Int.J.Curr.Microbiol.App.Sci (2017) *6*(3): 1599-1608

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 3 (2017) pp. 1599-1608**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.603.184

Seroepidemiology of Caprine Leptospirosis in South Gujarat Region of India

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Keywords

Goats, Leptospirosis, Microscopic Agglutination test, Seroprevalence.

Article Info

Accepted:
22 February 2017
Available Online:
10 March 2017

A B S T R A C T

A study was conducted to assess the prevalence of leptospirosis and prevalent serovars among goats in south Gujarat, India. A total of 459 serum samples were collected randomly from goats of different age groups and breeds of either sex. Goats were sampled from Navsari, Surat, Tapi, Valsad districts of south Gujarat. Sera were screened by Microscopic Agglutination Test (MAT) using seventeen antigens representing *Leptospira* sp. serovars. The result showed 25% (116/459) seropositivity among goats. The most common serovars in goats included serovars Pyrogenes, Tarassovi, Bankinang, Icterohaemorrhagiae, Shermani, Patoc1 and Pomona. Higher prevalence was noted in Valsad district (35.86 %) followed by Navsari (18.52 %), Tapi (7.89 %) and seroprevalence in these districts was dependent. Seropositivity was found to be 26.27% in females/does and 20.93% in male/bucks. Non-Descript breed showed higher seropositivity than Surti and Barberi breeds which are recognized breeds. Goats of 1-3 years age (26.76%) were more susceptible to leptospires than goats of above 3 years of age (25.19%) and below 1 year age (15.91%). However rate of seroprevalence was not dependent on age, breed and/or sex of goats. The high prevalence of antileptospiral antibodies among goats could constitute a threat to human and animals in areas of south Gujarat besides causing economic losses. Furthermore, this also warrants sero-surveillance in other species of domestic animals in this region to know exact epidemiology of disease and to combat public health risk.

Introduction

Leptospirosis, zoonotic and re-emerging disease with worldwide distribution, is caused by pathogenic spirochetes (*i.e. Leptospira interrogans*). It occurs both in tropical and

subtropical regions of the world (Levett 2001). Infection in humans or animals results from direct contact with infected urine or tissue from infected animals or through contact with water or soil previously contaminated by urine (Adler and de la Peña Moctezuma, 2010). The pathogenic *Leptospira* infects wide range of domestic animals and clinical manifestations of leptospirosis vary from acute to subacute or/and chronic infection in animals.

Among goats, in acute form of leptospirosis sick animals may exhibit pyrexia, depression, jaundice, anorexia and anemic or hemorrhagic syndromes. However subclinical and/or chronic forms are common in goats where in most infected animals show impaired fertility, abortion, stillbirth, and decreased milk production which results in heavy economic losses (Faine *et al.,* 1999; Lilenbaum *et al.,* 2008). Once infected, the animal shed leptospires in the environment and acts as continuous source of infection to other animals and humans (Monahan *et al.,* 2008).

In India, outbreaks of human leptospirosis have been reported from coastal Gujarat, Maharashtra, Kerala, Tamil Nadu, Andhra Pradesh, Karnataka and Andaman periodically (Vijayachari *et al.,* 2008). In south Gujarat leptospirosis is endemic and human outbreaks have been reported since past decade. Also among animals like buffaloes, cattle, goats and sheep seropositivity have been reported in past (Oza *et al.,* 1998; Srivastava and Kumar 2003; Savaliya and Pal 2008, Patel *et al.,* 2014). Looking to the zoonotic potential of disease, a basic knowledge on distribution of serovars and their maintenance hosts is necessary to understand the epidemiology of leptospirosis in a region.

Definitive diagnosis of leptospirosis relies on the detection of anti-leptospiral antibodies in serum (Radostits *et al.,* 2000), detection of leptospires in urine or detection of leptospiral DNA in blood/urine/tissue samples because cases of leptospirosis in domestic animals like goats usually go unnoticed due to the expression of nonspecific symptoms. The serological testing using Microscopic Agglutination Test (MAT), the "gold standard" test (Rajeev *et al.,* 2010) has been used since long back for diagnosing leptospirosis or to determine prevalent serovars though the assay is not suitable for routine laboratories because it requires the maintenance of live, hazardous cultures of different strains of organism (Thiermann, 1984; Dassanayake *et al.,* 2009) which is time consuming and difficult as well as costly task.

