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# Sero-monitoring of canine leptospirosis by microscopic agglutination test (MAT) in and around Navsari, South Gujarat, India

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**Abstract**
Leptospirosis is neglected zoonotic infection caused by bacterium Leptospira having public health significant due to increasing prevalence in the world. It is caused by pathogenic spirochetes that belong to genus Leptospira. In our study, A total of 410 serum samples were collected from different breeds of dog from Navsari, Surat and Valsad districts of southern Gujarat. Microscopic agglutination test (MAT) was conducted to screen these samples by using a battery of 20 live known leptospira serovars. A total of 45 samples were positive out of 410 samples at titre of 1:50 or above with seroprevalence rate of 10.98%. Serovars found in our study in ascending orders were Lai (2.20%), Icterohaemorrhagiae (2.20%) Javanica (4.40%), Ballum (11.10%), Panama (13.30%), Grippotyphosa (15.60%), Canicola (15.60%), Hurstbridge (17.80%), Djasiman (20.00%), Shermani (22.20%) and Pyrogenes (24.40%). Serovars found with high percent were Djasiman, Pyrogenes and Shermani while the least serovars found were Lai, Icterohaemorrhagiae and Javanica and in and around Navsari district, Southern Gujarat region. Though, the dogs are vaccinated, they can acquire infection from other serovars of leptospira that was not used in immunization and the serovars in current vaccine doesn't provide cross protection against other leptospiral serovars. So, it is better to incorporate the serovar/s specific vaccine which is/are endemic at a particular geographically area.

**Keywords:** MAT, sero-prevalence, sero-monitoring

## Introduction
Leptospirosis, also called Weil disease (Ahmad *et al*., 2005 and Vijayachari *et al*., 2008) [4, 37] caused by pathogenic Leptospira, which are distributed globally in various hosts (Abdullathief *et al*., 2018) [1]. Common animals that transmit Leptospirosis include farm animals such as cattle, pigs, and horses but can range from wild animals such as raccoons and porcupines to domesticated dogs. It is most often spread through exposure to the urine of infected animals either from direct contact or from contact with soil or water contaminated by the urine. The bacteria are highly motile by endoflagella made up of fine spirals with hook-shaped ends. Motility is gained by writhing and flexing movements while rotating along the long axis. The bacterium is an obligate aerobic spirochete, gram negative bacteria (Bharti *et al*., 2003) [11].
Leptospira species can be classified into 22 serogroups having more than 300 serovars using traditional serological classification (Picardeau, 2017) [28]. Domestic animals like cattle, pigs, goats, sheep, dogs and horses have been infected with this disease. In dogs, it is commonly caused by Canicola, Icterohaemorrhagiae, Pomona, Bratislava, Grippotyphosa and Australis serovars. Rodents act as reservoir host to spread this disease either directly or indirectly through contaminated urine in water, feed, and soil (Hartskeerl and Terpstra, 1996 and Schneider *et al*., 2015) [19, 32].
In India, leptospirosis is known to be an endemic and majority of leptospirosis outbreaks are recorded from the Andaman Islands, Gujarat, Maharashtra, Kerala, Karnataka, Orissa, Tamil Nadu and the coastal areas of West Bengal and (Himani *et al*., 2013) [20]. Canine leptospirosis is common found during the monsoon season when there is a lot of stranded water and swampy situations with humidity.

Stranded water, polluted urine, direct touch, contaminated water, plants, soils as well as contaminated food are the most common source for dogs to become infected. Though dogs are vaccinated, they are getting infected due to non-vaccinal serovars circulating in that particular region. The present study was carried out to investigate the Sero-monitoring of canine leptospirosis and prevalence of circulating serovars in and around Navsari region, Gujarat.

