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Sero-epidemiological prevalence of leptospirosis among animals in south Gujarat

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Abstract

Leptospirosis is one of the neglected tropical diseases affecting humans, as well as wild and domestic animals. Livestock and pets also serve as asymptomatic reservoir hosts and can be a source of infection for humans in contact with them. While leptospirosis is usually mild and often subclinical in cattle, it can lead to higher incidences of abortion, stillbirth, infertility, mastitis, weak progeny, decreased milk production, and ultimately economic losses in livestock. The present study was conducted with the primary aim of assessing the seroprevalence of leptospirosis and identifying the prevalent leptospira serovars in cows, buffaloes, goats, and sheep in the South Gujarat districts. Serum samples obtained from different animals in the South Gujarat districts were tested for leptospirosis using Microscopic Agglutination Test (MAT) in the Microbiology Department of Government Medical College, Surat, Gujarat, India, from January 2021 to December 2023. The data were entered into Microsoft Excel, and OpenEpi software version 3.01 was used to calculate the chi-square test and p-value to analyze the level of significance in the presence of Leptospira among different animals. Out of 2,845 serum samples tested, 358 (12.58%) demonstrated the presence of agglutinating antibodies against leptospires by MAT test. Serovar-wise analysis showed that the predominant serovars in cattle were Hardjo (16.2%), Icterohaemorrhagiae (15.7%), Autumnalis (10.6%), Hebdomadis (10.6%), and Pomona, (10.6%). In buffalo, the predominant serovars were Hardjo (24.6%), Icterohaemorrhagiae (18.1%), Pomona (13%), Patoc (12.3%), Batavia (8.7%), and Hebdomadis (8%). In goats, the predominant serovars were Autumnalis 26.6%), Australis (13.3%), Icterohaemorrhagiae (13.3%), Patoc (11.1%), Pomona (6.6%), and Hebdomanis(6.6%).Periodic surveillance for leptospirosis in animals plays a vital role in creating awareness among the people regarding the diseaseand assists the farmers and public health workers to devise various control strategies.

Keywords: *Leptospirosis, MAT, seroprevalence, south Gujarat, one-health*

Leptospirosis is a quintessential one health disease affecting both humans and animals, caused by pathogenic *Leptospira* spp. (Sykes *et al*., 2022). Originally considered an occupational zoonotic disease, it primarily affects individuals in contact with animal tissues or urine. Pathogenic *Leptospira* spp.is maintained in the kidneys of animals such as cattle, dogs, horses, pigs, and several wild species. These bacteria asymptomatically colonize the renal tubules of these animals

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and are shed into the environment through urine, where, under suitable conditions, they can survive for extended periods, ranging from weeks to months. Both humans and animals may develop fatal, life-threatening illnesses as a result. Leptospirosis can also lead to economic losses in livestock due to its negative effects on production and reproduction. Livestock and pets, which can also act as asymptomatic reservoir hosts, serve as sources of infection for people in contact with them (Pratt and Rajeev, 2018).

Acute leptospirosis should be suspected in animals exhibiting sudden onset of agalactia, icterus, and haemoglobinuria, especially in young animals, as well as signs of meningitis and acute renal failure. Chronic leptospirosis should be considered in animals with a history of abortion, stillbirth, premature birth of weak offspring, infertility, chronic renal failure, or chronic active hepatitis (WOAH Terrestrial Manual, 2021). Acute cases of leptospirosis in cattle are very rare and can be controlled through the identification and treatment of urinary carriers (which appear healthy), quarantine of affected animals, antibiotic treatment for those infected, and systematic immunization with commercial vaccines containing the circulating serovars present in the herd (Lilenbaum and Martins, 2014). Therefore, continuous surveillance and monitoring of leptospirosis in livestock are essential to detect the carrier status of animals (Sunder *et al*., 2024). This study was conducted to determine the seroprevalence of leptospirosis among cows, buffalo, goats, and sheep in the South Gujarat districts, with a focus on the frequency distribution of serovar-specific antibodies as determined by Microscopic agglutination test (MAT).

