



Seroprevalence of *Leptospira* in Cattle and Goats from Andaman and Nicobar Islands, India

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ABSTRACT

Background: Leptospirosis is one of the important zoonotic diseases prevailing in most tropical countries worldwide. The disease is endemic in Andaman and Nicobar Islands, India and affects humans and animals. Due to its zoonotic importance, continuous monitoring and surveillance are important. The present work was conducted to determine the seroprevalence and distribution of different serovars with respect to different ecoregions in cattle and goats.

Methods: A total of 100 samples each from cattle and buffalo were tested by using a microscopic agglutination test (MAT) using a panel of 12 serogroups as antigens. Samples showing a titer of more than 1:40 were considered positive.

Result: Leptospirosis is one of the important zoonotic diseases with high endemicity reported from the A and N islands. The present study reports the prevalence of multiple serovars in cattle and goats, however, there have not many changes in terms of the prevalence of serovars is concerned. Regular monitoring and surveillance of livestock are very much important and necessary to study the disease prevalence scenario in changing climatic conditions in these islands.

Key words: Cattle, Goat, Leptospirosis, Microscopic agglutination test, Seroprevalence.

INTRODUCTION

Andaman and Nicobar Islands (ANI), an archipelago located at the juncture of the Bay of Bengal and the Andaman Sea, are very rich in floral and faunal biodiversity and are the home of several animal genetic resources. Out of the total of 38 inhabited islands, 12 islands have no livestock and another 4 islands have a population of fewer than 200 numbers (Kundu *et al.*, 2010). Very few diseases like foot and mouth disease, contagious ecthyma, Brucellosis, leptospirosis, swine fever, etc. have been reported from the livestock of these islands (Sunder, 2014). Leptospirosis is a very common disease that occurs in humans and in animals from these islands. The first case of leptospirosis was reported from these islands by Taylor and Goyle in 1929 (Taylor and Goyle, 1931). Outbreaks and seroprevalence studies of leptospirosis have been reported by many workers in India (Srivastava *et al.*, 1983; Ratnam, 1994; Sehgal, 2000; Vijayachari *et al.*, 2008). As per the previous studies conducted at ANI, it is found that the prevalence of leptospirosis in livestock ranges from 0.9 to 15.7% (Sunder *et al.*, 2018; Jeyakumar *et al.*, 2002; Sharma *et al.*, 2003; Mitra *et al.*, 2015).

Leptospirosis is considered one of the neglected zoonotic diseases mostly reported by urban slums and rural subsistence farmers (Schafbauer *et al.*, 2019). Globally, approximately 1.03 million cases and 58,900 deaths are occurring due to leptospirosis (Polo *et al.*, 2019). The genus *Leptospira* consists of 66 species, with more than 300 serovars arranged in 30 serogroups (OIE Terrestrial Manual, 2021). The disease is of considerable economic importance in livestock due to manifestations like abortion, infertility and decreased production (Faine, 1994).

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In ANI, the common serogroup reported are *L. hebdomadis*, *L. icterohaemorrhagiae*, *L. australis*, *L. grippotyphosa*, *L. Pomona*, *L. hardjo*, *L. canicola*, *L. pyrogenes*, *L. autumnalis*, etc. (Sunder *et al.*, 2018). For diagnosis of leptospirosis, the microscopic agglutination test is considered the gold standard test (Wolff, 2013; Venkataraman, 1992). Criteria for presumptive diagnosis of leptospirosis is a single MAT test with a positive titer and IgM ELISA or immune chromatography test (ICT) is conducted. However, for confirmatory diagnosis, a PCR test should be conducted to rule out the negative MAT result.

Cattle acts as a reservoir host of the *Leptospira* which is the main natural reservoir of the organism and transmits the infection to humans through contaminated urine.

