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Seasonal analysis of leptospirosis in apparently healthy dairy cattle of Pananchery panchayat in Thrissur district, Kerala#

A. P. Sriji[1*], K. Vrinda Menon[2], C. Latha[3], Deepa Jolly[4] and K. Justin Davis[5]
Department of Veterinary Public Health
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651
Kerala Veterinary and Animal Sciences University, Kerala, India.

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Abstract

Leptospirosis is an economically important zoonotic bacterial disease of cattle with global distribution. The present study investigated the seroprevalence and distribution of leptospiral serovars in cattle population in Pananchery panchayat of Thrissur district, Kerala. From 52 apparently healthy dairy cattle, serum samples were collected in both pre-monsoon and post-monsoon seasons. All samples were subjected to microscopic agglutination test (MAT) against 13 serovars of Leptospira spp. Among the samples tested, 13 (25 per cent) samples in pre-monsoon and 19 (36.54 per cent) samples in post-monsoon were found to be positive. The predominant serovars detected in pre-monsoon were Hardjo (29.41 per cent) followed by Grippotyphosa (17.64 per cent), Bataviae and Pomona (11.76 per cent each). Similarly in post-monsoon, Hardjo (28.57 per cent) followed by Grippotyphosa (23.81 per cent), and Bataviae (19.05 per cent) were identified as the most common serovars. Higher seroprevalence of disease in apparently healthy cattle in post-monsoon compared to pre-monsoon indicate the influence of rainfall on occurrence of leptospirosis.

Keywords: C*attle, Microscopic Agglutination Test, Predominant serovars, Thrissur*

Leptospirosis is a neglected anthropo-zoonotic disease caused by pathogenic spirochetes of the genus Leptospira. Disease is commonly found in tropical and subtropical countries, but occurrence was found to be high after seasonal rainfall. According to the data of National Health Mission, 1690 confirmed human cases and 58 confirmed deaths were reported in Kerala in 2021. Pathogenic Leptospira species are categorized into 300 serovars and 28 serogroups based on the

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1. *M.V.Sc scholar*
2. *Associate Professor*
3. *Director (Academics and research)*
4. *Assistant Professor*
5. *Assistant Professor, Department of Veterinary Epidemiology and Preventive Medicine*

**Corresponding author: srijiap94@gmail.com, Ph: 7025878319.*

outer membrane lipopolysaccharide (Saito *et al*., 2013). Animals infected with host-adapted serovars are referred to as maintenance hosts, and clinical symptoms are usually mild. Cattle act as the maintenance host of *L. borgpetersenii* serovar Hardjo (Adugna, 2016). Even though highest concentration of leptospires is excreted by rats, cattle excrete much more urine, leads to higher environmental contamination. Cattle can excrete leptospires through their urine for 28 to 40 weeks (Leonard *et al*., 1992) with 6.3 X 10^8 cells per day (Barragan *et al*., 2017). The organism enters the susceptible animals through direct or indirect contact with urine of infected or carrier animals, aborted foetuses and uterine secretions. Farmers suffer economic losses as a result of leptospirosis in cattle, as the disease can cause abortion, infertility, and reduced milk production. Serogroups maintained by animals in that region are reflected in human leptospirosis. Thus, studying temporal dynamics of disease seroprevalence among animals in a region will help to understand the period of disease transmission to humans. The present study mainly focused on seasonal analysis of leptospirosis in apparently healthy cattle in Pananchery panchayat of Thrissur district and identification of predominant serovars present in the study area.