Materials and methods

This retrospective observational analysis was conducted after obtaining institutional ethical approval [GMCS/STU/RRC-1/Approval/16645/24]. We received 2,845 serum samples from various animals in South Gujarat districts with the consent of their owners, between January 2021 and December 2023. Testing was performed at the leptospirosis laboratory in the Department of Microbiology, Government Medical College Surat, Gujarat, India. The laboratory serves as the state referral center for leptospirosis testing in humans and provides diagnostic services for animal leptospirosis as part of a surveillance program in the South Gujarat districts. The serum samples were received from different animals, including cattle, buffalo, goats, and sheep from the Navsari, Surat, Tapi, and Valsad districts each year.

Serum samples were stored at -20˚C. The samples were tested by MAT for leptospirosis. Doubling dilutions of serum, ranging from1:25 to 1:200, were prepared by adding PBS (Phosphate Buffered Saline) in 96-well flat-bottom microtiter plates. All the 96 wells of the microtiter plate were filled with25 µL of PBS. Then, 23 µL of PBS and 2 µL of serum were added to the wells in column 2 (resulting in a dilution of 1:25). The mixture was mixed, and 25 µL was transferred from one well to the next, discarding the last 25 µL. We then added 25 µL of leptospira cultures to all wells. Column 1 contained only the antigen without the addition of antibody and served as the antigen control. The final dilutions after adding the antigen ranged from 1:50 to 1:400. The mixtures were thoroughly mixed on a micro-shaker and incubated at 37°C for 2 hours.

The serum-antigen mixtures were examined under a dark field microscope for agglutination. To observe, one drop of the mixture was transferred with a pipette from a well to a microscopic slide and examined under a dark field microscope with a 20x objective lens, without a cover slip. The reported titer was calculated as the reciprocal of the highest dilution that agglutinated at least 50% of the cells for each serovar, or a reduction in the number of leptospiral cells compared to the antigen control. A titer of 1:50 or higher was considered significant. The serovars tested were, Pyrogenes, Australis, Autumonalis, Grippotyphosa, Patoc, Pomona, Icterohemorrhagiae, Hebdomadis, Canicola, Hardjo, Ballum, and Batavia. Strains were obtained from the Regional Medical Research Center (WHO collaborating center for diagnosis, research, and training in leptospirosis, ICMR) in Port Blair, Andaman and Nicobar Islands. These serovars were maintained in semisolid EMJH media supplemented with 10% enrichment (Difco, USA) at 30°C in screw-capped test tubes.

Data collection and analysis

Demographic details of the animals were recorded from the data sheets sent by different districts. A master chart sheet with MAT results was created using Microsoft Excel software version 2013. Data were analyzed to determine the seroprevalence of leptospirosis in animals using MAT. OpenEpi software version 3.01 was used to calculate the chi-square test and P-value to analyze the level of significance regarding the presence of leptospires among different animals. The observed data were presented in the form of tables, bar diagrams, and pie charts as percentages.

Confidentiality was maintained throughout the study by avoiding the use of personal details of owners and other identifiers in the formulation of the report.

Results and discussion

Every year, serum samples were received from different animals, primarily cows, buffaloes, goats, and sheep, for surveillance of leptospirosis by MAT. The samples were collected from four major districts in South Gujarat, where the highest number of human leptospirosis cases were observed during the monsoon season. Leptospirosis has been endemic in South Gujarat since 1994 (Shivakumar, 2008). Major areas of the

South Gujarat districts are used for rice and sugarcane cultivation and are rich in natural vegetation, featuring plenty of marshy lands and small water logging areas, with neutral pH, suitable humidity, and temperature essential for the survival of leptospires. The prevalence in animals can be attributed to their habit of wallowing in water bodies contaminated with infected urine, which is one of the main sources of leptospira transmission (Patel *et al*., 2016). Many residents harbor animals at their homes or come into contact with them during the monsoon season. The total seroprevalence rates in animals were 13.52% (102/754) in 2021, 7.31% (73/998) in 2022, and 16.74% (183/1093) in 2023. The rates of MAT positivity observed in various districts of South Gujarat were depicted in Table 1.

