

SEROLOGICAL STUDY FOR DETECTION OF ANTILEPTOSPIRAL ANTIBODIES AMONG GOATS IN SOUTH GUJARAT REGION

Priti D. Vihol^{1*}, J.M. Patel¹, J.H. Patel², I.H. Kalyani³, J.K. Raval⁴, R.D. Varia² and
Pushpa H. Rathod⁵

¹Assistant Professor, Department of Veterinary Pathology, ²Department of Veterinary
Pharmacology & Toxicology, ³Department of Veterinary Microbiology, College of
Veterinary Sciences & Animal Husbandry, Navsari Agricultural University, Eru Cross Road,
Navsari-396 450, Gujarat, India

⁴Subject Matter Specialist, Krishi Vigyan Kendra, District: Tapi, Navsari Agricultural
University, Navsari-396 450, Gujarat, India

⁵Department of TVCC, College of Veterinary Sciences & Animal Husbandry, Navsari
Agricultural University, Eru cross road, Navsari-396 450, Gujarat, India.

E mail: drprietvet@gmail.com (*Corresponding Author)

Abstract: A study was conducted to assess the prevalence of leptospirosis among goats in various districts of south Gujarat. Total 414 serum samples were collected and subjected to Immunoglobulin G (IgG) Enzyme-Linked Immune Sorbent Assay (ELISA). Among goats, the overall seroprevalence was 23.67 %. Higher prevalence was noted in Valsad district (30.77 %) followed by Navsari (23.30 %), Tapi (7.58 %) and Surat (4.17 %) with significant statistical difference. Females showed higher seroreactivity than males. Non-Descript breed showed higher seropositivity than Surti and Barbari breeds. Goats of 1-3 years age (30.69%) were more susceptible to leptospire than goats of above 3 years of age (24.79%) and below 1 year age (6.59%). The rate of seroprevalence was dependent on age of goats. Increasing age and favorable environment in different areas may increases susceptibility to infection by increasing chances of exposure to *Leptospire*s. The prevalence of antileptospiral IgG antibodies among goats indicates carrier status of animals. This could constitute a threat to human and animals in areas of south Gujarat besides causing economic losses. In this region epidemiological studies including other domestic animals should be carried out to combat this disease.

Keywords: Goats, Immunoglobulin, Leptospirosis, Seroprevalence, Serum.

Introduction

Leptospirosis is a common global zoonotic disease affecting man and various farm animal species especially in sub-tropical and tropical regions of the world. Humans may be affected after direct contact with infected urine or more often indirectly via exposure to water or soil contaminated by the urine of infected animals (Vijayachari et al., 2008).

Leptospirosis in small ruminants may be present in acute form where sick animals display pyrexia, depression, jaundice, anorexia, and anemic or hemorrhagic syndromes. However,

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most infected animals show the chronic form with impaired fertility, abortion, stillbirth, and decreased milk production which results in important economic losses (Faine et al., 1999). In addition, infected animals can develop chronic renal infection and excrete the organisms in the urine, disseminating leptospire to other animals and constituting a potential zoonotic threat to those engaged in animal production and related industries (Gerritsen et al., 1994).

For diagnosis of leptospirosis, culture or direct detection of leptospire or detection of antileptospiral antibodies are main methods. Serological methods remain the mainstay of diagnosis for leptospirosis (Levett, 2001). The most commonly used and internationally accepted reference serological test for leptospirosis is the microscopic agglutination test (MAT). The technical skill required for MAT limits its use in many non-reference laboratories. Additionally, the need for a live panel of leptospire in MAT is a laboratory biohazard, and maintaining a geographically appropriate range of serovars may be difficult. Other limitations include a limited sensitivity during the early phases of illness, inter-laboratory variation due to subjective interpretation of agglutination and difficulty in standardization (Levett and Branch, 2002). In present days, besides MAT, for serology, ELISA offers a cheaper, simpler alternative method of serodiagnosis.

Leptospirosis is endemic in South Gujarat where since last decade, outbreaks of human leptospirosis have been reported. Because of severity of the disease in this region among humans and possible involvement of domestic animals as carriers, it is imperative that continuous efforts are made to screen the affected population for finding out the prevalence of the disease in livestock. A holistic knowledge of the transmission dynamics of the disease is required to formulate intervention strategies for a particular geographical area (Sharma et al., 2014). Most of the human cases were reported in farm workers and/or landless farmers in the region. So goats reared in these villages where human cases have been reported were screened in the present study for leptospiral antibodies using IgG ELISA.