**Materials and Methods**

Four hundred and ten clinical serum samples were collected from Navsari, Surat and Valsad districts of Southern Gujarat, during a period of Mid-September 2021 to first week of May 2022. They were sent to ICAR -NIVEDI, Bengaluru, Karnataka for screening of MAT for detection of anti-Leptospira antibodies (Balamurugan *et al*., 2018) [9] using twenty known live reference strains (serovars) of Leptospira covering 18 serogroups *viz*. Australis, Bankinang, Ballum, Bataviae, Canicola, Djasiman, Grippotyphosa, Hebdomadis, Hurstbridge, Icterohaemorrhagiae, Lai, Javanica, Mini, Panama, Pomona, Pyrogenes, Hardjo, Shermani, Kaup and Tarassovi. It was performed in doubling dilutions, starting from a dilution of 1 in 25. Positive samples were titrated up to end titre (Faine, 1982) [15]. In our sero-monitoring study, a MAT titre of more than or equal to 1:50 was considered as positive reactor to monitor with other associated clinical epidemiological factors. Selvaraj *et al*. (2010) [33] and Sunder *et al*. (2017) [35] had considered titre of 1:50 and 1:40, respectively as positive titre in MAT. A titre of 1:50 is used as the cut off because the titre 1:50 is used in sero-epidemiological surveys (Everard *et al*., 1985) [14]. In dogs, Altheimer *et al*. (2020) [6] considered ≥ 1:20 as cut-off titre to study prevalence in Thailand.

MAT was performed as per Faine *et al*. (1999) [16] using a battery of known twenty live leptospira serovars. The test was carried out in U-bottomed micropipette plates (Tarsons). A quantity of 960 µl of sterile PBS with pH 7.2 was added to each well of the plate. To it, a total 40 µl of each serum samples were added to all wells (1 - 11) except last row (12) in which 40 µl of PBS is added which acted as antigen control. The sera samples were diluted to 1:50 in PBS in test tubes. Mix thoroughly. Each serum sample of 50 µl was transferred to the Micro U bottom test plates (Tarson) from 1-11 row wise i.e., each row has one serum sample and the last row is antigen control. Add 50 µl of the well grown live leptospira antigen (8 different serovars) culture of 5-8 days old antigen row wise eg. A- Australis, B- Autumnalis, C-Canicola etc… making a dilution of 1:50, mix thoroughly by tapping for few seconds and cover the plates with aluminium foil. Keep the plate at 30º C in an incubator for 2-4 hours. At the end of incubation, 10 µl of each dilution was taken on a clean glass slide and examined under low power (10 X) of dark field microscope, without using a coverslip. The endpoint of agglutination reaction was taken as the highest point in which 50 percent of the leptospires had agglutinated. The reciprocal of the end point was taken as the titre. A titre of 50 and above was considered positive. Thereafter, those sera samples which demonstrated an agglutination at 1:50 dilution were again tested at 1:100, 1:200, 1:400, 1:800, 1:1600 sera dilutions (Figure 2). Some samples may show agglutination to more than one serovar that is called mixed agglutination.

**Results and Discussion**

Among 410 serum samples screened, 45 were positive at titre of 1:50 or above (Table 1) with seroprevalence of 10.98% recorded in and around Navsari, South Gujarat (Figure 1 and Table 4). In this region, couple of years ago, Desai *et al*. (2020) [12] and Godhani (2021) [17] reported 46.42% and 53.13% seroprevalence of leptospirosis in dogs, respectively. Kshirsagar (2015) [21] reported seroprevalence of leptospirosis in cattle (6.68%), buffalo (14.86%), goat (21.81%) and sheep (33.33%) in South Gujarat. Patel *et al*. (2017) [25] and Patel *et al*. (2018) [26] found 15.69% in cattle and 12.81% in buffalo, respectively in Southern Gujarat. Vihol *et al*. (2017) [36] concluded that seroprevalence of leptospirosis in sheep and goat was 12.20% and 23.67%, respectively. Among different states of India, varying seroprevalence rate had been recorded as per literature reviewed. A total of 23.60% in Bengaluru (Dharanesh *et al*., 2009) [13], 7.07% in Chennai, Izatnagar and Delhi, Paramilitary forces (Kumar *et al*., 2009) [22], 75.44% seroprevalence in Thrissur (Abhinay *et al*., 2012) [2], 71.12% in Kerala (Ambily *et al*., 2013), 33% in Pondicherry (Kumar *et al*., 2013) [23], 26.4% in Tiruchirappalli, Tamil Nadu (Meera, 2016) [24], 27% in Thrissur, Kerala (Akhila *et al*., 2018) [5], and 28% in Pune and Goa (Bale *et al*., 2021) [10].

