Veterinary World, EISSN: 2231-0916
Available at www.veterinaryworld.org/Vol.7/November-2014/18.pdf

RESEARCH ARTICLE
Open Access

Seroepidemiological pattern of leptospirosis in bovine of South Gujarat, India

J. M. Patel[1], P. D.Vihol[1], M. C. Prasad[1], I. H. Kalyani[2], J. K. Raval[1], K. M. Patel[3], Sushma R. A. Thirumalesh[4] and V. Balamurugan[4]

1. Department of Veterinary Pathology, Vanbandhu College of Veterinary Sciences & Animal Husbandry, Navasari Agricultural University, Navsari, Gujarat, India; 2. Department of Veterinary Microbiology, Vanbandhu College of Veterinary Sciences & Animal Husbandry, Navasari Agricultural University, Navsari, Gujarat, India; 3. Veterinary Dispensary, Patan District Panchayat, Department of Animal Husbandry, Varahi, Patan, Gujarat, India; 4. Project Directorate on Animal Disease and Survellance, Indian Council of Agricultural Research, Hebbal, Bengaluru, Karnataka, India.

Corresponding author: J. M. Patel, e-mail: dr.jams@rediffmail.com, PDV: drpriti@nau.in, MCP: drmcprasad1938@gmail.com, IHK: ihkvet@gmail.com, JKR: ravaljk2@gmail.com, KMP: kmplpt@yahoo.com, SRAT: srath@gmail.com, VB: balavirol@gmail.com

Received: 02-08-2014, **Revised:** 25-10-2014, **Accepted:** 03-11-2014, **Published online:** 27-11-2014

doi: 10.14202/vetworld.2014.999-1003. **How to cite this article:** Patel JM, Vihol PD, Prasd MC, Kalyani IH, Raval JK, Patel KM, Thirumalesh SRA, Balamurugan V (2014) Seroepidemiological pattern of leptospirosis in bovine of South Gujarat, India, *Veterinary World, 7*(11): 999-1003.

Abstract

Aim: Seroepidemiological study of leptospirosis in cattle of various South Gujarat district (Navsari, Tapi, Surat, Valsad).

Materials and Methods: Whole blood samples were collected randomly from different age groups, and breeds of cattle of either sex reared in different districts (Navsari, Surat, Tapi, Valsad) of South Gujarat. To obtain serum, whole blood was kept in slanting position in 9.0 ml plain vacutainers until serum extracted out of the whole blood. Then these 9.0 ml plain vacutainers were centrifuged at 7000 rpm for 10 min. The straw colored serum was then collected in 1.5 ml sterile cryo vials and aliquoted and stored at −20°C for microscopic agglutination test.

Results: In the present study, overall 12.81% (51/398) seroprevalence were recorded with highest seroprevalence (47.06%, 24/51) from Valsad followed by Navsari (9.14%, 18/197), Surat (6.90%, 2/29) and Tapi (5.79%, 7/121) among cattle. The seroprevalence rate of breed and sex wise did not differ significantly ($p \leq 0.05$). Maximum incidence of seropositivity was found above 4 years (16.32%, 39/239) of age group followed by animals between 1 and 4 years (9.68%, 12/124). Thus, the age was significantly influencing the seropositivity ($p \leq 0.05$). In cattle out of 398 sera screened, 51 were positive with one or more serovars. The highest seropositivity was recorded against serovar Pomona (28.89%).

Conclusions: Overall 12.81% seroprevalence of leptospirosis in apparently healthy and clinically ailing bovine of South Gujarat indicating potential zoonotic risk to farmers, labor, and animal owners.

Keywords: cattle, leptospirosis, seroepidemiology, zoonosis.

Introduction

Leptospirosis is worldwide distribution and occurs in man, cattle, buffaloes, pig, sheep, goat, dog, horse, etc. Due to its zoonotic nature, it is communicable from man to animals and *vice versa*. The earliest recorded outbreak of leptospirosis in India was reported among construction workers in the village of South Andaman in 1929 and was named as Andaman hemorrhagic fever [1]. It is still of common occurrences in South and North Andaman.

