. 2008. Seroprevalence of leptospirosis among domestic animals in Andhra Pradesh. *Indian Vet. J.* **85**(5): 551-552.

- Faine, S., Adler, B., Bolin, C.A. and Perolat, P. 1999. *Leptospira and leptospirosis*. (2nd Ed.). MediSci publishers, Melbourne, Australia, 272 p.
- Galton, M.M., Sulzer, C.R., Santa Rosa, C.A. and Fields, M.J. 1965. Application of a microtechnique to the agglutination test for leptospiral antibodies. *Appl. Microbiol.* **13**: 81-85.
- Himani, D., Suman, M.K. and Mane, B.G., 2013. Epidemiology of leptospirosis: an Indian perspective. *J. Foodborne Zoonotic Dis.* **1**(1): 06-13.
- Kuriakose, M., Eapen, C.K. and Paul, R. 1997. Leptospirosis in Kolenchery, Kerala, India: Epidemiology, prevalent local serogroups and serovars and a new serovar. *Eur. J. Epidemiol.* **13**(6): 691-697.
- Picardeau, M. 2017. Virulence of the zoonotic agent of leptospirosis: still terra incognita?. *Nat. Rev. Microbiol.* **15**(5): 297.
- Sathiyamoorthy, A., Selvaraju, G., Palanivel, K. and Srinivasan, P. 2017. Seroprevalence of canine leptospirosis in namakkal, tamilnadu by microscopic agglutination test. *J. Cell. Tissue. Res.* **17**(1): 5991-5996.
- Tilahun, Z., Reta, D. and Simenew, K. 2013. Global epidemiological overview of leptospirosis. *Int. J. Microbiol. Res.* **4**:9-15.
- Vijayachari, P., Sugunan, A.P. and Sehgal, S.C. 2001. Evaluation of microscopic agglutination test as a diagnostic tool during acute stage of leptospirosis in high and low endemic areas. *Indian J. Med. Res.* **14**: 99-106. ■

**C.N. Amrutha¹, K.C. Bipin², P.M. Deepa³,
K. Vijaykumar⁴, Siju Joseph⁵**

Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and animal sciences, Pookode, Lakkidi (PO), Wayanad 673 576.

**Part of MVSc thesis of first author submitted to KVASU, Kerala*

1. MVSc. Scholar
2. Assistant Professor
3. Assistant Professor & Head (i/c)
4. Professor & Head Department of Veterinary Epidemiology and Preventive Medicine, CVAS, Mannuthy
5. Assistant Professor, Department of Veterinary Microbiology, CVAS, Mannuthy