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Prevalence of *Brucella abortus* agglutinins in canines in and around Korutla town, Telangana

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Abstract

Brucellosis is one of the highly infectious and contagious zoonoses of livestock and domestic animals caused by the *Brucella melitensis*, *Brucella abortus*, *Brucella canis* and *Brucella suis*. Generally brucellosis in canines caused by *B. canis* but sometimes especially, when dogs come in contact with infected livestock, they may get infected by picking the *B. abortus* organisms. Further, because of its zoonotic importance, these dogs are also act as source of infection for human beings. Hence, the present study aims to determine status of *B. abortus* agglutinins in dogs in and around Korutla town. The sera samples (n=81) were aseptically obtained from apparently healthy dogs that were brought to the teaching veterinary clinical complex between June, 2022 and September, 2022 and the samples were subjected for screening for the presence of *Brucella abortus* agglutinins by using standard screening test, Rose Bengal plate agglutination test (RBT). The obtained results were statistically analyzed by employing chi-square test and Odds ratio analysis and identified the statistical significance of the association of various risk factors with occurrence of brucellosis in canines. The study displayed that the sero-positivity of brucellosis in canines as 6.1% (5/81). Further, the factors such as managemental practices (outdoor and indoor) (p= 0.033) and breeds of the dogs (p= 0.04) are considerably correlated with the occurrence of brucellosis in canines.

Keywords: *B. abortus* agglutinins, brucellosis in canines, sero-prevalence

Introduction

Brucellosis is a highly contagious and one of the most significant zoonoses induced by certain brucella species that are pathogenic to wide range of animal species and their associated humans (Momoh *et al.*, 2015)^[1]. Brucellosis in canines is commonly caused by *Brucella canis* (Carmichael 1990, Wanke 2004 and Santos *et al.*, 2021)^[2, 3, 4], that has been reported at global level, and is having high potential of public health significance as the humans are having close association with dogs, in addition to the occasional infections induced by *B. melitensis*, *B. abortus* and *B. suis*. In canines, the infection is mostly transmitted by ingesting the infected materials such as aborted fetus, placenta, contaminated tissues, feeding of raw milk/under pasteurized milk obtained from infected animals and also transmitted by sexual transmission (Woldemeskel, 2015 and Baek *et al.*, 2003)^[5, 6]. The disease spreads quickly between closely confined dogs and no significant disparity of sex in spread of disease. The first time infected female canines will show the pathognomic symptoms such as miscarriage during last trimester of gestation with any other abnormalities like still births, infertility, importantly continuous vaginal discharges for longer periods can be seen while during subsequent pregnancies. In male dogs, the pathognomic symptoms are epididymitis, orchitis, prostatitis due to which inflammatory conditions the dogs will be hesitant to mate (Santos *et al.*, 2021)^[4]. However, asymptomatic infections are not uncommon in dogs.

Canine brucellosis is can largely be diagnosed by using serological techniques, since the agent is a potent zoonotic pathogen which requires special infrastructure facilities like special biosafety cabinets for confirmation by the gold standard test, cultural isolation and identification in addition to the requirements of skilled personnel. However, the diagnosis of brucellosis in canines by serology based techniques is remaining a contesting method due to low diagnostic accuracy of existing tests (Shankarlingam *et al.*, 2022). The Rose Bengal plate agglutination (RBT) test is considered as an ideal screening test in endemic areas where there is no proper infrastructure and good laboratory settings and availability of other limited resources. It is a test based on the property of agglutination of specific antibodies and smooth lipopolysaccharides (LPS) and having overall sensitivity of 92.9%, hence it should carefully be considered in the endemic areas.