In Gujarat, the disease reemerged in Surat district during 1997/1998. In the year 2005, the disease was noted in 392 persons and deaths occurred in 81 patients due to leptospirosis from various districts of South Gujarat. Since then, cases of leptospirosis were very often reported from Navsari, Valsad, and Surat districts [2]. In neighboring state of Maharashtra, similar episodes have often been reported during postflood period especially in Mumbai [3,4].

Hence, it is very necessary to know the present status of bovine leptospirosis in the South Gujarat. The present study was planned to study seroepidemiogical pattern of leptospirosis among cattle in this region of Gujarat.

Materials and Methods

Ethical approval

The experiment was carried out according to the protocol of Institutional Animal Ethics Committee and the authors were permitted by animal owners for sampling.

Samples

A materials comprise whole blood/serum were collected randomly from different age groups and breeds of cattle (n=398) of either sex reared in different districts (Navsari, Surat, Tapi, Valsad) of South Gujarat (Tables-1-3). Whole blood samples were collected from the jugular vein of cattle in sterile 9.0 ml plain vacutainers. To obtain serum, whole blood was kept in slanting position in 9.0 ml plain vacutainers

until serum extracted out of the whole blood. Then these 9.0 ml plain vacutainers were centrifuged at 7000 rpm for 10 min. The straw colored serum was collected in 1.5 ml sterile cryo vials and aliquoted and stored at −20°C for future use.

Tests

All the sera were tested for antibodies against live antigens of *Leptospira* sp. Serovars Pyrogenes, Australis, Bankinang, Grippotyphosa, Patoc, Pomona, Icterohaemorrhagiae, Hebdomadis, Canicola, Hardjo, Bellum, Bataviae, Tarassovi, Shermani, Kaup, Hurstbridge, and Javanica by microscopic agglutination test (MAT) at Leptospirosis Reference Laboratory, Government Medical College, Surat [5] and Project Directorate on Animal Disease Monitoring and Surveillance, Bengaluru using standard procedure [6,7]. A MAT titre of 1:40 or above is taken as a positive reactor.

Statistical analysis

Chi-square test was used according to Web Agri Stat Package software developed by Jangam and Wadekar, ICAR research complex, Goa for statistical analysis of data [8].

Results and Discussion

The details of district, breed, sex, and age wise results of seroprevalence in cattle are given in Table 4.

In the present study, seroprevalence among cattle was recorded to be higher (12.81%, 51/398) than the reported rate of occurrence (6.65%) in certain state of India i.e. Andhra Pradesh, Assam, Gujarat, Himachal Pradesh, Orissa, Maharashtra, West Bengal, Kerala, and Tamil Nadu [9]. In contrast to present findings higher seroprevalence, 44.37% [10] and 42.5% [11] was recorded from Orissa and 22% from Uttaranchal, Tamil Nadu, Uttar Pradesh [12]. Mandal *et al.* [13] reported 16.84%, and Savalia and Mahendra [14] reported 14.77% seroprevalence of leptospirosis in West Bengal and Gujarat, respectively, which compare well with our present findings. Among different countries low to high seroprevalence rate has been recorded like 3.4% [15] in Turkey, 37.75% [16], 22.5% [17], 21.05% [18] in Iran, 27.7% [19] in Malaysian, 38.3% [20] and 50.71% [21] in Brazil.

District-wise (Navsari, Surat, Tapi, and Valsad) seroprevalence rate of occurrence revealed that the presence of leptospiral antibodies differed significantly ($p \leq 0.05$) among the districts. The highest

Table-1: Details of age-wise sample collection in cattle.

Species	Age groups	Districts				Total
		Valsad	Navsari	Tapi	Surat	
Cattle	<1 year	01	02	03	01	007
		00	11	13	04	028
	1-4 years	00	07	02	10	019
		08	60	34	03	105
	>4 years	03	04	00	02	009
		39	113	69	09	230
Total		51	197	121	29	398

Table-2: Details of breed-wise sample collection in cattle.