The *leptospira* transmits through contaminated urine by rats (*Rattus norvegicus*). The other source of transmission is through aborted fetuses or vaginal discharge of animals (Ellis, 2015). Reports of seroprevalence in the livestock show a high prevalence of leptospirosis in cattle (Sunder *et al.*, 2018; Sharma *et al.*, 2003; Mitra *et al.*, 2015). Continuous studies and monitoring of leptospirosis in livestock are very much important to detect the carrier status of animals. Therefore, the present study was conducted with the main aim to assess the seroprevalence and carrier status in cattle and goats of the ANI.

MATERIALS AND METHODS

Sampling plan and history

Samples were collected from the different ecoregions (tehsils) of ANI where the livestock concentration is more. Tehsils viz. Port Blair (68), Ferrargunj (42) from the South Andaman district and Rangat (45), Mayabunder (15) and Diglipur (30) from North and Middle Andaman districts were selected for the collection of samples. A total of 200 samples (100 cattle and 100 goats) were collected from January to March 2022. Blood samples were collected from cattle aged between 6 months to 2 years, of which 68 were female and 32 were male. In goat, all the samples were from adult goats. Serum was separated from the clotted blood and stored at -20°C for further use.

During sampling, the geographical coordinates of surveyed locations were collected using Global Positioning System (GPS) navigator (Garmin eTrex® Touch 25). The sampled data from different locations was analysed for total percentage of *leptospira* prevalence and different Serovar types in cattle and goat. The prepared data for total percentage of *leptospira* prevalence and serovar types in cattle and goat along with the corresponding geographical coordinates (latitude and longitude) was loaded into Microsoft Excel Comma Separated Values (CSV) file. This CSV file was added into ArcGIS 10.8.1 layer along with the shape file of Andaman and Nicobar Islands, which is accessed from <https://www.diva-gis.org/>.

Microscopic agglutination test

The samples were screened for the presence of anti-leptospiral antibodies by microscopic agglutination test (MAT) at the WHO Reference Laboratory for *Leptospira*, ICMR, Port Blair as per the standard method (Sharma *et al.*, 2003). The common *leptospira* prevalent in these islands were used as a panel for MAT. A total of 12 serogroups were used as a reference for MAT (Table 1). They are: Bankinang, Australis, Ballum, Canicola, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Lai Like, Pyrogenes, Pomona and Hardjo (Table 1). MAT was performed at doubling dilution starting from 1:20. Criteria for positive samples were considered as 1:40 or more. A titer of 1:40 is taken as the cut-off point because it is the closest dilution to the usual cut-off of 50 used in the seroepidemiological survey (Everard *et al.*, 1985). The data were collected and analyzed as per the standard statistical methods.

Statistical analysis

Seroprevalence was estimated with 95% confidence intervals (CI) using the Binomial (Clopper-Pearson) "exact" method based on the beta distribution (Brown, 2001). The differences in the characteristics of MAT-reactive animals (seropositive and seronegative) between cattle and goats were analyzed. In cattle variation for sex (male, female), age (calf, adult), tehsil wise (Port Blair, Ferrargunj, Rangat, Mayabunder, Diglipur, Nancowry) were assessed. In goat tehsil wise (Port Blair, Ferrargunj, Rangat) variations were assessed. All the seroprevalence variations between the categories were assessed using binomial logistic regression using Wald Chi-Square statistic for statistical significance ($p < 0.05$).

RESULTS AND DISCUSSION

Tehsil wise *Leptospira* seroprevalence in cattle and goat of ANI is spatially analysed to prepare maps (Fig 1 and 2). The result of seroprevalence among the cattle and goat species were similar. However, the percentage of positive samples was found to be more in goats compared to cattle. In cattle, no significant difference could be found between

Table 1: Panel of *Leptospira* serovars used in MAT.