The present study was planned to know the seroprevalence and frequency distribution of various leptospiral serovars among goats in south Gujarat with aim to understand epidemiology of leptospirosis in region.

Materials and Methods

Study area

The South Gujarat region lies in southern part of Gujarat including costline and adjoining areas of different districts. The latitude and longitude of south Gujarat is 21.17 N and 72.83 E. In this tropical region, climate is humid with average rainfall 1793 mm.

Collection of samples and study design

In present study, samples were collected from goats. A total of 459 blood samples (Male/Buck-86, Female/Doe-373) were collected randomly from goats of different breeds (Surti-292, Barberi-7 and Non-Descript-160) during 2011-12 from different districts (Navsari-108, Valsad-292, Vapi-76, Surat-24). Purposive sampling was done from different farms in villages of these districts

where cases of human leptospirosis were reported in past. Data related to age/breed/sex were collected during field visit at the time of sample collection by providing questionnaires to the animal owners and grouped accordingly. For analysis, goats divided into three age groups *i.e.* below 1 year, 1-3 years and above 3 years. It should be noted that vaccination programme against leptospirosis in goats is not practiced in Gujarat. In the field, by jugular venipuncture, five ml blood was collected in serum vaccutainers from each animal. Samples were brought to the Pathology Laboratory, centrifuged at 1800×g for 10 min. to harvest clear sera and stored at -20° C until further use.

All the sera were tested for antibodies against live antigens of *Leptospira* sp. serovars Pyrogenes, Australis, Bankinang, Grippotyphosa, Patoc1, Pomona, Icterohaemorrhagiae, Hebdomadis, Canicola, Hardjo, Bellum, Bataviae, Tarassovi, Shermani, Kaup, Hurstbridge and Javanica by Microscopic Agglutination Test at Leptospira Reference Laboratory, Government Medical College, Surat using standard procedure (Vijayachari *et al.*, 2001) and/or National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Indian Council of Agricultural Research, Hebbal, Bengaluru using standard procedure (Faine 1982; WHO 2013). Results were considered positive when 50% or more agglutination of leptospires at titre of 40 or more to any of the serovars was observed. A titre of 40 was used as the cut off because it was closest dilution to the usual cut off 50 used in seroepidemilogical surveys (Everard *et al.*, 1989). Leptospira species included in the antigen panel are listed in table 1.

Statistical analysis

For statistical analysis, Chi-square test was used and considered to be significant at <0.05. Data were analysed using Web Argi Stat Package (WASP) software developed by Jangam and Wadekar, Indian Council of Agricultural Research (ICAR), Goa, India (Jangam and Wadekar, 2012).

Results and Discussion

In this study a total of 116 out of 459 (25.27%) sera reacted against one or more leptospiral serovars in MAT. Among the seropositive goats, the highest seropositivity was recorded against serovar Pyrogenes (31.66 %) followed by Tarassovi (11.56 %), Bankinang/Autumnalis (11.06 %), Icterohaemorrhagiae (8.04 %), Shermani (8.04 %), Patoc1 (7.04 %), Pomona (5.53 %), Australis (4.52 %), Hardjo (4.02 %), Canicola (3.02 %), Grippotyphosa (1.51 %), Hebdomadis (1.51 %), Kaup (1.01 %), Bellum (1.01 %) and Bataviae (0.50 %) (Table 2).

The test results of district wise, breeed wise and age wise seroprevalence are shown in table 3. The sera were tested from four different districts viz., Navsari, Valsad, Tapi and Surat of South Gujarat and highest seroprevalence was recorded in Valsad (35.86 %) followed by Navsari (18.52 %), Tapi (7.89 %) and Surat (0.0 %). The difference was statistically highly significant (P<0.01). Higher seroprevalence rate was recorded in Non-Descript (29.38 %) breed followed by Surti (23.29 %) and Barberi (14.29 %) breeds (Table 3). Breedwise seroprevalence did not differ significantly (P < 0.05). Seropositivity was noted to be higher in females (26.27 %, 98/373) than in males (20.93 %, 18/96) but the findings did not differ significantly. In respect of age groups, 7 goats (15.91 %) in age group below 1 year age, 76 goats (26.76 %) in 1-3 years age group and 33 goats (25.19 %) in above 3 years age group were positive for antileptospiral antibodies but the seroprevalence was not associated with age as these findings were statistically no significant.