Species	Breeds	Districts				Total
		Valsad	Navsari	Tapi	Surat	
Cattle	HF	00	00	00	00	00
		07	37	20	01	65
	Jersey	00	00	00	00	00
		05	19	02	00	26
	Gir	02	00	00	00	02
		07	16	09	02	34
	HF cross	02	13	05	13	33
		21	103	72	13	209
	Jersey cross	00	00	00	00	00
		04	05	07	00	16
	Gir cross	00	00	00	00	00
		03	04	06	00	13
Total		51	197	121	29	398

HF=Holstein Friesian

Table-3: Details of districts and sex-wise sample collection in cattle.

Districts	Cattle		
	Male	Female	Total
Valsad	04	47	51
Navsari	13	184	197
Tapi	05	116	121
Surat	13	16	29
Total	35	363	398

Table-4: Seroprevalence of leptospirosis in cattle.

Attributes	Number of tested	Number of positive	Percent positive
Region			
South Gujarat	398	51	12.81
Districts			
Navsari	197	18	9.14
Valsad	51	24	47.06
Tapi	121	7	5.79
Surat	29	2	6.90
Total	398	51	12.81
χ^2=7.82 *(p<0.05)			
Breed wise			
HF	65	7	10.77
Jersey	26	4	15.38
Gir	36	9	25.00
HF cross	242	29	11.98
Jersey cross	16	0	0
Gir cross	13	2	15.38
Total	398	51	12.81
χ^2=11.07^{NS} (p<0.05)			
Sex wise			
Male	35	03	8.57
Female	363	48	13.22
Total	398	51	12.81
χ^2=3.84^{NS} (p<0.05)			
Age wise			
<1 year	35	00	00.00
1-4 years	124	12	9.68
>4 years	239	39	16.32
Total	398	51	12.81

χ^2=5.99* (p<0.05). ^{NS}Non-significant at p<0.05, *Significant at p<0.05, HF=Holstein Friesian

seroprevalence (47.06%, 24/51) was recorded from Valsad followed by Navsari (9.14%, 18/197), Surat (6.90%, 2/29) and Tapi (5.79%, 7/121). The highest seroprevalence rate in Valsad could be justified due to the location (temperate zone) of the district and comparatively higher rainfall. In Tapi, this could be attributed to the fact that most of the animals are maintained by individual household in low number and the owners pay personal attention about their cleanliness, hence infection from recovered/convalescent/sub-clinical carrier animals occasionally spread to others. Tapi district is at higher place compared to Surat and Navsari; hence, most of rain water is drained off resulting in less water reservoir and water logging. This may be another reason for low seroprevalence of leptospirosis.

In the present study, six breeds of cattle (two pure exotic, one pure indigenous, and three crossbred) were included. The rate of seroprevalence was the highest in pure indigenous breed: Gir (25.00%, 9/36) followed by pure exotic breed/cross bred i.e. Jersey (15.38%, 4/26), Gir cross (15.38%, 2/13), Holstein Friesian (HF) cross (11.98%, 29/242) and HF (10.77%, 7/65). Controversy exists about prevalence of leptospirosis among different breed of cattle. Varma *et al.* [22] and Agrawal *et al*. [23] opined that indigenous breeds are more susceptible than crossbreds and our present observations supported their finding. Contrary to this Balakrishnan *et al.* [24] noted that exotic pure breeds are more susceptible followed by indigenous pure breeds and cross breeds. They further mentioned that both HF and Jersey are equally susceptible to leptospirosis. Among Indian breeds, Red Sindhi was the most susceptible (66.66%) followed by Gir (40.00%), Kankrej (27.27%) and Sahiwal (25.00%). Thus, there appears to be the absence of unanimity in the literature about breed susceptibility.

Sex-wise seroprevalence in cattle did not differ significantly ($p \leq 0.05$) though the females showed higher seroprevalence (13.22%, 48/363) in comparison to male (8.57%, 3/35). This could possibly be due to number of sample tested from male and female cattle and supported the observations of Ramin and Azizzadeh [25] and Shafighi *et al*. [16] who too did not observed any sex bias in respect of seropositivity. Contrary to the above observations Mandal *et al*. [13] and Balakrishnan *et al*. [24] noted higher seropositivity in males than female cows/heifer.