Serovars found in our study in ascending orders were Lai (2.20%), Icterohaemorrhagiae (2.20%) Javanica (4.40%), Ballum (11.10%), Panama (13.30%), Grippotyphosa (15.60%), Canicola (15.60%), Hurstbridge (17.80%), Djasiman (20.00%), Shermani (22.20%) and Pyrogenes (24.40%) as shown in Table 2 The predominant serovars present in this study were Djasiman, Pyrogens and Shermani which was contrary to the research findings of Godhani (2021) [17] who reported Pomona and Grippotyphosa were found higher in and around Navsari. Our results data were in complied with Patil *et al*. (2014) [27] who reported that Pyrogenes serovar of leptospira was found to be the most prevalent serovar followed by the Icterohaemorrhagiae. While Amrutha *et al*. (2019) [8] emphasized that Pyrogenes was highly sero-prevalent followed by Australis, Grippotyphosa, Icterohaemorrhagiae, Tarasovi and Javanica. Moreover, Shaji *et al*. (2019) [34] stated that serovar Pyrogenes were found commonly in Wayanad, Kerala. In contrary to previous workers, Meera (2016) [24] who reported that Javanica was the most principle serovar in Tiruchirappalli, Tamil Nadu. Abdullathief *et al*. (2018) [1] observed that *L. interrogans* serovar Australis was prime serovar followed by serovar Autumnalis and Canicola in Mannuthy, Kerala whereas Akhila *et al*. (2018) [5] stated that predominant serovars identified in Thrissur, Kerala were Australis and Autumnalis. Globally, Habus *et al*. (2020) [18] stated that most predominant serovar was Pomona, followed by Icterohaemorrhagiae, Grippotyphosa, Australis and Sejroe in Canada, Raj *et al*. (2021) [30] concluded that Copenhageni was found to be most predominant serovar followed by Canicola, Icterohaemorrhagiae, Australis, Bratislava, Sejroe, Saxkoebing in England, Piredda *et al*. (2021) [29] reported Icterohaemorrhagiae as the most predominant serovar followed by Bratislava, Canicola and Grippotyphosa serovars in Italy and Retnowati *et al*. (2022) [31] stated serovar Bataviae was the most prevalent followed by Icterohaemorrhagiae, Tarassovi and Javanica in Indonesia.

Based on the distribution of the aforementioned serovars from various countries around the globe, it is reasonable to conclude that serovar distribution varies from area to area and country to country.

At present Scenario in and around Navsari district, the dogs are protected against serovars Canicola, Icterohaemorrhagiae because they are regularly vaccinated against them.

According to Adesiyun *et al*. (2006) [3], it was necessary to immunize domestic animals against geographically prevalent serovars of leptospira as the immunity against disease was highly serovar-specific. Though, the dogs are vaccinated, they can acquire infection from other serovars of leptospira that was not used in immunization and the serovars present in vaccine doesn't provide cross protection against other leptospiral serovars. So, it is better to incorporate the serovar/s specific vaccine which is/are endemic at a particular geographical area.

This study shows the broad dispersion of serovars and the diversity of serovars detected is indicative of the possible existence of a variety of animal reservoirs involved.