Serogroup	Serovar	Strain	Genomospecies
Autumnalis	Bangkinag	Bangkinang 1	<i>Leptospira interrogans</i>
Australis	Australis	Ballico	<i>Leptospira interrogans</i>
Ballum	Ballum	Mus 127	<i>Leptospira borgpetersenii</i>
Canicola	Canicola	HondUtrecht IV	<i>Leptospira interrogans</i>
Grippotyphosa	Grippotyphosa	CH-31	<i>Leptospira interrogans</i>
Grippotyphosa	Grippotyphosa	Moskva V	<i>Leptospira interrogans</i>
Hebdomadis	Hebdomadis	Hebdomadis	<i>Leptospira interrogans</i>
Icterohaemorrhagiae	Icterohaemorrhagiae	RGA	<i>Leptospira interrogans</i>
Icterohaemorrhagiae	Lai Like	AF 61	<i>Leptospira interrogans</i>
Pyrogenes	Pyrogenes	Salinem	<i>Leptospira interrogans</i>
Pomona	Pomona	Pomona	<i>Leptospira interrogans</i>
Sejroe	Hardjo	Hardjoprajitno	<i>Leptospira interrogans</i>

age, sex and region in the seroprevalence of *Leptospira* or for any of the serovars. In the case of goats, overall seroprevalence across the regions was similar while the seroprevalence of serovars Icterohaemorrhagiae AF61,

Icterohaemorrhagiae GFA were significantly ($p < 0.05$) high in Rangat Tehsil of North and Middle Andaman district. Seroprevalence of Grippityphosa CH31 was significantly high ($p < 0.08$) in the Ferrargunj Tehsil of the Andaman and

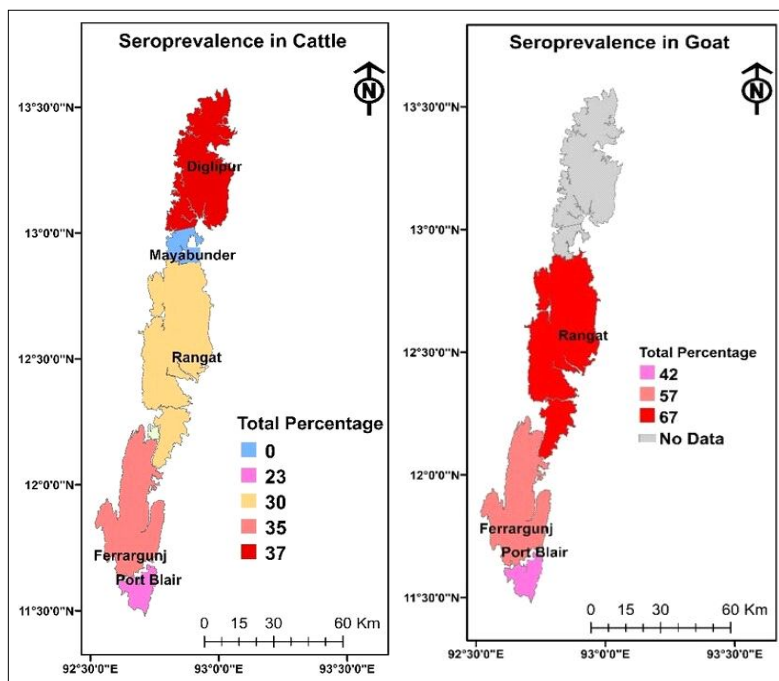


Fig 1: Map of South Andaman showing Tehsil wise prevalence of *Leptospira* in cattle and goat.