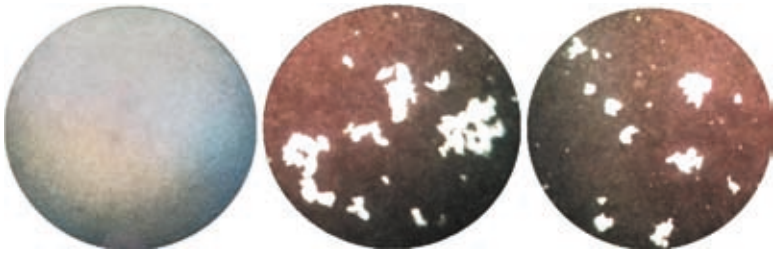
Materials and methods

In the present study, 52 apparently healthy dairy cattle were selected from Pananchery panchayat, Thrissur district, Kerala and blood samples were collected from the same cattle in both pre-monsoon (August-September) and post-monsoon (December-January) seasons. Serum was separated from the clotted blood by centrifugation at 2000 rpm for two minutes (Bojiraj *et al*., 2018) and stored at -20°C. Serum samples were subjected to Microscopic agglutination test (MAT) using a panel of 13 live leptospiral antigens including Autumnalis, Australis, Bataviae, Canicola, Djasiman, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes and Tarassovi. The MAT was performed according to the procedure of Faine (1982), from a dilution of 1:50 to 1:6400 in Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy, Kerala. The final titration was determined as the dilution where the 50 per cent agglutination or 50 per cent reduction in the number of free leptospires in comparison to control was observed. Statistical analysis was performed using McNemar test for identifying the temporal influence on occurrence of leptospirosis in the study area.

Results and Discussion

In the present study, all the serum samples were collected from apparently healthy dairy cattle. An antibody titre of 1:100 in non endemic and 1:400 in endemic areas were considered as positive for leptospirosis in animals (OIE, 2018). Favero *et al.* (2017) observed that, a titre of 1:50 indicated animal exposure to the etiological agent, and 1:100 or higher is suggestive of clinical illness. Since the study was conducted in apparently healthy cattle, a titre of 1:50 and above was considered as positive in the present study.

Out of 52 serum samples analysed by MAT, 13 (25 per cent) samples in pre-monsoon and 19 (36.54 per cent) samples in post-monsoon were found to be positive at dilution between 1:50 and 1:200. On analysis of the data by McNemar test revealed that, there was no significant difference between the occurrence of disease between pre-monsoon



Negative control **1:50 dilution** **1:100 dilution**

Fig. 1. Microscopic agglutination test (MAT)

Table 1. MAT antibody titre observed from dairy cattle

Sl. No	Titre value	Pre-monsoon	Post-monsoon
1.	1: 50	12(92.31)	12(63.16)
2.	1: 100	1(7.69)	5(26.31)
3.	1: 200	0	2(10.53)
Total		13^{ns}	19^{ns}