Significant level of seroprevalence of leptospira with involvement of emerging serovars in dogs suggests that leptospirosis could be a major public threat and other animal concerned. It needs immediate and appropriate attention in respective geographical areas of study in Southern Gujarat to minimize the occurrence of this disease having zoonotic importance. Not only traditional techniques like Isolation and identification of infecting serovars to be emphasized but also latest advancement of serodiagnosis to be performed for better understanding the prevalent or emergent serovars for collective control strategies or regiment. Scientific awareness of neglected zoonotic leptospiral disease among medical health communities, workers and the public is urgently needed.

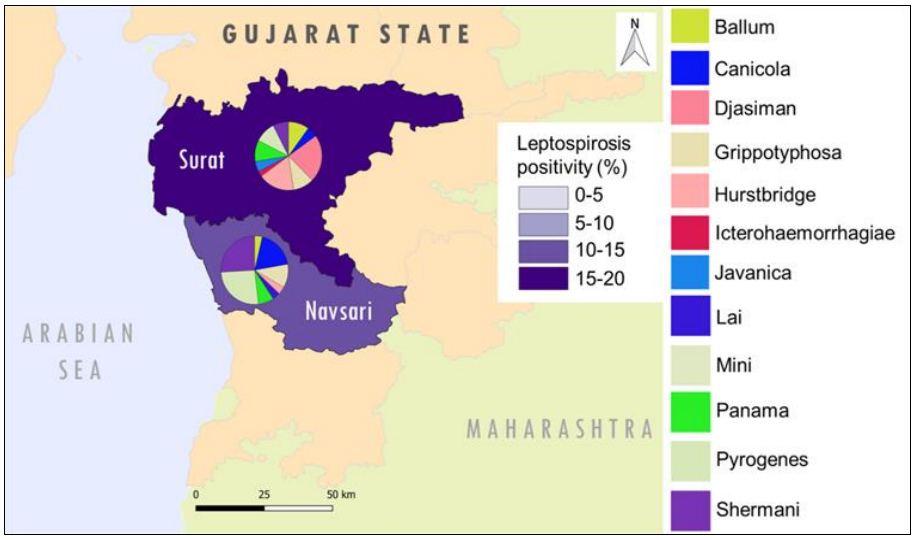


**Fig 1:** Map showing District wise distribution of serovars of leptospira

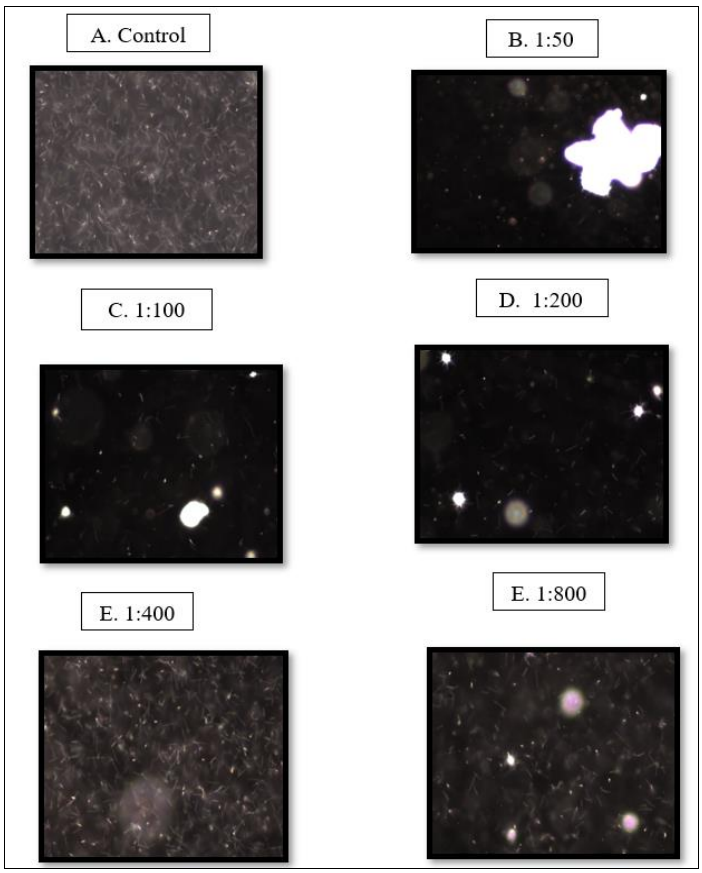


**Fig 2:** Microscopic Agglutination Test by DFM (20x) showing agglutination of leptospires. Note: Titre 1:200 showing ≥ 50% agglutination to particular serovar