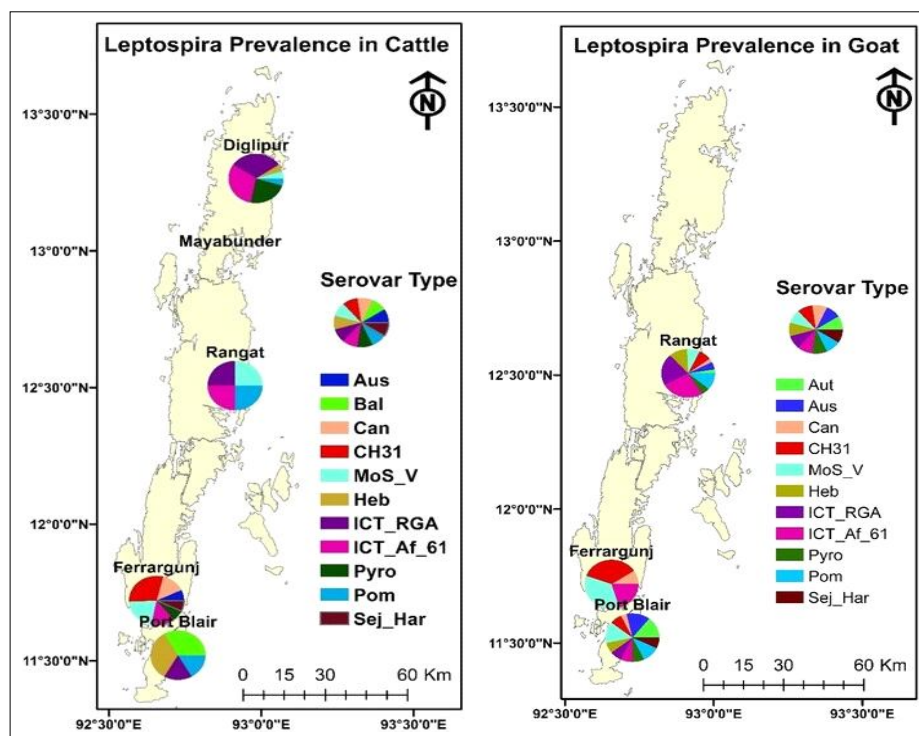


Fig 2: Map of South Andaman showing prevalence high to low concentration of seroprevalence of different serovars of *Leptospira* in cattle and goat.

Nicobar Islands. A total of 62 sera samples showed more than 1:40 titer (CI: 24.7-37.9%) in both cattle and goats (Table 2). However, the goat sera samples showed the highest seroprevalence compared to cattle. The MAT titer ranged from 1:40 to >1:320, with geometric mean titer ranging from 1:40 (Hardjo) to 1:240 (Hebdomadis) (Fig 3 and 4). In the case of cattle, the frequency of percent positive

was found to be highest against *Icterohaemorrhagiae* (19.05%) followed by *Pyrogenes* (11.9%), *Grippotyphosa* (11.9%), *Hebdomadis* (7.14%) and *Pomona* (7.4%). Other serovars showed less than a 5% prevalence rate. In goats, the highest frequency of seroprevalence was found against *Icterohaemorrhagiae* (16.67%), followed by *Grippotyphosa* (13.89%), *Pomona* (9.72%), *Australis* (8.33%), *Hebdomadis*

Table 2: Seroprevalence of leptospirosis in cattle and goat.

Serovar	Seropositive (N)	Seroprevalence (%)	Confidence interval (%)
Bangkinang	5	2.5	(0.8-5.7)
Australis	7	3.5	(1.4-7.1)
Ballum	2	1	(0.1-3.6)
Canicola	5	2.5	(0.8-5.7)
Grippotyphosa (CH31)	13	6.5	(3.5-10.87)
Grippotyphosa (MoSV)	15	7.5	(4.3-12.1)
Hebdomadis	9	4.5	(2.1-8.4)
<i>Icterohaemorrhagiae</i> (RGA)	16	8	(4.6-12.7)
Lai like	20	10	(6.2-15.0)
<i>Pyrogenes</i>	9	4.5	(2.1-8.4)
<i>Pomona</i>	10	5	(2.4-9)
Hardjo	3	1.5	(0.3-4.3)
Overall	62	31	(24.7-37.9)