* Figures in brackets indicates percentage

ns- no significant difference

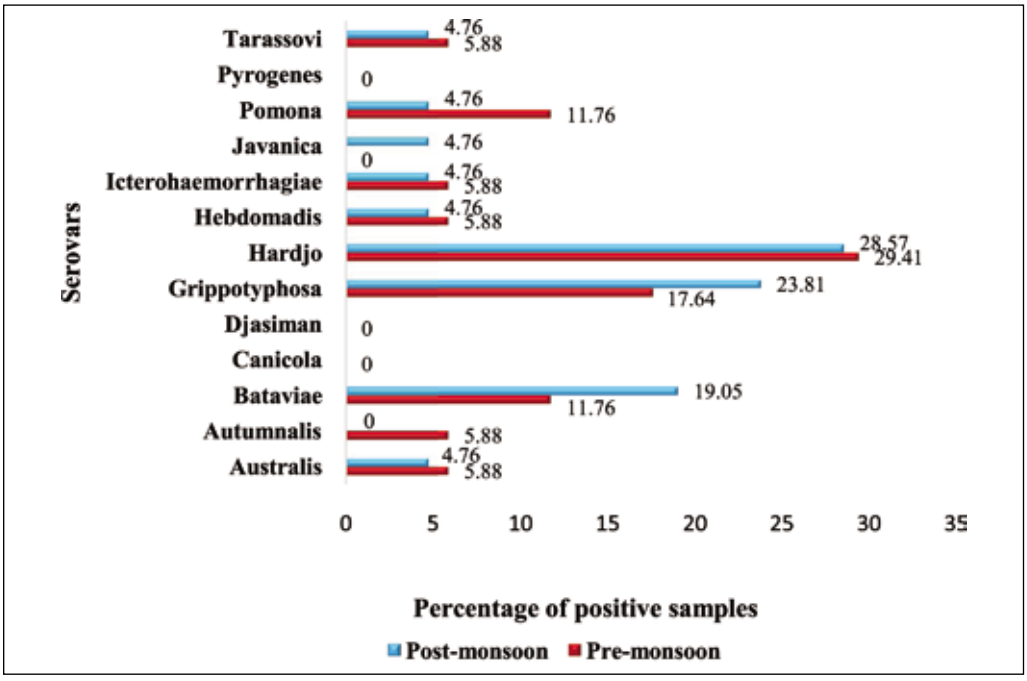


Fig. 2. Serovars in dairy cattle based on MAT

and post-monsoon (p value > 0.05) seasons. An antibody titre of 1:50 was observed in 12 (92.31 per cent) samples and 1:100 in one (7.69 per cent) sample in pre-monsoon. In post-monsoon, among the positive samples, 12 (63.16 per cent) were positive at 1:50, five (26.31 per cent) at 1:100 and two (10.53 per cent) at 1:200 dilutions as shown in the Table 1. The predominant serovars detected in pre-monsoon were Hardjo (29.41 per cent) followed by Grippotyphosa (17.64 per cent), Bataviae and Pomona (11.76 per cent each), Australis, Autumnalis, Hebdomadis, Icterohaemorrhagiae and Tarassovi (5.88 per cent each). The nine serovars namely Hardjo (28.57 per cent) followed by Grippotyphosa (23.81 per cent), Bataviae (19.05 per cent), Pomona, Australis, Hebdomadis, Icterohaemorrhagiae, Javanica and Tarassovi (4.76 per cent each) were identified as the predominant serovars in cattle in post-monsoon (Fig.2).

In the present study, 25 per cent and 36.54 per cent seropositivity was found in apparently healthy cattle in pre-monsoon and post-monsoon respectively. Balakrishnan *et al.* (2011) reported a similar seroprevalence (34.74 per cent) in Gujarat. Various seroprevalence studies of bovine were conducted in Thrissur district and a seroprevalence of 47 per cent (Soman, 2004), 20.68 per cent (Divya *et al.,* 2021), and 44.24 per cent (Murugavelu *et al*., 2022) were observed. Sreekutty *et al.* (2020) found a higher seropositivity (52.2 per cent) in apparently healthy cattle in Alappuzha. Balamurugan *et al.* (2018) found a seroprevalence of 65.15 per cent, in contrary to the present study, but samples were taken from cattle with a history of reproductive problems in various endemic states in India. In

cattle suspected with leptospirosis in Andhra Pradesh, Alamuri *et al.* (2019) found 70.8 per cent seropositivity. This clearly shows that investigations in animals with suspected symptoms showed a higher percentage of seropositivity than those in apparently healthy cattle. The difference in seroprevalence between studies could be explained by differences in the cut-off titres considered positive for disease in each investigation, as well as change in the area of study.

Out of the 52 cattle in Pananchery panchayat, 10 (19.23 per cent) animals which were negative in pre-monsoon became seropositive in post-monsoon season. The seroprevalence was found to be high in post-monsoon than that of pre-monsoon. This was in accordance with Senthilkumar *et al.* (2021) in Tamilnadu, where they found 25.8 per cent seropositivity in pre-monsoon and 30.5 percent in post-monsoon cattle serum samples. Similarly, Hinjoy (2016) and Cunha *et al.* (2022) found that amount of rainfall and incidence of leptospirosis were positively correlated. Rainfall, wet weather, water source contaminated with sewage and urine of infected or carrier animals could be the reason for greater seropositivity in post monsoon. Following rainfall, flood may occur, which will replace rodents into habitats near residential areas increasing risk of exposure to domestic animals. In contrary to the present study, Agrawal *et al.* (2018) reported a higher seroprevalence in pre-monsoon (27 per cent) compared to post-monsoon (21 percent) in New Delhi.