The number of samples from each species of livestock tested by MAT, along with the positive results in the four main districts, is shown in Table 2. The highest prevalence was recorded in goats (17.5%), followed by buffalo (14.9%) and cattle (9.3%) in Navsari district. In Surat district, cattle (21.2%) demonstrated the highest levels of agglutinating antibodies, compared to buffalo (15.4%) and goats (10%). The samples from cattle and buffalo were primarily from Tapi district, which showed a prevalence of 9.4% and 12.8%, respectively. Valsad district showed a prevalence of 14.7% for cattle and 11.5% for buffalo. Overall prevalence rates were 12%, 14.1%, and 16.2% among cattle, buffalo, and goats, respectively.

Table 1. Year and district-wise positivity rate observed in Microscopic Agglutination Test

District	2021		2022		2023	
	Total	Positive	Total	Positive	Total	Positive
Navsari	551	77(14%)	555	18 (3.2%)	470	76 (16.2%)
Surat	40	12 (30%)	160	36 (22.5%)	183	28 (15.3%)
Tapi	70	0	160	18 (11.3%)	270	33 (12.2%)
Valsad	133	13 (9.8%)	123	1 (0.8%)	170	46(27.1%)
Total	754	102 (13.5%)	998	73 (7.3%)	1093	183(16.7%)

Table 2. District and species-wise distribution ofsamples andpositivity rate in the Microscopic Agglutination Test from 2021 to 2023

District	Total	Positive	Cattle		Buffalo		Goat		Sheep	
			Total	Positive	Total	Positive	Total	Positive	Total	Positive
Navsari	1536	171 (11.1%)	1054	98 (9.3%)	349	52 (14.9%)	120	21 (17.5%)	13	0
Surat	383	76 (19.8%)	311	66 (21.2%)	52	8 (15.4%)	20	2 (10%)	0	0
Tapi	500	51 (10.2%)	381	36 (9.4%)	117	15 (12.8%)	2	0	0	0
Valsad	426	60 (14.1%)	348	51 (14.7%)	78	9 (11.5%)	0	0	0	0
Total	2845	358 (12.6%)	2094	251 (12%)	596	84 (14.1%)	142	23 (16.2%)	13	0