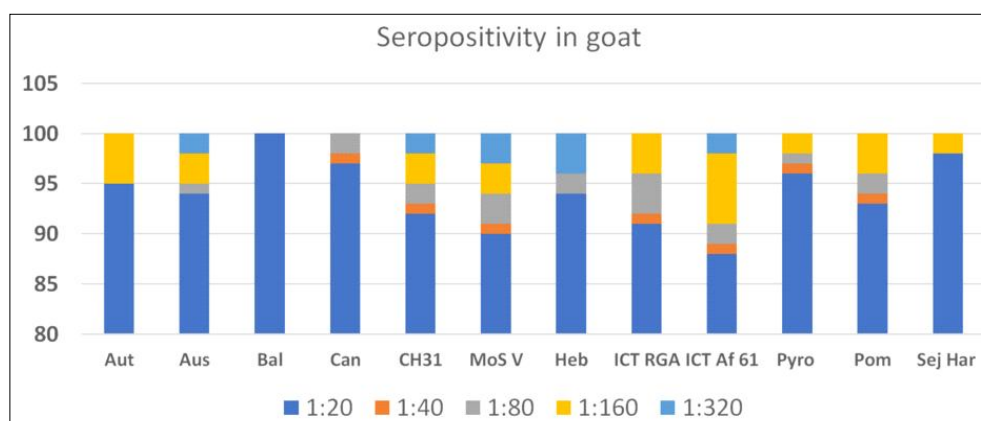


Fig 3: Seropositive samples in goats with titre ranging from 1:40 to 1:320.

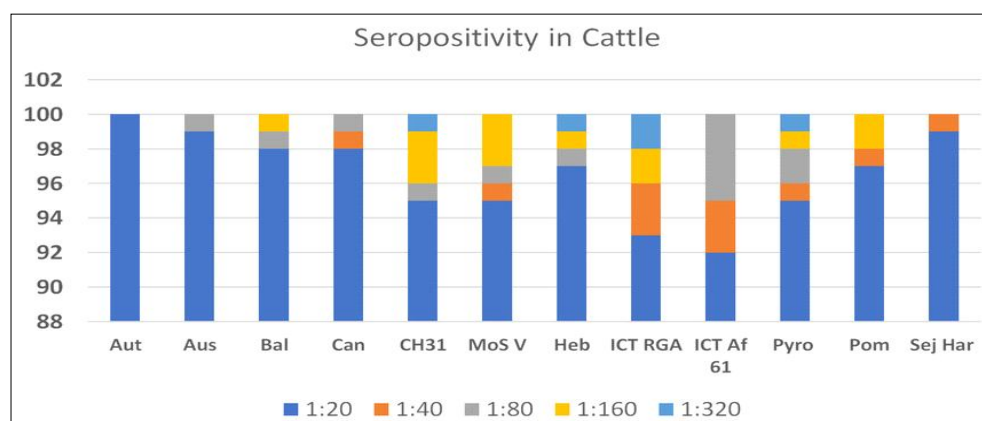


Fig 4: Seropositive samples in cattle with titre ranging from 1:40 to 1:320.

(8.33%), Autumnalis (6.94%) and Pyrogenes (5.56%) (Table 3). In cattle, none of the samples showed positive against Autumnalis and Australis, while Ballum showed less than 5% prevalence. In goats, none of the samples showed positive against serovar Ballum. The most reactive serovars were Lai-like (6.2% to 15%), followed by Icterohaemorrhagiae (4.6% to 12.7%), Grippytyphosa Moskva V (4.3% to 12.1%), Grippytyphosa CH-31 (3.5% to 10.87%), Pomona (2.4% to 9.0 %), Pyrogenes (2.1% to 8.4%), Hebdomadis (2.1% to 8.4%), Australis (1.4% to 7.1%), Canicola (0.8% to 5.7%), Bangkinag (0.8% to 5.7%), Hardjo (0.3% to 4.3%) and Ballum (0.1% to 3.6%) in both cattle and goat.

Samples having multiple reactions with more than 2 serovars were more in goats (57) than cattle (25) with overall multiple seroreactivity of 71.92% (Table 4). The highest seroreactivity was found in one sample in goat from Rangat tehsil which showed multiple reactivities with 8 serovars. The serovars which showed maximum reactions were Icterohaemorrhagiae Lai-like (20), Grippytyphosa (16) and Icterohaemorrhagiae RGA (15). Multiple reactivities were observed to be more in goats compared to cattle. Multiple reactivities to more than 3 serovars were detected in 15.79% of the goat samples.