In India, semi intensive method of grazing is most commonly practiced by the livestock farmers. Brucellosis caused by *B. abortus* is one of the most frequent ignored zoonotic diseases of livestock from India. These organisms excreted through raw milk, aborted foetus, and placenta of infected livestock. In semi intensive system of grazing these secretions may contaminate the water sources and pasture lands, hence they may act as infection sources for susceptible animals. During roaming, the dogs may get infected by picking the *B. abortus* organisms. Further, because of its zoonotic potential, these dogs are also act as source of infection for human beings. Recently Sonali *et al.*, 2022 [17] reported 4.4% sero-prevalence of brucellosis in apparently healthy bovines in Korutla town and in this area the livestock have enough contact with canines. Further, the availability of the data about the prevalence of *B. abortus* agglutinins in canines from this area is meagre. Therefore, the present study aims to determine status of *B. abortus* agglutinins in dogs in and around Korutla town, Jagtial district Telangana, India.

Materials and Methods

The blood samples (n=81) were aseptically collected from apparently healthy dogs that were brought to Teaching Veterinary Clinical Complex, College of Veterinary Science, Korutla town, Jagtial district, Telangana. The samples were collected from apparently healthy canines of both sex of different age groups and information on management (Indoor or outdoor) of dog was also collected from owners.

Approximately, 5 milliliter of venous blood (cephalic vein) was obtained in evacuated glass tubes with the help of needle and adopter aseptically. The sera (n=81) were extracted from collected blood samples after allowing sufficient time for clotting in slanting position and proper centrifugation at 1500 rpm for 10 min. Further the sera were properly labeled and stored at -20 °C till they were analyzed by RBT to identify the presence of antibodies against *Brucella*.

RBT

It is a standard screening test recommended by the OIE for screening the brucellosis at individual and herd level (Saavendra *et al.*, 2019) [16]. The protocol described by Diaz *et al.*, 2011 [7] was followed in this study for screening of the sera samples. The RBT antigen was procured from IAH&VB, Bengaluru. The obtained results were recorded and coded using Microsoft® Excel for Windows 2010 and statistical analysis was performed for Chi-square test and odds ratio by using SPSS software version 22.0 (IBM Corp., N.Y., USA) to determine the significance of the association of the risk factors (sex, age, breed, parity, and health status of the animals) for occurrence of the disease. Differences among groups of each factor were considered significant at $p < 0.05$ for all parameters tested.

Results

Five serum samples were positive out of 81 sera tested, giving 6.1 percentage of sero-positivity. On Chi-square analysis, it was found that out of four risk factors, the breed of the animal ($p=0.04$) and management of dogs ($p=0.03$) were associated with the presence of *B. abortus* agglutinins (Table 1). The odds ratio results revealed that the chances of occurrence of *B. abortus* agglutinins in indigenous breeds were 11.2 times higher than crossbred dog breeds. Similarly, the dogs raised in outdoor management system were showing 12 times more possibility of depicting positive for the presence of *B. abortus* agglutinins than the dogs raised in indoor system of management. The percentage of positivity of *B. abortus* agglutinin was 7.5 in female dogs, which is higher than male dogs (4.9 %) with the presence of *B. abortus* agglutinins. The percentage of positivity of *B. abortus* agglutinin was 9.3 in dogs with less than 2 years of age, which is higher than dogs with more than 2 years age (5.3%). Further, the association of age and sex factors was not significant with the presence of *B. abortus* agglutinins in canines.

Table 1: Univariate Analysis of risk factors associated with seropositivity of *B. abortus* Agglutinins

Variable	Total Samples	Positive by RBT	Percentage of positivity	Chi square	p value	OR (odds ratio)
Sex						
Female	40	3	7.5	0.0008	0.97	1.58
Male	41	2	4.9			
Breed						
Indigenous breeds	24	4	16.7	4.16	0.04	11.2
Crossbred	57	1	1.8			
Age						
Less than 2 years	43	4	9.3	0.25	0.61	2.9
More than 2 Years	19	1	5.3			
Management						
Outdoor management	23	4	17.4	4.53	0.033	12
Indoor management	58	1	1.7			

