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SCREENING OF BRUCELLOSIS IN DOGS USING ROSE BENGAL PRECIPITATION TEST (RBPT) AND CANINE BRUCELLOSIS ANTIBODY RAPID DETECTION TEST (GENOMIX)

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Abstract: Serological screening of canine brucellosis was conducted using Rose Bengal Precipitation Test (RBPT) and Genomix Canine brucellosis Antibody Rapid Detection Test Kit. A total of 91 canine serum samples (31 from asymptomatic and 60 from dogs with clinical signs suggestive of brucellosis) were screened with RBPT and Canine brucellosis Antibody Rapid Detection Test (Genomix). The results showed an overall positivity of 4.4 and 1.1 per cents by RBPT and Canine brucellosis Antibody Rapid Test (Genomix) respectively. High percentages of positive cases were seen in n ≥5 year's age group, females and exotic breeds. Also, high positive cases were seen in dogs with infertilility and skeletal problems compared to apparently normal. The study arrived at the conclusion that the screening of brucellosis in dogs could be done with RBPT and Canine brucellosis Antibody Rapid Detection Test (Genomix) for *Brucella abortus* and *Brucella canis* respectively.

• Introduction

Brucellosis is of particular concern in India because nearly 80% of the Indian population resided in rural areas in close contact with livestock like cattle, sheep, goat etc (3). Hence, human population stands at a greater risk of acquiring zoonotic diseases including brucellosis (14). Brucellosis is an infectious disease affecting domestic and wild animals which impose serious health implications to both human and animals. Brucellosis causes considerable economic losses at the capacity of livestock farmers and also has a reasonable health impact, particularly among occupational groups since it is anthropozoonotic in nature and associated with male and female infertility. The disease has been identified as a vital public health problem in various parts of the world. Almost all the species of livestock including cattle, goats, pigs,horses and dogs plays significant role in the transmission of brucellosis to man (3).

Diagnosis of *B. canis* infection is very challenging. Although the dog is the most common host of *B. canis*, canine infections with other *Brucella* spp. such as *B. suis* (10) and *B. abortus* (17) may occur. Importantly, *B. canis* is serologically distinguished from *B. melitensis, B. abortus,* and *B. suis,* which carry a smooth Lipopolysaccharides (LPS), and therefore their antigens do not react with anti-*B. canis* antibodies (4). However, none of the serological tests currently used for the diagnosis of canine brucellosis are completely satisfactory. Serologic diagnosis of *B. canis* infection is challenging, and a combination of different tests is highly recommended (11). Since the *Brucella* spp. infects not only their preferred hosts but also other domestic and wild animal species, which in turn can act as reservoirs of the disease for other animal species and humans. The present study aim to screen brucellosis in dogs using RBPT to detect *B. abortus* antibodies which carry a smooth LPS and Canine Brucellosis Antibody Rapid Detection Test (Genomix) which is specific in detecting *B.canis* antibodies which carry a rough LPS.

• Material and method

Experimental design

This study was designed to screen for brucellosis in dogs by RBPT and Canine Brucellosis Antibody Rapid Detection Test (Genomix) from small animal clinic, obstetrics and gynecology and orthopaedics units of Madras Veterinary College teaching hospital, MVC, Chennai. The study area and animals were sampled using simple random sampling to represent the target population. The samples were collected randomly from dogs with history of abortion, infertility, spondylitis and apparently healthy ones of different ages and sexes.

Sample collection

Sera samples were collected from randomly selected dogs from small animal clinic, obstetrics and gynecology and orthopedics units of Madras Veterinary College teaching hospital, MVC, Chennai, India. About 3 ml blood sample was collected from each of the 91 dogs by cephalic vein puncture, into sterile test tubes of 5 ml capacity. The tubes were left undisturbed until the sera cleared, and centrifuged at 2000 rpm for 15 minutes. All the sera samples were numbered and stored at – 20^{o}C until further use.

Serological tests

Rose Bengal plate test (RBPT)

The colored antigen required for RBPT was obtained from the Division of Biological products, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, the test was performed as per the standard protocol of agglutination test (20). Briefly, a drop of serum (30 µl) was placed on clean grease-free glass slide and an equal quantity of antigen was added and mixed thoroughly using an inoculation loop.The mixture was observed for clumping / agglutination for one minute and the results were recorded as agglutination (+) or no agglutination (-).