The study was conducted with the main objective to evaluate the prevalence rate of leptospirosis in cattle and goats in ANI. As representative samples, a total of 100 samples each from cattle and goats were screened for the presence of leptospiral antibodies against 12 different serovars. The result indicates that there is a moderately high

seroprevalence (31%) of leptospirosis with single or multiple serovars. Leptospirosis is highly endemic in ANI with reports of a high sero-prevalance rate as well as isolation of the causative organisms (Sehgal, 2000; Sunder *et al.*, 2018; Sharma *et al.*, 2003; Mitra *et al.*, 2015). Livestock, mainly cattle and goats plays an important role in the epidemiology of the disease in ANI. The seroprevalence in both species (cattle-28%, goat -34%) was found to be very high. The goat population is highest in these islands and is distributed to all three districts (Sunder *et al.*, 2018). However, the cattle population is mainly concentrated in the South and North and Middle Andaman districts of these islands. Cases of *Leptospira* have been reported mainly from the North and Middle Andaman followed by South Andaman districts (Vijayachari *et al.*, 2003; Sharma *et al.*, 2006). No significant differences were noted in terms of age, sex and region in the case of cattle. Spatial distribution indicated that there was significantly high seroprevalence recorded in the ecoregions of Ferrargunj and Rangat Tehsils. The high seroprevalence in cattle and goats from these regions might be due to several factors such as the high concentration of cattle and goat population, agricultural activities, common grazing area and exposure to rodents. Rat plays a significant role in the transmission of the disease in animals and humans. The carrier animals transmit the organisms through the contaminated urine to the other animals. Earlier reports also support that there is seroprevalence of leptospirosis in rats (Sharma *et al.*, 2003). The topography of the ANI is undulating and due to high rainfall, there is stagnation of water after the monsoon. The favorable climatic conditions facilitate the survival of leptospires and act as a source of infection to humans as well as to animals. All these factors along with weather attributed to the high sero-prevalance of leptospirosis in the ANI.

The most common positive serovars in both cattle and goats were found to be Icterohaemorrhagiae Lai like (17.54%), Grippytyphosa (14.03%) and Icterohaemorrhagiae RGA (13.15%). The results are in agreement with the previous studies, in which the most common serovars were found to be Grippytyphosa (Sharma *et al.*, 2003), Autumnalis, Hardjo (Mitra *et al.*, 2015), Grippytyphosa, Icterohaemorrhagiae, Hebdomadis (Sunder *et al.*, 2018). In all the previous studies, the common serovar was found to be Icterohaemorrhagiae and Grippytyphosa. Serogroup Grippytyphosa is considered one of the most common serogroups infecting human beings. The high seroprevalence of Grippytyphosa from cattle and goat in the

Table 3: Frequency distribution (%) of different serovars in cattle and goat.

Serogroup	Cattle	Goat
Autumnalis	6.94	0
Australis	8.33	2.38
Ballum	0	4.76
Canicola	4.17	4.76
Grippytyphosa (CH 31)	11.11	11.9
Grippytyphosa (Mos V)	13.89	11.9
Hebdomadis	8.33	7.14
Icterohaemorrhagiae (RGA)	12.5	16.67
Icterohaemorrhagiae (AF 61)	16.67	19.05
Pyrogenes	5.56	11.9
Pomona	9.72	7.14
Sejroe	2.78	2.38

Table 4: Samples showing multiple reactivities.

Positive serovars (n)	No samples showing multiple reactivities (n)		Overall n (%)	Serovars with a maximum reaction
	Cattle	Goat		
1	17	15	32 (28.07)	Icterhaemorrhagiae Lai like (20),
2	16	18	34 (29.82)	Grippytyphosa (16), Icterhaemorrhagiae
3	9	21	30 (26.32)	RGA (15)
>3	0	18	18 (15.79)	

present studies is alarming as the livestock acts as a carrier of *Leptospira* and sheds organism and contaminate the environment (Jeyakumar *et al.*, 2002; Sunder *et al.*, 2005). Sharma and co-workers also reported high sero-prevalance of leptospirosis in cattle (34%) and goats (29%) (Sharma *et al.*, 2006). They also reported that the most common serovars were Icterohaemorrhagiae, Hebdomadis and Grippotyphosa from the cattle. In the present study also, a high seroprevalence rate of leptospira in cattle and goats was reported. In our studies also, similar results were obtained as the seroprevalence in cattle was 28% and the goat was 34% respectively. This suggests that there have not many changes in the serovars affecting the livestock.