Table.1 Panel of *Leptospira* serogroups and serovars used in MAT

Sero groups	Serovars
Pyrogenes	Pyrogenes
Australis	Australis
Autumnalis	Bankinang
Grippotyphosa	Grippotyphosa
Semeranga	Patoc1
Pomona	Pomona
Icterohaemorrhagiae	Icterohaemorrhagiae
Hebdomadis	Hebdomadis
Canicola	Canicola
Sejroe	Hardjo
Bellum	Bellum
Bataviae	Bataviae
Tarassovi	Tarassovi
Shermani	Shermani
Tarassovi	Kaup
Hurstbridge	Hurstbridge
Javanica	Javanica

Table.2 Details of serovar reactivity in MAT

Serovars	No. of samples reacted	Per cent Positive
Pyrogenes	63	31.66 %
Tarassovi	23	11.56 %
Bankinang/ Autumnalis	22	11.06 %
Icterohaemorrhagiae	16	8.04 %
Shermani	16	8.04 %
Patoc 1	14	7.04 %
Pomona	11	5.53 %
Australis	9	4.52 %
Hardjo	8	4.02 %
Canicola	6	3.02 %
Grippotyphosa	3	1.51 %
Hebdomadis	3	1.51 %
Bellum	2	1.01 %
Kaup	2	1.01 %
Bataviae	1	0.50 %
Hurstbridge	0	0.00 %
Javanica	0	0.00 %
Total	**199/459**	

Table.3 Details of analysed results of samples screened for leptospirosis in goats

Attributes	Number Tested	Number Positive	Per cent Positive
South Gujarat	459	116	25.27
Districts			
Navsari	108	20	18.52
Valsad	251	90	35.86
Tapi	76	6	7.89
Surat	24	0	0
Total	459	116	25.27
			$\chi^2 = 37.766$**
Breedwise			
Surti	292	68	23.29
Barberi	7	1	14.29
Non descript	160	47	29.38
Total	459	116	25.27
			$\chi^2 = 2.484^{NS}$
Sexwise			
Male	86	18	20.93
Female	373	98	26.27
Total	459	116	25.27
			$\chi^2 = 1.055^{NS}$
Agewise			
<1 year	44	7	15.91
1-3 years	284	76	26.76
>3 years	131	33	25.19
Total	459	116	25.27
			$\chi^2 = 2.371^{NS}$

Note: ^{NS}-Non significant at P < 0.05, ** - Highly significant at P < 0.01

Seropositivity of caprine leptospirosis in the present study was noted to be 25.27 % and supported the findings of earlier workers (Oza *et al.,* 1998). However in another study from the same region positivity was only 15.38 % (Savaliya and Pal, 2008). These findings clearly indicated that the rate of seroprevalence is not consisted and varies at different time intervals supporting the earlier findings reported from Nigeria (Agunloye 2002), Bolivia (Ciceroni *et al.,* 1997), Egypt (Maronpot and Barsoum 1972), Brazil (Martins *et al.,* 2012; Higinoa *et al.,* 2013), Iran (Hassanpour *et al.,* 2012) and Malaysia (Bahaman *et al.,* 1987; Samsi Suhaila *et al.,* 2013).

It was reported that goats located under tropical climates have almost three times more chances to be seroreactive than those from temperate climates (Lilenbaum *et al.,* 2008). Leptospiral antibodies remain in serum for considerable period of time. Presence of antibody in serum is indicative of present or past exposure to organism. Present result pointed out presence of leptospiral antibodies among goats and that too with high prevalence rate. Possible reason could be favorable environmental conditions of south Gujarat. It is a tropical region with high humidity, alkaline soil, water logging and high rainfall. These factors are ideal for survival and propagation of leptospires. As

reported earlier, suitability of the environment for survival of leptospires appears to be an imperative factor in maintaining the infection among animals or humans (Haji Hajikolaei *et al.,* 2006). Leptospires can survive for long periods of time in favorable environmental conditions, thus increasing the probability of infecting a susceptible host (Trueba *et al.,* 2004). Another factor could be agricultural practices routinely followed in this region mainly cultivation of paddy crops. As paddy cultivation needs water filled fields which favour the possibility of water contamination through urine of rodents in an area. For grazing, the goats are let loose in lush green areas near such fields, so might get exposed to infection. These factors might have contributed high rate of prevalence among goats in the region.

