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Sero occurence of brucellosis in dogs of Telangana state

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Abstract
The present study was undertaken to know the sero occurence of brucellosis in dogs from Telangana state. A total of 400 (171 North and 229 South Telangana) blood samples were collected from dogs and sera samples subjected to four serological tests namely RBPT, LFA, STAT and ELISA. Out of 400 serum samples from dogs of Telangana state, the prevalence of *Brucella* was 11 (2.75%), 12 (3.00%), 9 (2.25%) and 13 (3.25%) by RBPT, LFA, STAT and ELISA respectively. The prevalence in dogs in less than 1 year was zero by all the methods, in 1-5 years age group it was 5 (2.92%), 4 (2.34%), 4 (2.25%) and 5 (2.92%) and whereas in above 5 years age it was 6 (3.19%), 8 (4.26%), 5 (2.66%) and 8 (4.26%) by RBPT, LFA, STAT and ELISA respectively. The prevalence in 1-5 years age group of North Telangana was 2.78% by all methods and in above 5 years it was 2 (2.44%), 3 (3.66%), 2 (2.44%) and 3 (3.66%) by RBPT, LFA, STAT and ELISA respectively. The prevalence in 1-5 years from South Telangana was 3 (3.03%), 2 (2.02%), 2 (2.02%) and 3 (3.03%) and in above 5years it was 7 (3.06%), 7 (3.06%), 5 (2.18%) and 8 (3.49%) by RBPT, LFA, STAT and ELISA respectively. Out of 130 male dogs from Telangana state 2 (1.54%), 2 (1.54%), 1 (0.77%) and 2(1.54%) and out of 270 females 9 (3.33%), 10 (3.70%), 8 (2.96%) and 11 (4.07%) were positive by RBPT, LFA, STAT and ELISA respectively.

Keywords: Dogs-brucellosis-serodiagnosis-Telangana state

Introduction
Brucellosis is an important contagious disease causing infertility and abortion in dogs. Incidence is higher in stray dogs than in pets [1]. Dogs can be infected by four species of *Brucella i.e, Brucella canis, Brucella abortus, Brucella melitensis* and *Brucella suis* [2, 3, 4]. *Brucella canis* is infrequently associated with human disease [4]. Dogs can acquire infection by the consumption of infected aborted placentas and fetuses, vaginal discharge or milk. Veneral transmission appears to occur most frequently when infected animals are bred to susceptible [3, 4]. Socio-economic deprivation, as well as the changes in the urban and peri-urban environment due to the development of slums and informal communities, has resulted in increased dog populations and thus a dramatic increase of canine roamers in these communities [5]. Infection acquired through environmental contamination is also possible, especially in areas where dogs often urinate or where vaginal discharges are deposited [6]. Furthermore, dogs living together are at higher risk of infecting each other, with urine being the most important source of infection in these cases, especially from male dogs.
Diagnosis of canine brucellosis is difficult because of unstable serum antibody titers that vary from individual to individual as well as between different methods used for their detection [7]. Serology is still the most commonly used method to diagnose *Brucella*, but must be used in combination with more specific methods like the Tube Agglutination Test (TAT) and repeated blood culturing is necessary to confirm diagnoses [8]. The different serological tests differ in sensitivities and specificities, which might lead to false positive and negative results. The absence of *Brucella* antibodies may also be a possibility during the early stages of the infection as well as in chronically infected animals. Blood is the best material to use because it is easy to collect and sterile and will allow for uncontaminated cultures [9].
Much information is not available on the prevalence of Brucellosis in dogs not only in Telangana state but also in other States of India. Hence, the present work was undertaken to study the sero occurence of *Brucella* spp in dogs of Telangana State

Material and Methods

Collection of samples

For collection of samples Telangana state was divided into North Zone (Adilabad, Nizamabad, Karimnagar, Warangal, Khammam districts) and South Zone (Hyderabad, Rangareddy, Mahaboobnagar, Nalgonda and Medak districts) based on erstwhile districts. The blood samples were collected from the Dogs maintained in the Kennel, cases coming to veterinary institutions and pet dogs maintained by individuals. Human blood samples were collected from workers and staff in the kennels, staff in the veterinary institutions and persons closely associated with the pet dogs, using adequate equipment and handled according to OIE and WHO specifications. The samples were carefully collected and packed, avoiding and possibility of leakage or cross-contamination. Individually identified containers were placed in large and strong outer containers and packed with enough absorbent material to protect from damage and packed in a cooler bag with ice packs and kept cool during transport from the place of collection to the laboratory as recommended in the OIE Manual (2000).