**Table 1:** Reactivity titre of serum samples with different reference leptospiral serovars by MAT

| Reactivity Titre | Serogroup | No. of serum samples |
|---|---|---|
| <1:25 | - | 297 |
| 1:25 | Aus, Ban, Bal, Can, Dja, Gri, Hur, Lai, Jav, Min, Pan, Pom, Pyr, Har, She, Kau. | 68 |
| 1:50 | Can, Pyr, She, Bal, Hur, Dja, Gri, Pan, Jav, Cyn, Ict. | 40 |
| 1:100 | Pyr, Gri, She. | 03 |
| 1:200 | She, Gri. | 02 |
| Total | | 410 |

**Note:** Aus- Australis, Ban- Bankinang, Bal- Ballum, Can- Canicola, Dja -Djasiman Gri- Grippotyphosa, Hur- Hurstbridge, Lai- Lai, Jav- Javanica, Min- Mini, Pan- Panama, Pom- Pomona, Pyr- Pyrogenes, Har- Hardjo, She- Shermani, Kau- Kaup, Ict- Icterohaemorrhagiae.

**Table 2:** Percent positivity of Serovars by MAT in dogs for leptospirosis

| Serovars | Percent Positivity |
|---|---|
| Pyrogenes | 24.40 |
| Shermani | 22.20 |
| Djasiman | 20.00 |
| Hurstbridge | 17.80 |
| Canicola | 15.60 |
| Grippotyphosa | 15.60 |
| Panama | 13.30 |
| Ballum | 11.10 |
| Javanica | 04.40 |
| Icterohaemorrhagiae | 02.20 |
| Lai | 02.20 |
| Australis | 00.00 |
| Bankinang | 00.00 |
| Bataviae | 00.00 |
| Hebdomadis | 00.00 |
| Mini | 00.00 |
| Pomona | 00.00 |
| Hardjo | 00.00 |
| Kaup | 00.00 |
| Tarassovi | 00.00 |

**Note:** *Serovars found having MAT reactivity titre ≥ 1:50 was considered positive

**Table 3:** District wise distribution of serovar of Leptospira

| Serovars | Navsari | Surat | Valsad |
|---|---|---|---|
| Australis | - | - | - |
| Bankinang | - | - | - |
| Ballum | 01 | 04 | - |
| Bataviae | - | - | - |
| Canicola | 05 | 02 | - |
| Djasiman | - | 09 | - |
| Grippotyphosa | 03 | 04 | - |
| Hebdomadis | - | - | - |
| Hurstbridge | 01 | 07 | - |
| Icterohaemorrhagiae | - | 01 | - |
| Lai | 01 | - | - |
| Javanica | - | 02 | - |
| Mini | - | - | - |
| Panama | 02 | 04 | - |
| Pomona | - | - | - |
| Pyrogenes | 07 | 04 | - |
| Hardjo | - | - | - |
| Shermani | 07 | 03 | - |
| Kaup | - | - | - |
| Tarassovi | - | - | - |

**Table 4:** District wise seroprevalence of leptospirosis in dogs (n=410)

| District | No. of the samples screened | No. of the sample positive | Percentage positivity/seroprevalence | p-value |
|---|---|---|---|---|
| Navsari | 188 | 16 | 08.51 | 0.261* |
| Surat | 219 | 29 | 13.24 | |
| Valsad | 003 | 00 | 00.00 | |
| Total | 410 | 45 | 10.98 | |

**Note:** $^{NS}$ - Non significant at $p>0.05$

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