In India, seroprevalence of leptospirosis among goats has been reported since long back from different states (Ball and Sheikh, 1958; Sharma *et al.,* 2003; Meenakshisundaram and Chellapandian, 2010; Agrawal *et al.*, 2005; Srivastava, 2008; Prakssh Krupakaran *et al.,* 2009; Balakrishnan, 2012; Vamshi Krishna *et al.,* 2012; Patel *et al.,* 2014). However, in India including South Gujarat, vaccination strategy for leptospirosis is not followed in domestic animals. As mentioned in literature, disease can affect wide range of hosts and the reservoir hosts vary with serovars and the geographical areas. So the possible role of goats in epidemiology of the disease could not be neglected.

In the present study, out of total 17 serovars tested, reactivity was noted with 15 serovars where serovars Pyrogenes, Tarassovi, Bankinang, Icterohaemorrhagiae, Shermani, Patoc1 and Pomona reacted predominantly. This result is in the agreement with previous report (Savaliya and Pal, 2008) made from south Gujarat. However in their study they have noted higher reactivity with Pomona and Hardjo. This indicates circulation of different serovars in the region. In India, different serovars have been reported from goats in various states viz., Bankinang in Kerala (Vamshi Krishna *et al.,* 2012), Javanica, Bankinang and Grippotyphosa in Andaman and Nicobar Island (Verma *et al.,* 2001) and Pomona in Tamil Nadu (Natarajaseenavasan and Ratnam, 1997; Balakrishnan *et al.,* 2008; Meenakshisundaram and Chellapandian, 2010). In contrast to these Koteeswaran (2006) reported serovar Australis as predominant serovar among small ruminants in Tamil Nadu. Similarly different serovars have been reported from different countries of the world viz., Poi and Pomona in Bolivia (Ciceroni *et al.,* 1997), Autumnalis in Egypt (Maronpot and Barsoum, 1972) and Poi in Italy (Ciceroni *et al.,* 2000) and Pomona in Malaysia (Bahaman *et al.,* 1987). Thus, it can be concluded that the serovars distribution of leptospires not only differs from country to country but also from state to state of the same country, time to time and workers to workers depending upon the sources of materials.

Studies conducted in the south Gujarat, in recent past reports Pomona, Hardjo, Canicola (Patel *et al.,* 2014) and Autumnalis/ Bankinang as well as Australis (Panwala and Mulla 2015) as predominant serovars in cattle and human, respectively. Similarly, in our study, serovars reacted including predominant serovars Pyrogenes, Tarassovi, Bankinang/ Autumnalis, Canicola and others. Previously, serovars Pyrogenes and Tarassovi have been not reported among goats in the region. In recent past, seroreactivity against serovar Pyrogenes was noted but serovar Tarassovi was not found among cattle (Patel *et al*, 2014). On the same line, serovars Pyrogenes, Hebdomadis, Autumnalis, Pomona and Grippotyphosa were noted among rats in south Gujarat (Panwala *et al.*, 2015). These

findings indicate that there is circulation of reported serovars in region and role of rodents in transmission of the disease could not be neglected as the serovars present in rodents in this region are also reported in goats and in cattle living in that same region. It is noteworthy to indicate that exposure to serovar Pyrogenes appears to be more common and rodents might have been the source of infection to goats and others in this region. Furthermore serovar Tarassovi was reported among goats in this study. It was reported that pigs may harbor and act as maintenance host for serovar Tarassovi (Bolin, 2000). So presently, the source of exposure/infection among goats to serovar Tarassovi seems to be indirect contact with infected pigs through contaminated environmental sources like water. These findings are of public health concern which indicates that infected animals can contaminate the environment and spread infection to humans/animals. It also warrants large scale study on serovar distribution in the area including cattle, buffaloes, pigs, rats, sheep and humans as this would help in better understanding of epidemiology of the disease in south Gujarat region. In addition to this, the systemic screening will also help to select serovars panel in microscopic agglutination test, too.