Materials and methods

A total of 414 serum samples were collected randomly from three different breeds of goats (Surti-256, Barbari-6 and Non-Descript-152) of either sex reared in villages of various districts (Navsari, Surat, Tapi, Valsad) of South Gujarat. Data related to age were also collected and on the basis of age, three groups were made i.e. below 1 year, 1-3 years and above 3 years. No vaccination programme against leptospirosis was carried out in these animals. Blood was collected by jugular vein puncture directly in sterile 9.0 ml plain vacutainers. After collection, the samples were transported under cold chain system to our

pathology laboratory. To obtain serum whole blood was kept in slanting position in 9.0 ml plain vacutainers until serum was extracted out. The 9.0 ml plain vacutainers were centrifuged at 7000 rpm for 10 minutes, if needed. The straw coloured serum was collected into two sets of 1.5 ml sterile cryo vials and aliquoted. Samples were stored at -20°C for serological analysis. An Enzyme Linked Immunosorbent Assay (ELISA) was performed to screen sera for antibodies against pathogenic leptospires in goats using Goat *Leptospira* antibody (IgG) ELISA kit (CUSABIO[®] Catalog no. CBS-EQ027948GO). The assay was performed as per the protocol outlined in the user manual supplied with kits as mentioned below. In two wells each of ELISA Assay plates, 100 μl negative control and 100 μl standard serum were dispensed separately while one well was used as substrate blank in which substrate (100 μl) was added by micropipette. In all other remaining wells, 100 μl of each sample (1:100 dilution) per well was dispensed. The plate was covered with the adhesive strip provided and incubated for 60 minutes at 37°C . Post incubation all the wells were aspirated of the content and washed by diluted (1X) wash buffer using multichannel pipette. Each well was filled by diluted wash buffer (300 μl) using multi-channel pipette and left for 20-40 seconds. Complete removal of wash buffer was carried out by decantation at each step. This was repeated four times. After the washing step, complete removal of wash buffer was carried out by inverting the plate following decantation and blotting with clean paper towels. In all the wells except substrate blank, 100 μl of conjugate was added and ELISA Assay plate was covered with new adhesive strip and incubated for 30 minutes at 37°C . Washing of plate was done as described in step (ii) above. This was followed by adding 100 μl of substrate to each well (including substrate blank well) and incubated for 30 minutes at 37°C . Stopping solution (100 μl) was added to all the wells and the plate was gently tapped to mix the content properly. The plate was read in ELISA reader (Cyberlab) within 60 minutes at 405 nm against substrate blank. (Reference wavelength between 620 nm and 690 nm). The cut off ranges were fixed by multiplication of the mean value of the measured standard OD as follows:

$\text{OD} = 0.979 \times \text{mean absorbance value of positive control or standard with upper cut-off}$

$\text{OD} = 0.784 \times \text{mean absorbance value of positive control or standard with lower cut-off}$

While $\text{OD sample} \geq \text{upper cut-off}$: Positive while $\text{OD sample} < \text{lower cut-off}$: Negative

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, Goa, India (Jangam and Wadekar, 2012).

As per CPCSEA guidelines, study involving clinical samples does not require approval of Institute Animal Ethics Committee. However, samples were collected as per standard sample collection methods without any harm or stress to the animals.

Results and discussion

In present study, the test serum samples showing OD values more than 0.4 were considered positive for leptospiral antibodies. Out of 414 samples, 98 (23.67 %) were scored positive for leptospiral antibodies in indirect IgG ELISA (Table 1), this suggests apparent prevalence of leptospiral antibodies among goats in the region. Presently reported higher seroprevalence rate in goats concurred with the findings of earlier workers (Oza et al., 1998). They reported 26.31 % seroprevalence among goats from south Gujarat. Savalia and Pal (2008) from the same region reported 15.38 % seropositivity. This indicates rate of seroprevalence is not consisted and varies at different time intervals. Caprine leptospirosis has been reported from other Indian states too (Balakrishnan, 2012; Srivastava, 2008; Srivastava and Kumar, 2003) and different countries of the world (Al-Badrawi et al., 2010; Hassanpour et al., 2012; Martins et al., 2012; Higinoia et al., 2013). The prevalence rate reported by these workers varied between 8.7 to 13.3 % where they used microscopic agglutination test. Seroreactivity among goats in this region where vaccination is not practiced for leptospirosis, is indicative of natural infection to these animals.

As reported by Radostits et al. (2000), the first serological response to infection is the production of immunoglobulin M (IgM), which rapidly increases but then diminishes to undetectable concentrations around the fourth week after infection. Within one or two weeks of infection, immunoglobulins G (IgG) appear and after three months constitute 80% of the antibodies. The titer peaks between 11 and 21 days after infection, ranging from 3,200 to undetectable concentrations, and gradually decreases for approximately 11 months, but the persistence is variable. The MAT can detect both IgM and IgG antibodies but can not distinguish it. Boonyod et al. (2005) and Sankar et al. (2008) opined that MAT is considered to be the standard reference test for the diagnosis of leptospirosis but this test requires maintenance of live cultures of strains of *Leptospira* organisms as a source of antigens. So ELISA, which is easy to conduct, is also preferred by researchers in laboratories where culture facilities are not available. In present study IgG ELISA was used to detect carrier animals. On the same line, Sankar et al. (2008) noted 25.83 and 21.66 % seropositivity in

goats using LipL32 and LipL41 recombinant ELISA, respectively at Indian Veterinary Research Institute, Izatnagar. Bashirua et al. (2013) used IgG indirect ELISA to study seroprevalence among sheep in Nigeria and reported 7.1 % rate of prevalence. Similarly ELISA has also been successfully used to detect antileptospiral antibodies in goats by various workers (Pinzauti et al., 2006) from other parts of the world.