Discussion

India is one of the high burden countries that endemic to bovine brucellosis and having no stringent legislation for the sanitary disposal of animal carcasses and infectious animal tissues or materials, hence there is a high potential for transmission of disease from ruminants to dogs (Athira *et al.*, 2021) [9]. Canine brucellosis holds the nickname, 'The great Imposter' as the clinical signs of infection could mimic many other diseases. In India, the first report of *Brucella* infection in dog was reported by Pillai *et al.*, 1991 [10] from Chennai. In the present study, 6.1 per cent of sero-prevalence of *B. abortus* agglutinins was recorded by using Rose Bengal Plate test as screening test. Serologically, antibodies of *B. abortus*

and *B. canis* are known not to cross react (Corgel *et al.*, 1979 and Osinubi *et al.*, 2004) [11, 12]. The five reactors observed in the present study are therefore indicative of true presence of *B. abortus* agglutinins, confirming the previous exposure to the *B. abortus* organism. The present study results were also in agreement with the findings of Lingam *et al.*, 2020, who reported 2.75 % of sero-positivity in canines in Telangana by using RBT as screening test. Further, the similar percentage of sero-prevalence of brucellosis in canines was observed in the states adjoining to the Telangana *i.e.*; 9.8% in Punjab (Sharma 2014) [15], 2.18% in Tamil Nadu (Pillai *et al.*, 1991) [10] and 7.69 % in Uttarakhand (Maansi and Upadhyay, 2015) [14].

Further, the risk factors like the management and type of breed were significantly associated with the occurrence of *B. abortus* agglutinins in canines. The higher percentage of *B. abortus* agglutinins was seen among the dogs kept outdoors (17.4%) than those kept indoors (1.7%). Similar results are reported by Momoh *et al.*, 2015^[1] in Zaria, Nizeria. They observed higher sero-prevalence of canine brucellosis in dogs maintained outdoors (37.1%) than those maintained indoor (13.9%). There have been earlier reports of *B. abortus* infection in cattle in the present survey area (Sonali *et al.*, 2022)^[17]. The most of the dairy farmers belongs to present study region were following the semi intensive method of rearing, where the animals are not only exposed to sunlight but also they can move and rest at their will. Further, *B. abortus* organisms reported to be persist in vaginal discharges for up to 42 days after abortion or parturition. It is reasonable to speculate that aborted material and infected vaginal discharges from cattle may contaminate the pasture (Baek *et al.*, 2003)^[6]. In outdoor management system, Dogs have every possibility of coming in contact with these contaminated pastures and may acquire the infection from cattle posing owners and handlers of these dogs to the risk of infection.

The chances of occurrence *B. abortus* agglutinins were 11.2 time more in the cross bred dogs than in indigenous dogs. Similar results were reported by the Athira *et al.*, 2021^[9], who observed 27.7 percent of seroprevalence of *B. abortus* agglutinins in dogs of various breeds like Labrador, Rottweiler, Beagle, Spitz and German shepherd from Thrissur, Kerala. Rhyan *et al.*, 2000^[18] stated that many exotic breeds of dogs are imported to our country, if they are not screened before entry into the country because most of the times these dogs are used for breeding, may contribute the re-emergence of brucellosis. Cosford (2018)^[19] advised that all adult dogs should routinely be screened serologically for presence of brucellosis before mating and in *Brucella* tested positive cases, it is recommended to neuter the infected canines despite of their high genetic value.

Further, in the present study all the serum samples were collected from apparently healthy dogs and no dog was presented any type of clinical signs related to reproductive system infection. This indicates that the owners and handlers of these dogs are at high risk of getting infection. Therefore, adequate precautions should be taken by the clinicians, animal handlers and veterinary students while handling the apparently healthy animals and owners of the dogs should go for routine clinical examination of their dogs (Osinubi *et al.*, 2004)^[12].

Conclusion

The *B. abortus* infection in dog population appears to be an emerging zoonotic problem and it appears that dogs of sexually active age group are more predisposed to *Brucella* infection. This study established risk factors such as outdoor management and breed were significantly associated with the occurrence of brucellosis. As Brucellosis is a potent zoonotic disease and human population is equally susceptible along with other animals, it is very necessary to create awareness on canine brucellosis and organize the enlightenment programmes with emphasis on its zoonotic importance to control the spread of brucellosis.

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