Sera were tested from the cattle of three age groups *viz.*, below 1 year, 1-4 years and above 4 years. Maximum incidence of seropositivity was found in cattle of above 4 years (16.32%, 39/239) of age group followed by animals between 1 and 4 years (9.68%, 12/124). None of the cattle below 1 year showed seropositivity. Thus, age was significantly influencing the seropositivity ($p \leq 0.05$). These findings indicated that increasing age of cattle makes them more prone to leptospiral infection and supported the findings of Kocabiyik and Cetin (2004) who also opined that seropositivity markedly increase with age and Balakrishnan *et al.* (2011) mentioned that seropositivity was comparatively higher in the cattle of above 4 years of age. Contrary to this, Agrawal *et al.* (2005) studied the seroprevalence in cattle, buffaloes and goats and reported that the seropositivity among cattle of 6-8 year age was 10% which decreased to 3% at the age of above 9 years.

In cattle out of 398 sera screened, 51 were positive for with one or more serovars. The highest seropositivity was recorded against serovar pomona (28.89%) followed by Hardjo (15.56%), Canicola (12.22%), Patoc (5.56%), Icterohemorrhagiae (5.56%), Hebdomadis (5.56%), Pyogenes (4.44%), Bellum (4.44%), Bataviae (4.44%), Autumnalis/Bankinang (3.33%), Australis (2.22%), Hurstbridge (2.22%), Javanica (2.22%), Grippotyphosa (1.11%), Shermani (1.11%), and Kaup (1.11%). Serovar tarassovi could not be detected from any of cattle sera sample (Table-5).

In the present study, the most prevalent serovar was Pomona (28.89%) as against earlier observations made by Balakrishnan *et al.* [24] from this region who noted Serovar Hardjo to be most prevalent among cattle. In addition a number of serovars from time to time were reported from other states of India such as serovars Hardjo, Bataviae, Canicola, and Australis from West Bengal [13], Grippotyphosa, Pomona and ictrohemorhagiae from Andaman and Nicobar [22], Pomona, Hebdomandis, Medanensis, Hardjo, Andamana and Saxkoebing from Andhra Pradesh [26,27], Icterohaemorrhagiae and Grippotyphosa from Uttar Pradesh [28].

Conclusions

Seroepidemiological study of leptospirosis in South Gujarat region revealed that this region is endemic for leptospirosis. It's prevalence in apparently healthy and clinically ailing bovine irrespective of breed, age, and sex indicating potential zoonotic risk. This study also determines the need for continuous monitoring of leptospirosis in animal and humans to combat this zoonotic infection.

Authors' Contributions

JMP and PDV conduct experiment. MCP and IHK provide the necessary guidance during study period. JKR and KMP help in sample collection from a different district of South Gujarat. VB provided help for MAT. [JMP and MCP drafted and revised manuscript] All authors read and approved the final manuscript.

Acknowledgments

The authors wishes to thank Dr. Sumaiya Mulla, Professor & Head, Department of Microbiology, Government Medical College, Surat, Gujarat, Indiafor their help in screening of serum samples.

The authors are highly thankful to the Dean and Principal, Vanbandhu College of Veterinary science &

Table-5: Leptospiral serovar distribution in cattle of South Gujarat.