Districtwise higher seropositivity was noted be 30.77 % (68/221) in Valsad followed by 23.30 % (24/103) in Navsari, 7.58 % (5/66) in Tapi and 4.17 % (1/24) in Surat (Table 2). There was highly significant ($P < 0.01$) difference in seropositivity among goats in various districts. Present findings are in agreement with earlier reports (Oza et al., 1998; Savalia and Pal, 2008). The highest seroprevalence rate in Valsad could be justified due to comparatively higher rainfall and the location (temperate zone) of the district. 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. In Navsari most of the animals are maintained by individual household/landless farmers or farm workers in low number and this district faces high rainfall and water logging in most of the areas because of adjacent sea costline. While Tapi is at higher place geographically hence, most of rain water is drained off resulting in less water reservoir and water logging. In villages of Surat district goats are reared in very low numbers and in comparatively clean areas so infection from recovered/convalescent/ sub-clinical carrier animals occasionally spread to others. However it is important to note here that in all the districts, prevalence of leptospirosis among goats was noted.

In this study, seropositivity in females and males was 24.77 % and 19.28 % respectively (Table 2). Present findings supported the reports of Agunloye et al. (1997) and Agrawal et al. (2005). Contrary to this Agunloye (2002), Ngan and Tien (2002) and Balakrishnan (2012) reported higher prevalence in male goats (bucks) than female goats (does). However in the present study, the seropositivity among male and female goats was not dependent. The results indicated that gender of animal had not a significant effect on the frequency of leptospirosis.

Goats of 1-3 years age group showed 30.69 % seroreactivity compared to 24.79 % in more than 3 years and 6.59 % in less than 1 year age groups. There was statistically highly significant ($P < 0.01$) association of age and seropositivity was noted among goats in this region. In current study results concurred with the findings of Agrawal et al. (2005). Increasing age of the animals was supposed to provide frequent chances of infection from contaminated surroundings.

Breedwise seroreactivity was noted to be higher in Non-descript breed (28.29 %) followed by Surti (21.09 %) and Barbari (16.67 %). Breedwise prevalence did not differ significantly ($P < 0.05$). On the same line, Balakrishnan (2012) reported higher seroprevalence among non-descript breed of goats.

The reported higher percentage of positivity among the goats may be due to their habitation and husbandry method followed in India. Most of the landless or marginal farmers in India rear goats in small numbers as a source of additional income. They let loose the goats for grazing in bushy/jungle area and near small ponds/water loggings areas. Leptospirosis is endemic in the south Gujarat region and possibly goats might be infected with leptospire from contaminated areas. The environmental conditions of south Gujarat, tropical region with high humidity, alkaline soil, water logging and high rainfall are favourable for leptospire. These factors are ideal for survival and propagation of leptospire. Leptospire can survive for long periods of time in favorable environmental conditions, thus increasing the probability of infecting a susceptible host (Trueba et al. 2004). Infected animals act as carrier of leptospire and can eliminate the organism in environment for long periods (Lilenbaum et al. 2008).

So it may be concluded that presence of antileptospiral antibodies in sera of goats is indicative of natural infection of these grazing animals as well as existence of foci of infection in the region. It was also found that increasing age of animal favors the susceptibility to infection. Detection of IgG antibodies indicates past infection or carrier status of seropositive goats. So preventive and control strategies to combat this zoonotic disease may be implicated in the region. Further future detailed study on the disease including other domestic animals is requisite to understand epidemiology of the disease in this region.

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Table 1: Details of districtwise seroprevalence of leptospirosis among goats in South Gujarat

Attributes	No. of samples analysed	No. of samples positive	% positive
Valsad	221	68	30.77
Navsari	103	24	23.30
Tapi	66	5	7.58
Surat	24	1	4.17
Total	414	98	23.67
			$\chi^2 = 20.687^{**}$

Note: ** - Highly significant at $P < 0.01$

Table 2: Details of seroprevalence of leptospirosis among goats in south Gujarat

Attributes	No. of samples analysed	No. of samples positive	% positive
Genderwise			
Female	331	82	24.77
Male	83	16	19.28
			$\chi^2 = 1.110^{NS}$
Age-wise			
< 1 year	91	6	6.59
1-3 years	202	62	30.69
>3 years	121	30	24.79
			$\chi^2 = 20.286^{**}$
Breed-wise			
Surti	256	54	21.09
Barbari	6	1	16.67
ND	152	43	28.29
			$\chi^2 = 2.898^{NS}$

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