Environment plays a major role in the epidemiology of leptospiral infection. The hot, humid and high rainfall conditions of the ANI highly favours the multiplication of *Leptospira* in this region. Previous studies from these islands as well as from different parts of the India and world suggest that the disease is mainly prevalent in the island and coastal regions (Schafbauer *et al.*, 2019; Dhivahar *et al.*, 2019). The seasonal outbreak of leptospirosis is usually reported from the coastal areas of Tamil Nadu, Kerala, Gujarat, Maharastra and ANI (Karande and Thakarae, 2002; Sehgal *et al.*, 1995; Arumugam *et al.*, 2011). Generally, the antibodies remain in the blood for a longer period after the systematic infection. The detection of antibodies did not mean that the animals were infected or were recently infected. However, these animals may act as reservoir host or carrier host and usually transmit the leptospires to other healthy animals. In the present study, more than 31% of the surveyed animals were found to be exposed to leptospires. The animals affected with leptospires continue to excrete bacteria through the urine for up to several months (Sharma *et al.*, 2003). The high sero-prevalance is an indicator that the *Leptospira* are in circulation in cattle and goats and there is always a high risk of contracting the infection from animals to the human being. Therefore, continuous and routine examination of the animals is very much important to develop suitable preventive measures to contain and control leptospirosis in both humans and animals.

The present study reports the prevalence of multiple serovars in cattle and goats, as 20.97% of the animals showed the presence of more than 3 serovars. Our findings are in accordance with the earlier report (Sharma *et al.*, 2003), wherein, 15.13% of the total tested samples showed multiple reactivities to more than 2 serovars. They also reported the common serovars as Grippotyphosa, Icterohaemorrhagiae, Pomona and Australis. In another study, similar observation of multiple reactivity in 36.5% of the samples were reported (Schafbauer *et al.*, 2019). Cross-reactivity in *Leptospira* is very common since many serovars share a common antigen and there is a high probability of cross-reaction. The distribution of similar serovars in both cattle and goats indicates that the same strains are circulating within the same ecoregions. The epidemiology of leptospirosis is very complex, as the animal/human will show multiple infections with different serovars. In our

present study, goat samples showed more cross-reactivity compared to cattle. This might be due to the recent infection and production of IgM antibodies. Reports suggest that IgM antibodies contributed to a certain degree of cross-reactivity (Schafbauer *et al.*, 2019). Sometimes, after the infection, there is a high rise of IgM antibodies which may persist for a longer period in the blood. Due to this, the MAT titer might be more, however, without exhibiting any clinical symptoms. The high titer does not necessarily indicates infection, however, the animals might have been exposed to leptospires (Blanco *et al.*, 2016). Mixed infections with different serovars were also reported by many researchers (Sunder *et al.*, 2018; Tripathy *et al.*, 1985; Balakrishnan, 2012; Vihol *et al.*, 2016). They have reported multiple infections in goats with serovars viz., Australis, Hardjo, Hebdomadis, Icterohaemorrhagiae and Pomona.