Blood and Serum

About 2 ml of blood was aseptically collected from the Dogs into vacutainer tubes (AcCuvet, Quantum Biologicals Pvt Ltd, Chennai) with Heparin. Further, 5 ml of blood was collected in a vacuette with serum clot activator (BD). The vacuettes were kept in upright position at room temperature for about 2 h. The separated sera was collected in a screw capped plastic vials and transported to the laboratory. The serum samples were heat inactivated at 56 ºC for 30 min and Merthiolate (1:10,000) was added in all vials as preservative. The sera and blood samples with anticoagulant were stored at -20 ºC till further use. Serum was separated by centrifugation within 24 hours of collection. A part of sera samples were stored at -20˚ C until further analysis for serological diagnosis.

Serological diagnosis

1. Rose Bengal Plate Test

The Rose Bengal Plate Agglutination Antigen was obtained from Institute of Animal Health and Veterinary Biologicals, Hebbal, Bengalure. With the help of micropipette, one drop (0.03ml) of serum is placed on glass slide. The antigen bottle was shaken to ensure homogenous suspension and one drop (0.03ml) was added to the serum on the slide. The antigen and serum were mixed with a spreader to area about 2.5 cm diameters and then the slide was manually rotated for four min. The results were observed immediately after four minutes. The test was examined for agglutination in bright light. Any degree of agglutination was taken positive and no agglutination was taken as negative.

2. Standard tube agglutination test

The Antigen obtained from obtained from Institute of Animal Health and Veterinary Biologicals, Hebbal, Bengalure was used. All serum samples were tested up to minimum five dilutions. For high titre sera, more dilutions were prepared in order to achieve end point titre. Five tubes were placed in rack. 0.8 ml of 0.5% phenol saline was taken in first tube and 0.5 ml in rest of the tubes. 0.2 ml of serum was added in the first test tube and mixed the contents. 0.5 ml of this mixture was added to the second tube. The process was continued up to fifth tube and 0.5 ml was discarded from the last tube after mixing. 0.5 ml antigen was added to each tube and mixed. This will provide dilutions of 1:10, 1:20, 1:40, 1:80, and 1:160 and so on. Considering the significance of 50 per cent end point a control tube was set up to simulate 50 per cent clearing by mixing 0.5 ml antigen with 1.5 ml of 0.5 ml phenol saline in an agglutination tube.

All the tubes were incubated 37 ^{0}C for 20 h before result was read. The degree of agglutination was judged by opacity of supernatant fluid. The highest serum dilutions showing 50 per cent or more agglutination (50% clearing) was considered as the titre of serum. The titre so obtained was expressed in unit system by doubling of the serum titre as International Unit (I.U) per ml of serum. 40 I.U per ml or above considered as a positive for brucellosis.

3. Lateral flow assay (Immuno-chromatographic assay)

The testing device was removed from the foil pouch by treating at the “notch” and placed on a level surface. Holding the sample dropper vertically, 10µl of specimen was added without air bubbles into the sample well and add one drop of sample diluents marked with an arrow on the testing device. Waited for the purple colored test band to appear and then the results were read. Positive results were read as soon as it appears; whereas Negative results were confirmed in 20 minutes. The background of the test window was taken white before interpreting the results.

4. ELISA

Brucella antibody test kit, (*Brucella* Serum) along with the user manual was procured from IDDEXX CHEKIT, USA. The test was performed as per the instructions of supplier manual.

Results and Discussion

A total of 400 blood samples of dogs were collected from two regions of Telangana state i.e. 171 samples from North Telangana region and 229 from South Telangana region and tested for prevalence of *Brucella spp.* using four serological tests i.e. RBPT, LFA, STAT and ELISA. The prevalence of Brucellosis in dogs was done by sex wise (Table 1) and age wise (Table 2).