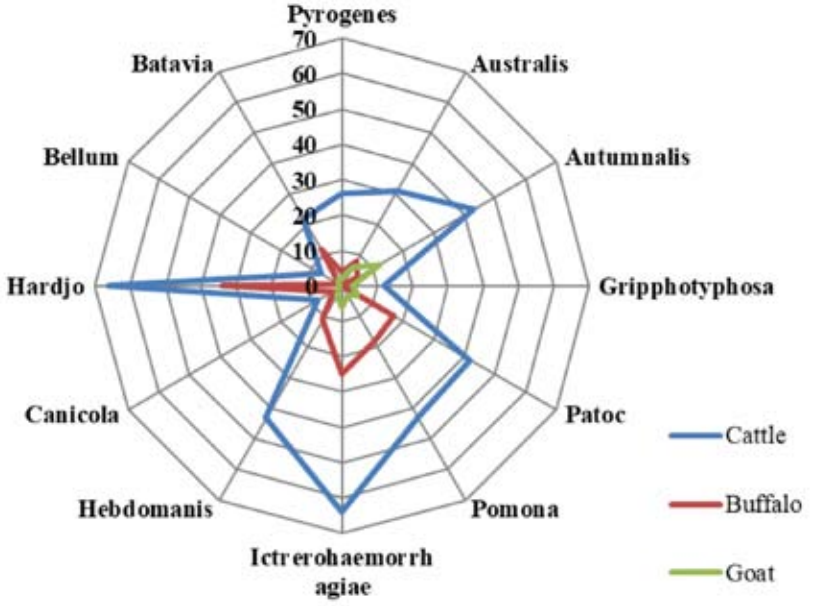

Fig. 1: Frequency distribution of pathogenic serovars in different animals

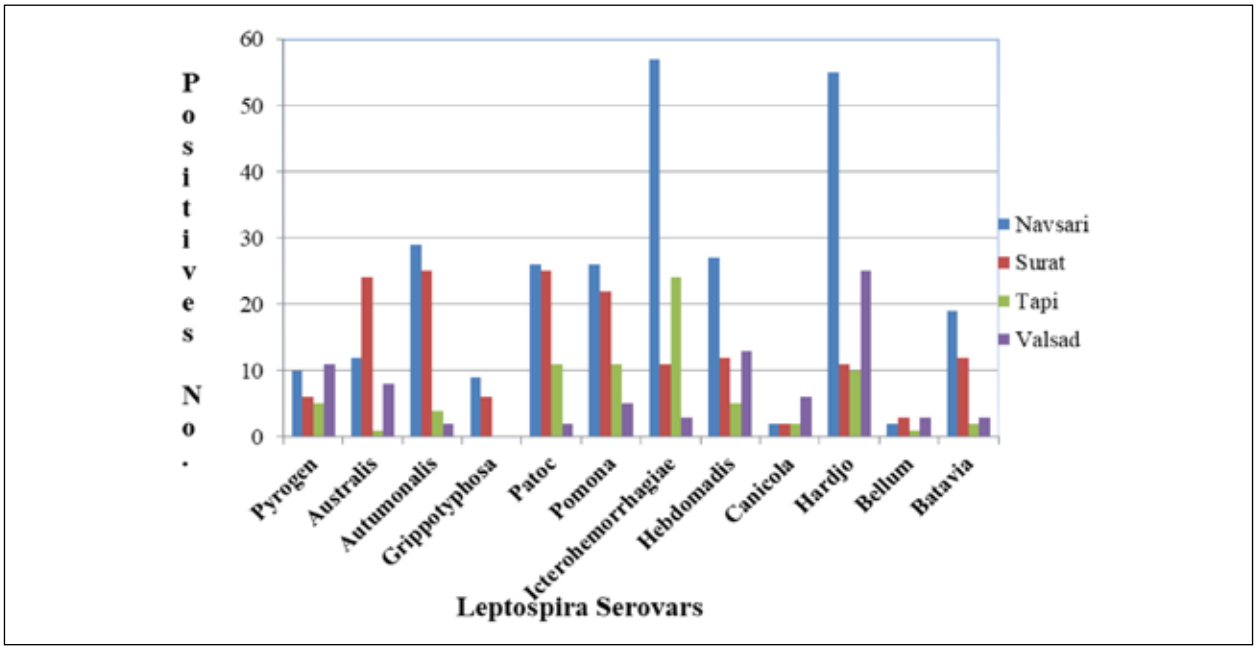

Fig. 2: District wise distribution of reacting leptospira serovars among positive samples

Different animals showed antibodies against various leptospira serovars. The most common serovars in cattle were Hardjo (16.2 %), Icterohaemorrhagiae (15.7%), Autumnalis (10.6%), Hebdomanis (10.6%), Pomona (10.6%), Patoc (10.3%), Australis (7.6%), Pyrogenes (6.4%), and Batavia (5.4%). In buffalo, the common serovars included Hardjo (24.6%), Icterohaemorrhagiae (18.1%), Pomona (13%), Patoc (12.3%), Batavia (8.7%), Hebdomanis (8%), and Australis (5.8%). Goats demonstrated agglutinating antibodies against the serovars Autumnalis (26.6%), Australis (13.3%), Icterohaemorrhagiae (13.3%), Patoc (11.1%), Pomona (6.6%), and Hebdomanis (6.6%) Fig 1.

