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Detection and Prevalence of Canine Leptospirosis from Navsari District of South Gujarat, India

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DD, PM, DP performed the experiment, analyses of the study and literature searches. Authors Sudhir Mehta, Suresh Mavadiya, JV and SP collected samples and gathered the information. Authors DD and PM performed statistical analysis. Author DD wrote the first draft of the manuscript. Authors JS and IK supervised the study, reviewed and finalized the draft. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Leptospirosis is endemically prevalent in coastal area of south Gujarat. Generally, pets are vaccinated against *Leptospira interrogans* but whosoever left unvaccinated, are prone to infection. The present study was conducted to detect canine leptospirosis and its prevalence from south Gujarat.
Place and Duration of Study: A total of 46 serum samples and 33 urine samples were collected from 56 dogs suspected of having leptospira infection. The study was done from September 2019 to May 2020.

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Methodology: Serum samples were diagnosed by IgG based ImmunoComb solid phase dot ELISA test for antibody against genus specific Leptospira interrogans. Urine samples were visualized under dark field microscopy (DFM) following laboratory manual on leptospirosis of WHO. Sex and age of dogs were noted to find out the percent positivity and prevalence. Chi square test was applied to find out any significant difference among the age groups.
Results: Out of 46 serum samples, 17 (36.95%) found positive by dot ELISA and out of 33 urine sample, 13 (39.39%) found positive by DFM. Sex wise prevalence found 42.10 percent (16/38) in Male while 55.55 percent (10/18) in female dogs. Age group wise prevalence was recorded 63.63 percent (07/11) in 1- ≤ 3 years group. 27.77 per cent (05/18) in > 3- ≤ 6 years group whereas, 51.85 percent (14/27) in > 6- ≤ 12 years group.
Conclusion: Detection and prevalence of canine leptospirosis were studied first time from Gujarat. Overall 46.42 percent (26/56) dogs were diagnosed positive for canine leptospirosis. Dogs having age group of 1- ≤ 3 years found more susceptible. Prevalence was found more in female than male dogs.

Keywords: Leptospira interrogans; canine leptospirosis; immunocomb solid phase dot elisa; dark field microscopy; Gujarat.

1. INTRODUCTION

Leptospirosis is an emerging global public health problem because of increasing incidence in both developing and developed countries [1-3]. It is caused by pathogenic spirochetes that occur all over the world with numerous hosts and it is re-emerging as an important zoonotic disease. Different serovars of *Leptospira interrogans* are ubiquitously present in sub-clinically infected wild and domestic animal reservoir hosts [4]. In 1886, Adolf Weil reported clinical syndrome characterized by splenomegaly, nephritis and jaundice [5,3], commonly referred as Weil's disease that became synonymous of leptospirosis. Reservoir hosts are a source of infection for human and other incidental hosts. Leptospira organism does not multiply outside of host. Their survival is very crucial and depends on environmental conditions in which they are found. Leptospira are sensitive to drying, high temperature and pH changes can be fatal [6].

Leptospirosis is known to be endemic disease in India [7-10,3]. Most of the outbreaks of leptospirosis in India are frequently reported from the coastal area of the states of West Bengal, Orissa, Kerala, Tamil Nadu, Karnataka, Maharashtra, Gujarat and the Andaman Islands [10]. Highest rates occur during monsoon season in these parts. Incidence of canine leptospirosis higher in rainy season when there is abundant standing water and swampy conditions. Dogs generally get infection through direct contact, standing water, contaminated urine, contaminated water, vegetation, soils and contaminated food. To prevent the disease, vaccines are available for dogs; however, vaccine does not contain relevant serovars always and duration of immunity is only for 12 months. Practice of vaccination routinely followed in pet dogs. However, whosoever left unvaccinated is prone to disease. Even, stray dogs are seldom vaccinated against leptospira.

Mostly for diagnosis of leptospirosis, dark field microscopy (DFM), microscopic agglutination test (MAT), ELISA and PCR techniques are used [1]. DFM is most economic and rapid technique to demonstrate organisms under the microscope [11,12-14], however it is less sensitive in detection [1,15]. ELISA which are based on IgM or IgG detection are usually employed. PCR technique is used for diagnosis of leptospira at molecular level. MAT is a gold standard technique to identify different serovars from the sample either organism or antibody determination. Hence, it is required to maintain reference serovars and sera to identify leptospira organisms at species level which is required established laboratory set up with trained staff and biosafety measurements that could not possible at low cost lab set up and in limited resource. Therefore, in present study DFM and ELISA are employed for primary diagnosis from urine and serum samples respectively.