Animal	Place of leptospira reference laboratory	Number of samples tested	Number of samples positive	Percent positive	Different serovars: Pyrogenes (%)	Australis (%)	Autumnalis/ Bankinang (%)	Grippotyphosa (%)	Patoc	Pomona	Icterohemorrhagiae (%)	Hebdomadis (%)	Canicola (%)	Hardjo (%)	Bellum (%)	Bataviae (%)	Tarassovi (%)	Shermani (%)	Kaup (%)	Hurstbridge (%)	Javanica (%)	Total (%)
Cattle	GMC	298	45	15.10	4 (5.13)	1 (1.28)	3 (3.85)	0 (00)	3 (3.85)	24 (30.77)	5 (6.41)	5 (6.41)	11 (14.1)	14 (17.95)	4 (5.13)	4 (5.13)	NT	NT	NT	NT	NT	78 (100.00)
	NIVEDI	100	6	6.00	0 (0.0)	1 (8.33)	0 (0.0)	1 (8.33)	2 (16.67)	2 (16.67)	0 (0.0)	0 (0.0)	0 (0.00)	0 (0.0)	NT	NT	0 (0.0)	1 (8.33)	1 (8.33)	2 (16.67)	2 (16.67)	12 (100.00)
	Total	398	51	12.81	4 (4.44)	2 (2.22)	3 (3.33)	1 (1.11)	5 (5.56)	26 (28.89)	5 (5.56)	5 (5.56)	11 (12.22)	14 (15.56)	4 (4.44)	4 (4.44)	0 (0.0)	1 (1.11)	1 (1.11)	2 (2.22)	2 (2.22)	90 (100.00)
Grand total		500	67	13.4	4 (3.54)	2 (1.77)	4 (3.54)	4 (3.54)	7 (6.19)	29 (25.66)	5 (4.42)	5 (4.42)	13 (11.50)	16 (14.16)	4 (3.54)	6 (5.31)	0 (0.0)	1 (0.88)	5 (4.42)	3 (2.65)	5 (4.42)	113 (100.00)

GMC=Government Medical College, Surat, NIVEDI=National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru, NT=Not tested

Animal Husbandry, Navsari Agricultural University, Navsari for providing facilities and fund under budget head: 12049 for this study.

Competing Interests

The authors declared that they have no competing interests

References

1. Taylor, J. and Goyle, A.N. (1931) Leptospirosis in Andamans (Suppl. Indian J. Med. Res. Mem. No. 20), Indian Research Fund Association, Thacker, Spink and Co., Calcutta.
2. Shivakumar, S. (2008) Leptospirosis - Current Scenario in India. *Med. Update*, 18: 799-809.
3. Bharadwaj, R., Bal, A.M., Joshi, S.A., Kagal, A., Pol, S.S., Garad, G., Arjunwadkar, V. and Katti, R. (2002). An urban outbreak of leptospirosis in Mumbai, India. *Jpn. J. Infect. Dis.*, 55(6): 194-196.
4. Maskey, M., Shastri, J.S., Saraswathi, K., Surpam, R. and Vaidya, N. (2006) Leptospirosis in Mumbai: Post-deluge outbreak 2005. *Indian J. Med. Microbiol.*, 24(4): 337-338.
5. Vijayachari, P., Suganan, A.P. and Sehgal, S.C. (2001) Role of microscopic agglutination test (MAT) as a diagnostic tool during acute stage of leptospirosis in low and high endemic areas. *Indian J. Med. Res.*, 114: 99-106.
6. World Organization for Animal Health (Office International des Épizooties-OIE). (2013) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Ch. 2.1.9. OIE, Paris. p251-264.
7. Faine, S. (1982) Guidelines for the Control of Leptospirosis. Vol. 27. WHO, Offset Publication 67, Geneva, Italy.
8. Jangam, A.K. and Wadekar, P. (2012) Web Based Agricultural Statistics Software Package. ICAR Research Complex for Goa.
9. Singh, S.P. and Uppal, P.K. (1983) Present position of prevalence of leptospira agglutinin in Indian cattle. *Indian Vet. J.*, 60: 147-149.
10. Tripathy, S.B. (1977) Serological prevalence of leptospirosis in cattle, sheep and goat. *Indian Vet. J.*, 54: 584-585.
11. Balamurugan, V., Thirumalesh, S.R.A., Sridevi, R., Mohandoss, N., Govindaraj, G., Hemadri, D., Gajendragad, M.R. and Rahman, H. (2013) Seroprevalence of bovine leptospirosis in Odisha, India. *World J. Vet. Sci.*, 1: 1-7.
12. Mariya, R., Srivastava, S.K. and Thangapandian, E. (2007) Seroprevalence of leptospiral antibodies in bovine. *Indian Vet. J.*, 84: 547-548.
13. Mandal, S., Joardar, S.N., Chakraborty, D. and Sardar, N. (2008) Seroepidemiological study of bovine leptospirosis in West Bengal. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.*, 29(1&2): 42-44.
14. Savalia, C.V. and Mahendra, P. (2008) Studies on the reservoir status of leptospirosis in Gujarat. *Indian J. Field Vet.*, 4(1): 7-9.
15. Kocabiyik, A.L. and Cetin, C. (2004) Bovine leptospirosis in south Marmara region of Turkey: A serological survey. *Rev. Méd. Vét.*, 155(12): 606-608.
16. Shafighi, T., Abdollahpour, G., Zahraei Salehi, T. and Tadjbakhsh, H. (2010) Serological and bacteriological study of leptospirosis in slaughtered cattle in North of Iran (Rasht). *Afr. J. Microbiol. Res.*, 4(20): 2118-2121.
17. Bahari, A., Abdollahpour, G., Sadeghi-Nasab, A., Sattari Tabrizi, S., Yavari, M. and Dadmehr, B. (2011) A serological survey on leptospirosis in aborted dairy cattle in industrial farms of Hamedan suburb, Iran. *Iran J. Vet. Res.*, 12(4): 337-339.
18. Hamali, H. Jafari Joozani, R. and Abdollahpour, G.R. (2012). Serodiagnosis and molecular survey on leptospiral abortions in the dairy cattle of Tabriz. *Iran J. Vet. Res.*, 13(2): 120-125.
19. El Jalii, I.M. (2008) Comparison between ELISA and the