Out of 2,845 animal serum samples tested, 358 (12.58%) were positive for the presence of agglutinating antibodies against leptospirosis as determined by MAT. Lall *et al*. (2017) observed a seroprevalence of 15.1% in cattle and goat samples from Andaman during 2013-14, while Balamurugan *et al*. (2016) and Lata *et al*. (2019) documented seroprevalence of 41.04% and 59.09% among the bovine population in Konkan, Maharashtra, and Chhattisgarh, respectively. Suwancharoen *et al*. (2013) and Suepaul *et al*. (2011) reported seropositivity rates of 11.1% and 14.5% among the livestock population in Thailand and Trinidad respectively. Although a high burden of the disease is common in coastal regions, dissemination in animals varies widely. Saranya *et al*. (2021) conducted a comparative study on leptospirosis in animal herds from the northeastern regions (NER) of Assam, Meghalaya, and Mizoram, as well as Tamil Nadu, and documented seroprevalence rate of 36.8% and 30.7% respectively.

The serovars Icterohaemorrhagiae (20.8%), Hardjo (20.1%), Autumnalis (10.6%), Hebdomanis (4.6%), Pomona (9.5%), Patoc (9.5%), Batavia (6.9%), and Australis (4.4%) were most common in Navsari district. In Surat, the most prevalent agglutinating serovars were Autumnalis (15.7%), Australis (15.7%), Patoc (15.7%), and Pomona (13.8%). In the Tapi district, the serovars Icterohaemorrhagiae (31.6%), Pomona (14.5%), Patoc (14.5%), Hardjo (13.2%) were observed where as in Valsad, Hardjo (30.9%), Hebdomanis (16%), Pyrogenes (13.6%), and Australis (9.9%) were common. Overall, the most prevalent serovars observed in animals from the south Gujarat districts were Hardjo (17.1%) and Icterohaemorrhagiae (16.1%), followed by Pomona (10.8%), Patoc (10.8%), Autumnalis (10.2%), Hebdomanis (9.7%), Australis (7.6%), Batavia (6.1%), and Pyrogenes (5.4%). Fig 2. In the present study, the seroprevalence of leptospirosis in animal herds in the south Gujarat districtswere also re-estimated. In South Gujarat, Seroprevalence with MAT varied between 29% and 13%. Panwala and Mulla (2015) observed 29% of seroprevalence in 2012-13 with Ballum, Autumnalis, Icterohaemorrhagiae, Hardjo, Pomona and Hebdomadis as predominant serovars in cattle whereas Mistry *et al*. (2022) has reported 11% of seroprevalence with prevalent serovars as Icterohemorrahiae, Hardjo, Patoc, and Pyrogenes in cattle, Patoc and Hardjo in buffalo, Hardjo in bullock, Autumnalis and Australis in goats in 2020. Present study concludes the 12.8% of seroprevalence with Hardjo, Icterohaemorrhagiae, Autumnalis, Hebdomadis and Pomona in cattle

Hardjo, Icterohaemorrhagiae, Pomona, Patoc, Batavia, Hebdomadis and Australis in buffalo, Autumnalis, Australis, Icterohaemorrhagiae and Patoc in goat as dominant serovars in 2021 to 2023. So, overall there is remarkably reduction in seroprevalence by 17%

from 2013 to 2023. This reduction might be attributed to the continuous surveillance and preventive measures implemented vigorously by the state government and municipality. The prevalent serovarsobserved in the study by Panwala and Mulla (2015) has been shifted from Ballum, Autumnalis, Icterohaemorrhagiae, Hardjo to Hardjo, Icterohaemorrhagiae, Autumnalis, Hebdomadis over the decades as noticed in present study Changes in the seropositivity of serovars in animals over the decade might be influenced by a multitude of factors affecting transmission dynamics, including environmental drivers, the density of carrier and reservoir animal populations, antigenic changes in the pathogen, and variations in the proportion of the host population immune to specific serovars of the pathogen (Lall *et al*., 2017).

Conclusion

Leptospirosis is a leading cause of morbidity and mortality in humans and animals. Periodic surveillance for leptospirosis in animals plays a vital role in helping farmers and public health officials to become aware of the disease and devise various control strategies. Surveillance data plays an important role in determining the prevalence of leptospirosis in various species and identifying the panel of serovars which has to include in MAT across different geographical regions, facilitating an accurate diagnosis in both animals and humans.

Conflict of interest

The authors declare that there is no conflicts of interest.

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