Leptospirosis is endemic in coastal area of south Gujarat [16]. Human and domestic animals like cattle, buffalo, goat and sheep are infected frequently over the year. Incidence and prevalence studies are reported earlier in this region from human and domestic animals [17-25]. However, prevalence was not studied from dogs in Gujarat. Earlier studies were reported prevalence of leptospira from dogs in south India

[26-31]. Due to unclear disease occurrence pattern, sometimes disease remains undiagnosed and may be dog owner not aware of vaccination that's why complete coverage of vaccination generally not achieved. Therefore, cross-sectional epidemiological study was designed to diagnose canine leptospirosis and its prevalence, which could be helpful in future for epidemiological survey.

2. MATERIALS AND METHODS

2.1 Collection of Samples and Processing

A cross-sectional epidemiological study was designed to diagnose canine leptospirosis from September 2019 to May 2020. Non-vaccinated dogs having suspect of leptospira infection were included in study. A total of 79 serum and urine samples of 56 suspected dogs (Table 1) showing clinical signs like fever, inappetence, chronic vomiting, unable to urinate, hematuria and weakness, received at Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Navsari Agricultural University. DFM was employed to visualize organisms in urine and Immunocomb solid phase dot ELISA was used to demonstrate IgG antibodies in serum samples. Systematic details of suspected dogs were recorded. Each details of samples viz. sex and age of dogs were noted to find out the percent positivity and prevalence of canine leptospirosis in and around of Navsari district of south Gujarat, India. Chi square test was applied to find out any significant difference among the groups.

2.2 Dark Field Microscopy (DFM)

Dark field microscopy (DFM) was performed by following laboratory manual on leptospirosis of WHO [32]. Briefly, urine sample was centrifuged at 3000 *g* for 10 min. A drop of deposit was placed on clear glass slide and covered with cover slip to prepare wet film which was further examined under DFM by using low and high power magnifications [11].

2.3 IgG based Immuno Comb Solid Phase Dot ELISA Test (Dot ELISA)

ImmunoComb Canine Leptospira Antibody Test kit (cat. no. 50CLC301) was procured from Biogal Galed Laboratories Acs Ltd., Israel. Kit is designed to detect antibodies of different pathogenic serovars of *Leptospira interrogans* which are *L. ichterohaemorrhgiae* (copenhageni and RGA), *L. canicola, L. pomona* and *L. grippotyphosa*. But it does not distinguish the specific serotype. A positive result indicates current infection. There is main two component supplied with kit namely, developing plate, which is reagent containing twelve separated and protected hole with aluminum cover and comb, which having twelve tooth for performing 12 test. Kit components depicted in Fig. 1. Test was performed by following manufacturer's instruction manual. Briefly, 5 µl of serum was deposited into a well of row A of developing plate and mixed well by forward pipetting. Comb was inserted into opened well in row A and incubated for 5 minutes at room temperature (RT). For better mixing, comb was moved up and down 3-4 times. Then after by using of tweezers, foil of row B was pierced, subsequently, comb was inserted for 2 minutes and mixed by same way. After that, row C was opened and comb was inserted for 5 minutes and mixed. Then next row D was pierced, comb was added for 2 minutes. Row E well was pierced and comb was inserted for 2 minutes. Then, row F was pierced and comb was inserted and remained for 5 minutes. Upon completion of the colour development, comb was backed to row E for 2 minutes for colour fixation. Finally, comb was dried for 5 minutes before reading the result. Presence of double dots interpreted as positive result. Upper dot represent control and down one represent test sample. Absence of control dot and presence of test dot recorded as invalid result.

3. RESEULTS AND DISCUSSION

Summary of sample wise positivity for canine leptospirosis is presented in Table 2. Total 30 (37.97%) samples out of 79 were found to be positive for leptospira infection. Among these, eight samples (both, urine and serum) of each four dogs found positive by both tests. [28] reported 23.33 percent (35/150) positivity in blood, 12 percent positivity (18/150) in urine whereas 06 samples out of 150 found positive in both (serum and urine) by using PCR. In our study, from the same dog, urine sample was found positive for leptospira, whereas serum was detected negative by dot ELISA. This might be due to active and recent infection, where organism can be demonstrated from urine but IgG antibody might not have been develop as it takes two weeks for generation. In this case, IgM based ELISA would be useful, which detects IgM antibodies. IgM antibodies appear earlier and initially against infection, that is part of body's early immune response. On other side, some of