Sero prevalence of brucellosis in dogs

The sero prevalence of Brucellosis in dogs from Telangana state was presented in Table 1. Out of 400 dogs the prevalence of *Brucella* was 11 (2.75%), 12 (3.00%), 9 (2.25%) and 13 (3.25%) by RBPT, LFA, STAT and ELISA respectively. Out of 171 dogs from North Telangana region 4 (2.34%), 5 (2.92%), 4 (2.34%) and 5 (2.92%) were positive by RBPT, LFA, STAT and ELISA respectively. Out of 229 dogs from South Telangana region 7 (3.06%), 7 (3.06%), 5 (2.18%) and 8 (3.49%) were positive by RBPT, LFA, STAT and ELISA respectively.

Sero prevalence of brucellosis in dogs by age wise

The distribution of 400 dog’s age wise was 41, 171 and 188 below 1yr, 1-5yrs and above 5yrs respectively. The prevalence of *Brucella* in dogs in less than 1yr was zero by all the four serological methods used in this study, similar type of findings were reported like zero prevalence of Brucellosis of dogs in age group of 0-6 months by RBPT, SAT, STAT and ELISA methods [10]. Higher prevalence of 2.3%, 4.48%, 4.34% and 33.3% in less than one year age group dogs was reported in Peru by AGID test [11], in Colombia [12] by AGID test, in Europe [13] by Agglutination test and in Nigeria [14] by

SAT respectively. Higher prevalence of Canine Brucellosis in the age group of 0-6 months (25% by RBPT and 20% by C-ELISA) and 6-11 months age group (29% by RBPT and 22.2% by C-ELISA) [15].

Table 2: Prevalence of brucellosis in dogs by age wise

Region	Age	No. Of samples tested	Rbpt		Lfa		Stat		Elisa	
			No of +ve	%	No of +ve	%	No of +ve	%	No of +ve	%
North Telangana	Below 1 year	17	0	0.00	0	0	0	0	0	0
	1 - 5 years	72	2	2.78	2	2.78	2	2.78	2	2.78
	Above 5 years	82	2	2.44	3	3.66	2	2.44	3	3.66
	Total	171	4	2.34	5	2.92	4	2.34	5	2.92
South Telangana	Below 1 year	24	0	0.00	0	0.00	0	0.00	0	0.00
	1 - 5 years	99	3	3.03	2	2.02	2	2.02	3	3.03
	Above 5 years	106	4	3.77	5	4.72	3	2.83	5	4.72
	Total	229	7	3.06	7	3.06	5	2.18	8	3.49
	Grand total	400	11	2.75	12	3.00	9	2.25	13	3.25

The prevalence of *Brucella* in 1-5 yrs age group was 2.92%, 2.34%, 2.25% and 2.92% by RBPT, LFA, STAT and ELISA respectively in the present study, which was less than the prevalence of 14.81% by RBPT, 11.11% by ELISA and 7.4% by SAT and STAT methods [10] Lower prevalence of 0.78% in the age group of more than one year in Colombia [17] and 1.69% in the dogs below 5yrs in Iran [12, 16] whereas in Nigeria [14] reported higher prevalence of 22% in the age group of less than 2 years. Higher prevalence of 5.6% by RSAT test and 6% by RBT test in the age group of less than 3 years, 5.9% in the age group of 1-4 years by AGID test, 7.56% in the age group of 3-5 years by Agglutination test in Europe and 30-33% in the age group of above 11 months by RBPT and ELISA in Nigeria [11, 13, 15 and 21] respectively.

The prevalence of Canine Brucellosis in the age group of 1-5 years was higher than the age group of less than 1 year in the present study. Dogs of sexually active (11 months and above) were more predisposed to Canine Brucellosis [15]. On contrary to the present study findings, higher prevalence of Canine Brucellosis reported (33.3%) in the age group of less than 1 year compared to 22% in the age group of less than 2 years [14].

The prevalence of canine brucellosis in the age group of above 5 yrs was 3.19%, 4.26%, 2.66% and 4.26% by RBPT, LFA, STAT and ELISA respectively. Higher prevalence of 9.3% in Iran by ICA test, 8% in the age group of 5-8year and no prevalence in the age group 9-15 years, 13.7% to 16.2% by RSA and RBT test and 5.55 to 6.03% in the age group of above 6 years was reported [11, 13, 16 and 17] respectively.