The most predominant serovars found in pre-monsoon were Hardjo (29.41 per cent) followed by Grippotyphosa (17.64 per cent), Bataviae and Pomona (11.76 per cent each). Similarly, Hardjo (28.57 per cent), Grippotyphosa (23.81 per cent), and Bataviae (19.05 per cent) were the most common serovars found in the post-monsoon season. The findings were similar to those of Murugavelu *et al.* (2022) in Thrissur, where Hardjo (28.76 per cent) and Grippotyphosa (21.91 per cent) were reported as the most common serovars. Balamurugan *et al.* (2014) and Natarajaseenivasan *et al.* (2011) found similar observation in various Indian states, noticed Hardjo as the prevalent serovar in cattle. Predominant serovars reported by Sreekutty *et al.* (2020) in Alappuzha were Grippotyphosa (34.04 per cent) and Hardjo (25.53 per cent) and Autumnalis (21.3 per cent). Cattle are considered to be the maintenance host for serovar Hardjo and other members of the serogroup Sejroe (Loureiro and Lilenbaum, 2020). In contrary to the above findings, Bojiraj *et al.* (2017) found Australis and Autumnalis as the predominant serovars in cattle in Tamil Nadu. Furthermore, a comparison of the current study with various studies from different researchers showed that predominant serovars of Leptospira vary between locations over time.

The majority of the cattle (92.31 per cent in pre-monsoon and 63.16 per cent in post-monsoon) in this investigation showed a titre value of 1:50, indicating that they had been exposed to *Leptospira* spp. Murugavelu *et al.* (2022) obtained a similar result, with 61.64 per cent positive samples showing a titre of 1:50. Since the cattle used in this research work were apparently healthy, the chance of finding a high titre is less.

In pre-monsoon, on analysis by MAT, 10 samples (76.92 per cent) were positive for one serovar, two samples (15.38 per cent) were positive for two serovars and only one sample (7.69 per cent) was positive for three serovars out of the 13 positive samples. Hardjo-Grippotyphosa, Autumnalis-Tarassovi and Hardjo-Pomona-Icterohaemorrhagiae were the serovar combinations found in pre-monsoon. Among the 19 positive sera samples in post-monsoon, 17 (89.47 per cent) were positive for only one serovar, whereas two samples (10.53 per cent) were positive for two serovars including Hardjo-Bataviae and Pomona- Icterohaemorrhagiae. In the study of Murugavelu *et al.* (2022) in Thrissur, 86.30 per cent bovine serum samples showed the presence of only one serovar, whereas 9.59 per cent, 2.74 per cent and 1.37 per cent samples showed the presence of two, three and four serovars respectively. Kirkimbayeva *et al.* (2015) in Kazakhstan found positive titres for more than one serovar only in 4.71 per cent of cattle samples. The serum from an animal reacted with various serovars, and this may be due to cross-reaction among various serovars and animal being infected with more than

one serovars (Chirathaworn *et al.*, 2014).

The present study detected leptospiral antibodies in the sera of cattle even in the absence of disease, indicating that cattle can act as the reservoir of these organisms. The occurrence of disease in cattle was found to be high in post-monsoon season. They can excrete leptospires through urine and pose significant threat to susceptible animals and humans which get exposed to contaminated environment.

Conclusion

The role of apparently healthy cattle in the maintenance and transmission of leptospirosis can be better assessed by investigating the prevalence of disease in cattle and identifying the predominant serovars that lead to infection. The temporal influence on disease should be considered while adopting preventive measures. This study showed that the humans and other susceptible animals are at significant risk of infection because cattle can act as carriers of leptospires even if they show no clinical indications. As a result, proper intervention techniques should be established for disease control and prevention, which demands a multidisciplinary one health approach.

Conflict of interest

The authors declare that they have no conflict of interest.

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