Table 1. Details of samples processed for Canine leptospirosis

Sample ID	Sex	Urine samples only	Serum samples only	Both samples
L1	M		√	
L2	M		√	
L3	M	√		
L4	M	√		
L5	M			√
L6	M		√	
L7	M	√		
L8	M		√	
L9	M		√	
L10	M			√
L11	F			√
L12	F	√		
L13	M			√
L14	M	√		
L15	F	√		
L16	F	√		
L17	F			√
L18	M		√	
L19	F		√	
L20	M			√
L21	M			√
L22	F		√	
L23	M	√		
L24	M		√	
L25	F		√	
L26	F		√	
L27	F		√	
L28	F		√	
L29	M	√		
L30	F		√	
L31	M		√	
L32	M			√
L33	M			√
L34	M		√	
L35	M		√	
L36	M			√
L37	M			√
L38	M			√
L39	M			√
L40	M			√
L41	M			√
L42	M			√
L43	M	√		
L44	M			√
L45	F		√	
L46	M			√
L47	F			√
L48	F			√
L49	M			√
L50	F			√
L51	M		√	
L52	M		√	
L53	F		√	
L54	M		√	
L55	F		√	
L56	M			√

"√" = Present

the serum samples tested positive by dot ELISA, whereas urine sample of the same dog found negative in present study. This happen due to chronic infection where organism and clinical signs may relapse and reappear but IgG detection persists and also due to poor sensitivity

of DFM which are the major reason could be drawn. Therefore, employing two tests is minimum required to detect the infection, which is more reliable for definitive diagnosis.

As, there was no vaccination done in dogs, 26 dogs out of 56 diagnosed positive for canine leptospirosis. Overall 46.42 percent prevalence of leptospirosis from dogs were recorded in our study. Representative photos of dot ELISA test results portrayed in Fig 2. Senthil et al. [31] reported 44.4 percent prevalence in dogs at around of Namakkal, Tamil Nadu state of India, which is in agreement with our study. Bhatia et al. [11] reported overall 35.33 percent prevalence of canine leptospirosis in Bidar, Karnataka state of India. Prameela et al. [29] reported 36 percent of prevalence by testing urine and serum sample in Tirupati city of Andhra Pradesh, India. Meera [27] conducted study on prevalence of leptospirosis among the pet, pet owner, butchers and associated risk group and 26.41 percent positivity reported among dogs in Trichy, Tamil Nadu, India. Rani [30] conducted seroepidemiological study on leptospirosis in Andhra Pradesh and found 15.15 percent (15/99) positivity in dogs by MAT. Contrarily, [25] found 71.12 percent seropositivity from serum by using MAT at Kerala, India, which was higher than our study.

Sex wise prevalence was recorded to find out any significant difference in prevalence between the sexes (Table 3). In our study, no statistical significant difference observed. However, female dogs (55.55%) affected more than the male dogs (42.10%). Senthil et al. [31] found 21 percent and 20.9 percent prevalence in female and male respectively from Namakkal, Tamil Nadu state of India. On other hand, [28] concluded higher prevalence in male dogs (34.73%) than the female (25.45%) from Bidar, Karnataka state of India. This might be due to randomized study, age difference and different susceptibility Age group wise prevalence was recorded to find out influence of age upon leptospirosis occurrence and it is described in Table 3. Though there was no significant difference found, highest prevalence was recorded 63.63 percent (07/11) in 1- ≤ 3 years group followed by 51.85 percent (14/27) in > 6- ≤ 12 years group whereas 27.77 percent (05/18) was recorded in > 3- ≤ 6 years. Nandini [28] found highest prevalence (46.34%) in 1-3 years of age group of dogs followed by 12 years and above age group (45.45%) whereas 20 percent in 3-6 years of age group studied from Bidar, Karnataka which is in accordance to our findings. Contrarily, [31] reported highest prevalence (37.2%) in 3-4 years of age group followed by 2-3 years of age group (28.9%) and 1-2 years of age group (26.3%) from Namakkal, Tamil Nadu. Our study showed that this disease is affecting very young (below 3 years) and very aged dogs (6-12 years). It might be due to compromised health status of dogs at these age groups.