Out of the total positive samples, the distribution of leptospira with a titer of 1:160 was more (41.23%), followed by the titer of 1:80 (28.07%), 1:40 (15.79%) and 1:320 (14.91%) respectively. In the previous study conducted on these islands, the highest titer was recorded as 640 from the Andaman Islands and 160 from the Nicobar Islands (Sharma *et al.*, 2003). However, in the present study, the samples showing a titer of more than 1:320 was found to be more. Vaccination against leptospirosis in animals in these islands is not practiced. The high titer of more than 1:320 indicates a recent infection. As per the OIE Terrestrial Manual 2021, a titer of 1:100 is taken as positive, however, because of the high specificity of the test, we have considered 1:40 as the positive titer with evidence of previous exposure. In our study, the percentage of positives in 1:160 and 1:320 was more in goats (63.89%) compared to cattle (42.86%). Most of the MAT titers were low (1:40), which might be due to exposure to earlier infection or circulation of antibodies in the sera without showing any clinical manifestation of the disease. Serotypes that showed a high titer of more than 1:320 were Grippotyphosa, Hebdomadis, Icterohaemorrhagiae and Pyrogenes. The high titer could be due to reinfection with leptospira and the continuous production of antibodies in the sera. On the island, vaccination is not practiced, so the question of high antibodies due to the vaccinal antibody response is ruled out. As MAT is generally very useful in the diagnosis of acute leptospirosis infection in animals. Animals with a titer of 1:40 (15.79%) are considered low titers or low states of infection. The animals with low titer generally act as a carrier and are the source of infection to other healthy animals. In the present study, none of the animals exhibited any clinical manifestation of the typical leptospira infection. Usually, the carrier animals are regarded as apparently healthy animals without showing any clinical symptoms. Serological test, especially the microscopic agglutination test is considered the gold standard test for the diagnosis of leptospirosis by OIE. Local strains which are prevalent in this island have been used in this study. The use of local strains may have increased the sensitivity of the test. The specificity of the test is also good, as the leptospira usually does not cross-react with other bacteria. However, there is

cross-reactivity among the different serovars. Therefore, in our study, multiple reactivities with more than two serovars are reported in goat and cattle samples. As per the reports, only a few serovars will be endemic in a particular zone or region and each serovar tends to be maintained in a specific maintenance host. *L. hardjo* infection is mainly reported and maintained in cattle hosts. In ANI, the common serovars which are reported from this regions are *Icterohaemorrhagiae*, *Pomona*, *Grippityphosa*, *Hebdomadis* and *Hardjo* (Vijayachari *et al.*, 2003; Sharma *et al.*, 2006). Serogroup *Grippityphosa* has been reported as the commonest infecting *leptospira* in ANI.

Surveillance and monitoring are considered important strategies for the prevention and control of any diseases. In ANI, leptospirosis is endemic and for many decades the disease has been reported in animals and humans. Cases of leptospirosis have been confirmed through clinical symptom, isolation, identification, molecular diagnosis, serology, MAT, etc. MAT is still used as the standard test for the diagnosis of *leptospira* infection. However, a single MAT titer does not necessarily confirm the current clinical infection as past infection against the circulating serovars could develop microscopic agglutinating antibodies which are long-lasting (several years). A MAT titer of 200/400 would provide the presumptive diagnosis of leptospirosis with compatibility of clinical signs and symptoms. However, negative MAT results <1 in 20 do not necessarily rule out the disease as microscopic agglutinating antibodies usually appear in the blood at a detectable level during the end of the first week or early second week of the infection. Therefore, MAT negative samples need to be tested using RT-PCR to confirm or rule out the disease. Serogroups *Grippityphosa* and *Icterohaemorrhagiae* have been reported as the commonest serogroup in cattle and goats. An earlier study also supports the findings (Sharma *et al.*, 2003; Raj *et al.*, 2018). The same serogroup is also responsible for leptospirosis in humans. Considering the potential to infect multiple species, the one health approach would be taken as the topmost priority in routine surveillance, monitoring and thereby appropriate action plan to contain and control the infection.

CONCLUSION

Leptospirosis is one of the important zoonotic diseases with high endemicity reported from the ANI. Regular monitoring and surveillance of livestock are very much important and necessary to study the disease prevalence scenario in changing climatic conditions in these islands. However, the present study indicated that there have not many changes as far as the prevalence of serovars is concerned. As the serovars affecting both humans as well as livestock are more or less the same. The findings indicate that leptospires are exchanged regularly among humans, cattle and buffalo. Strict biosecurity measures, including good livestock practices, regular screening and treatment are the

suggestive measures for control of leptospirosis from these islands.

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Conflict of interest

All authors declared that there is no conflict of interest.

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