It is important to note that serovars Hurstbridge and Javanica were not reported presently which indicates that these serovars have not been responsible for leptospirosis among goats in the region at the time of sampling.

None of the serum sample from Surat district was found to be positive that might be due to very small sample size screened in the present study. Highest seroprevalence (35.86 %) was seen in Valsad district followed by Navsari (18.52 %) and Tapi (7.89 %) and this variation in prevalence in different districts might be due to high rain fall or humidity and other contributing factors including human clinical cases in the district in comparison to others in question. Similarly, previously many researchers have reported higher prevalence of leptospirosis in Valsad district (Oza *et al.,* (1998); Savalia and Pal (2008); Patel *et al,* 2014). The seroprevalence in different districts viz., Valsad, Navsari and Tapi was dependent which might be due to almost similar environmental conditions, livestock rearing and agricultural practices as well as socioeconomic factors.

Seroprevalence was noted in all the breeds of goats from which samples were collected. Higher prevalence was noted in Non-Descript breed followed by Surti and Barberi. Our result supported the earlier findings reported from goats reared in Tamil Nadu state of India (Balakrishnan, 2012). Seropositivity in these breeds indicates exposure of animals to contaminated environment but the seroprevalence among goats was not associated with breeds. However, this study highlights the susceptibility of these three breeds to leptospirosis.

Female goats/does showed higher seropositivity than male goats/bucks and supported the findings of earlier workers (Agrawal *et al.,* 2005; Agunloye *et al.,* 1997). Contrary to this few reports indicated higher prevalence in male goats/bucks than female goats/ does (Agunloye, 2002; Balakrishnan, 2012; Ngan and Tien, 2002). The explanation put forth by them included high metabolic and sexual activity of male goats and lack of nutritious feed given to male compared to females. In our study, however, difference in samples size between male and female goats was the possible explanation of sex difference. In Gujarat and elsewhere in India females goats are maintained in more number and for longer period in comparison to males which are mostly used for breeding purpose only. As the females are maintained for

relatively longer time than males so the chances of exposure to contaminated environment increases for females and this could be the possible reason for higher prevalence in females than male. However, the seroprevalence among goats was not dependent on sex of goats.

Higher seropositivity was noted in goats of 1-3 years and above 3 years of age groups in comparison to goats of less than 1 year and concurred with the findings of earlier workers (Agrawal *et al.,* 2005). Increasing age of the animals was supposed to provide frequent chances of infection from contaminated surroundings.

In conclusion, seropositivity in goats indicates its possible role in the epidemiology of leptospirosis and represents a threat to public health in South Gujarat. Moreover, timely addition of reported and new major serovars in MAT panel will aid in diagnosis of leptospirosis cases and to decide prevention and control strategies in the region. Furthermore a detailed study on epidemiology in this region should be carried out including livestock, wild animals and humans to know prevailing serovars and their maintenance hosts. So that effective future control strategy to combat this zoonotic infection can be implemented.

Acknowledgement

Authors are very thankful to Dr. Sumaiya Mulla, Professor and Head and In-charge Leptospirosis Reference Laboratory at Government Medical College, Surat and Dr. A. R. Rehman, Director, National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Indian Council of Agricultural Research, Hebbal, Bengaluru for technical support. We are also thankful to Dr. N. H. Kelawala, Dean and Principal, Vanbandhu College of Veterinary Science and Animal Husbandry, Navsari Agricultural College, Navsari for financial assistance.

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How to cite this article:

Priti D. Vihol, Jignesh M. Patel, Jatin H. Patel, Mahesh C. Prasad, Vijendra S. Dabas, Irsadullakhan H. Kalyani and Kuldeep K. Tyagi. 2017. Seroepidemiology of Caprine Leptospirosis in South Gujarat Region of India. *Int.J.Curr.Microbiol.App.Sci.* 6(3): 1599-1608.
doi: https://doi.org/10.20546/ijcmas.2017.603.184