microscopic agglutination test for the diagnosis of bovine leptospirosis. *Rev. Élev. Méd. Vét. Pays Trop*., 61(2): 73-75.
20. Mineiro, A.L.B.B., Vieira, R.J., Costa, E.A., Santos, R.L., Gonçalves, L.M.F., Carvalho, S.M., Bomfim, M.R.Q. and Costa, F.A.L (2011) Serology, polymerase chain reaction and histopathology for leptospirosis in samples collected at slaughter from dairy cows of Parnaiba region, State of Piaul, Brazil. *Pesq. Vet. Bras*., 31(10): 859-866.
21. Tomich, R.G.P., Bomfim, M.R.Q., Koury, M.C., Pellegrin, A.O., Pellegrin, L.A., Albert Icksang Ko., A. and Barbosa-Stancioli, E.F. (2007) Leptospirosis serosurvey in bovines from Brazilian pantanal using IGG ELISA with recombinant protein Lipl32 and microscopic agglutination test. *Braz. J. Microbiol.*, 38(4): 674-680.
22. Varma, A., Rai, R.B., Balakrishnan, P., Gupta, A. and Naveen K.A. (2001) Seroprevalence of leptospirosis in animas of Andaman and Nicobar islands. *Indian. Vet. J*., 78: 936-937.
23. Agrawal, R, Kumar, M., Kumar, M. and Srivastava, S.K. (2005) Epidemiological pattern of leptospirosis in livestock of Uttaranchal state. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.*, 26(2): 109-113.
24. Balakrishnan, G., Roy, G.P., Govindarajan, R., Ramaswamy, V. and Murali Manohar, B. (2011) Seroepidemiological studies on leptospirosis among bovines in organized farm. *Int. J. Agro Vet. Med. Sci.*, 5(6): 511-519.
25. Ramin, A.G. and Azizzadeh, F. (2013) Seroepidemiological detection of antibodies against *Leptosp*ira spp. using microscopic agglutination test in Urmia cows and sheep. *Acta Vet. Beograd*, 63(1): 53-61.
26. Somasundara Rao, G. and Surendran, N.S. (1970) Incidence and serotypes of bovine Leptospira in Andhra Pradesh. *Indian Vet. J*., 47(4): 296.
27. Mrunalini, N., Ramasastry, P. (2000) A note on seroprevalence of subclinical leptospirosis in organised farms in Andhra Pradesh. *Indian Vet. J*., 77(8): 713-714.
28. Sachan, N., Binita N., Singh, V.P., Sabrinath, T., Chaudhary, P. and Agarwal, R.K. (2011) Seroprevalence of leptospirosis in the animals in Rohilkhand region of Uttar Pradesh, India. *Int. J. Agro Vet. Med. Sci*., 5(6): 511-519.

****