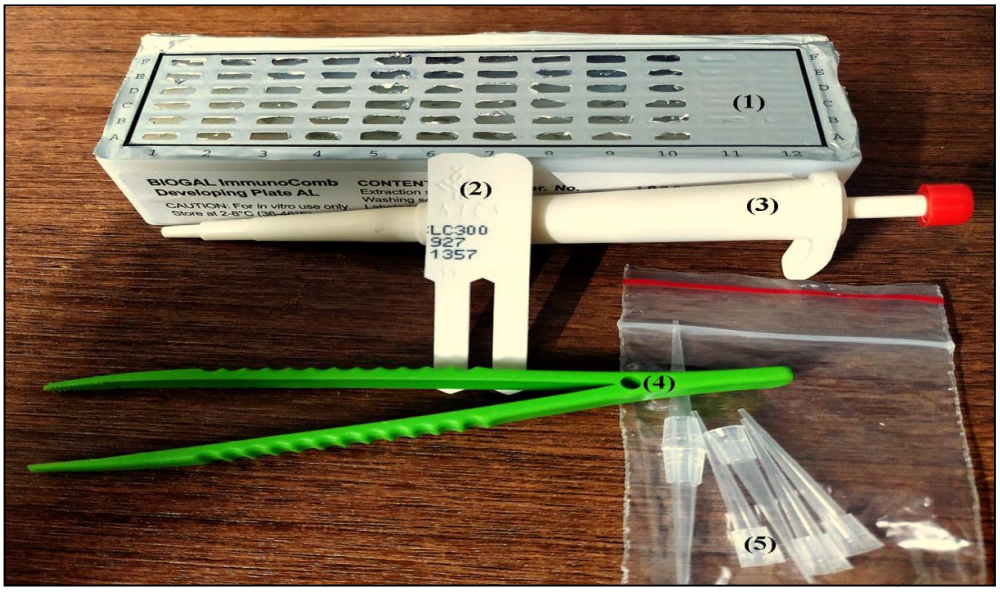

Fig. 1. Kit components namely, Developing plate (1), Comb having two tooth (2), 5 µl pipette (3), tweezers (4), tips (5)

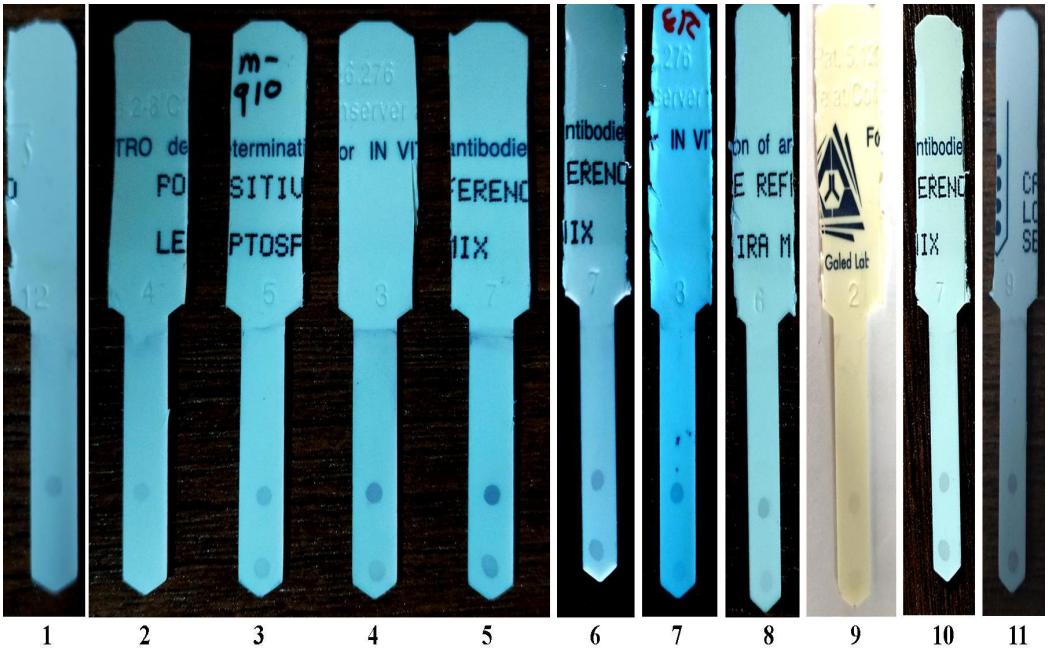

Fig. 2. Representative photograph of comb tooth showing results, Negative samples (1 and 2), Positive sample (3 to 11)

Table 2. Sample wise positivity for canine leptospirosis

Type of sample	Total samples	Percent positivity
Urine	33	13 (39.39%)
Serum	46	17 (36.95%)
Total Sample	79	30 (37.97%)
		χ^2 = p>0.05

Table 3. Sex wise and Age group wise prevalence of canine leptospirosis

		Total suspected dogs	Positive
Sex wise	Male	38	16 (42.10%)
	Female	18	10 (55.55%)
Age group	1- ≤ 3 years	11	07 (63.63%)
	> 3- ≤ 6 years	18	05 (27.77%)
	> 6- ≤ 12 years	27	14 (51.85%)
	χ^2 = p>0.05		
Total		56	26 (46.42%)

4. CONCLUSION

Leptospirosis is one the potential zoonotic threat globally. Leptospirosis is endemic in coastal area of south Gujarat. Dogs are generally most preferred pet animal in all household. Pet dogs are major source of transmission. They can act as bridge between the organism and host. Pet can easily get infection due to their sniffing behavior and contact with steady contaminated water. Therefore, regular vaccination must be required to breach transmission. We reported overall 46.42% leptospira infection in dogs, which may useful in epidemiological survey. Dogs having age group of 1- ≤ 3 years found more susceptible for leptospirosis. More studies are required to find out prevalence of dominant serovars and genetic underlying changes if any which might be helpful in vaccination strategies.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our

area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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