The prevalence was slightly higher in the age group of above 5 years compared to the prevalence in the age group of 1-5 years in the present study. *Brucella* in dog above 5 years was higher (9.3%) in comparison with dogs less than 5 years (1.69%) [16]. *Brucella* infection increases with age and that most diseased animal carries the infection throughout their life [19]. 2.3% prevalence reported in below 1 yr age group, 5.9% in 1-4 yrs age group and 8% in 5-8 yrs age group, which indicated that the prevalence increased as the age advances but it was zero prevalence in 9-15 yrs age group in Peru [19].

Sero prevalence of brucellosis in Dogs sex wise

Out of 400 dogs from Telangana state included in this study, 130 are males and 270 are females. Out of 130 males from Telangana state 2 (1.54%), 2 (1.54%), 1 (0.77%) and 2 (1.54%) were positive by RBPT, LFA, STAT and ELISA respectively. Out of 270 females from Telangana state 9 (3.33%), 10 (3.70%), 8 (2.96%) and 11 (4.07%) were positive by RBPT, LFA, STAT and ELISA respectively.

The sero prevalence in male dogs from Telangana state in this study ranged from 0.77% to 1.45% by various serological tests used. Higher prevalence of canine brucellosis in males of 2.6% in Japan by MAT test, 2.68% in Colombia, 3.36% in USA by agglutination test, 4.5% in Peru by AGID, 4.65% in Iran by ICA test, 5.13% in Europe by agglutination test, 7% in urban areas and 17% in rural areas in Zimbabwe by ELISA test, 15.9% by RBPT and 28.6% by C-ELISA in Nigeria, 18.4% to 21% in Turkey by IELISA, MPAT and 2 ME-TAT and 29.2% by SAT in Nigeria was reported [11, 12, 13, 14, 15, 16, 20, 21, 22, 23] respectively.

Table 2: Prevalence of brucellosis in dogs by sex wise

Region	Sex	No. of Samples tested	RBPT		LFA		Stat		Elisa	
			No of +ve	%	No of +ve	%	No of +ve	%	No of +ve	%
North Telangana	Male	59	1	1.69	1	1.69	0	0	1	1.69
	Female	112	4	3.57	4	3.57	4	3.57	4	3.57
	Total	171	5	2.92	5	2.92	4	2.34	5	2.92
South Telangana	Male	71	1	1.41	1	1.41	1	1.41	1	1.41
	Female	158	5	3.16	6	3.80	4	2.53	7	4.43
	Total	229	6	2.62	7	3.06	5	2.18	8	3.49
	Grand total	400	11	2.75	12	3.00	9	2.25	13	3.25

The seroprevalence in female dogs from Telangana state in this study ranged from 2.96% to 4.07% by various serological tests used, which was similar to seroprevalence of 3.87% reported [21] in USA using agglutination test. Higher prevalence of canine brucellosis in females of 4.82% in Colombia using ICT, 5.1% in Iran using ICA test, 5.3% in Peru using AGID, 6.18% in Europe by agglutination test, 9% in urban areas and 24 % in rural areas in Zimbabwe by ELISA test, 11.5% using RSA test and 12.2% using RBT test in Nigeria, 22.7% to 27.8% using I-ELISA, 2ME-TAT and MPAT in Turkey and 29.3% to 42.2% using C-ELISA and RBPT in Nigeria was reported [11, 12, 13, 15, 16, 17, 22, 23] respectively.

The prevalence of Canine Brucellosis was slightly higher in female dogs compared to male dogs in this study. This observation was similar to the findings [15, 16, 17 and 18] observed like higher prevalence in females which was not significant. This may be because a champion stud is more attractive to breeders and being a source of income, such stud is usually mated with many females and therefore putting the females at risk of getting infected [18]. Similarly, in uncontrolled mating among stray dogs; there is always the alpha male which mates all the female dogs in heat. Hence, the alpha male could eventually become infected with brucellosis and then transmits same to the females.

Sexual transmission is believed to be important since the organism is secreted in significant numbers in the semen of infected male dogs [24]. The differences of the infection prevalence between the sexes were due to variations of exposure to *Brucella* rather than to the biological diversity. A lower seroprevalence of *Brucella canis* antibodies in males than in females found in this study might be due to a decreased exposure to the agent. The decrease of male exposure to *Brucella* might be explained by a possible result of infection of the bitches [25]. By resorption of the fetuses, an infection of bones can rise to chronic Osteomyelitis and spondylitis in mother dogs leading a relatively higher occurrence of the infection in females than males.

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