Canine Brucellosis Antibody Rapid Detection Test Kit (Genomix) (Hyderabad)

The testing device from the foil pouch was removed by tearing at the "notch" and then placed the testing device on a level surface. 5µl of specimen was added without air bubbles into the sample well by holding the Sample dropper vertically followed by addition of 2 drops of Sample diluents marked with an arrow on the testing device. The appearance of distinct pink colored bands at the control and test line regions were taken as positive for *Brucella canis* antibody and the appearance of distinct pink colored band only at the control line region and no colour development at the test line region even after 20 minutes, the test were taken as negative for *Brucella canis* antibody (Fig. 1). Also, if there is no visible pink band at control region, the test was taken as invalid tests systems and the test was repeated with a new test device.

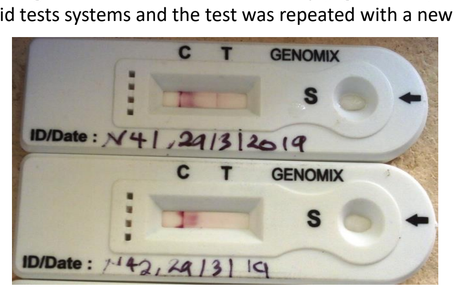

Fig.1. Canine brucellosis Ab Rapid Test (Genomix)

• Results and discussions

In this study, overall seropositivities of 4.4% and 1.10 % were observed respectively by RBPT and Canine Brucellosis Ab Rapid Test (GENOMIX®) (Table 1). Among the 91 sera samples screened by RBPT and Canine Brucellosis Ab Rapid Test (GENOMIX®), the highest percentage of positivity was observed in the ≥5 years of age group, with 3.30% and1.10% respectively. Sex distribution of the cases had high positivity in females (3.3%) compared to males (2.2%). Pedigreed dogs showed high seroposivitity with 4.40% in RBPT compared to non-descsripts with 1.10% in Canine Brucellosis Ab Rapid Test kit (Table 1). Base on health status of an animal, the study recorded 2.2% in each of infertility and spondylitis cases and 1.10% apparently healthy ones (Table 1).

Among the serum collected from clinical cases with various clinical signs (mainly, infertility and spondylitis cases) and also from apparently healthy ones, the RBPT and Canine brucellosis Ab test kit showed a positivity of 4.4 and 1.1% respectively. The variation in the positive percentage may be due to the fact that, the RBPT was performed using *Brucella abortus* colored antigen which could detect only *B. abortus* species and the canine brucellosis Ab Rapid Test kit could detect only *Brucella canis* antibodies in the serum.

Table 1
Percentage positivity of canine serum samples for brucellosis based on Canine brucellosis Ab Rapid Test and RBPT

Tests	Status of dog (91)				
	Infertility	Skeletal problems	General population	Total positives	Total % positives
Canine brucellosis Ab Rapid Test (Genomix)	-	-	1.10% (1/91)	1	1.10(1/91)
RBPT	2.2% (2/91)	2.2% (2/91)	-	4	4.4(4/91)

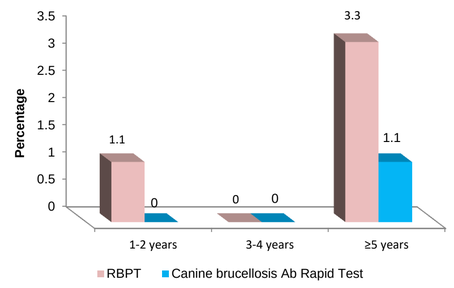

Fig. 2. Age wise distribution of brucellosis in dogs (%)

Another study, (5) reported a higher seropsitivity of 27.78 % than our findings using RBPT in epidemiological investigation for brucellosis in dogs of Thrissur, India. Also, (7) had reported a higher positivity of 12.72% by RBPT in a study on sero-epidemiological survey and risk factors associated with brucellosis in dogs. But, in the same study, sero positivity of 1.06 % in ELISA is very much similar to the 1.1% positivity of our study by employing canine brucellosis Ab Rapid Test. Similarly, (6) reported a seropositivity of 9.8 % by using canine brucellosis antibody test kit in a study on canine brucellosis in Punjab state of India and their public health significance. In Bangladesh (15) recorded an overall seroprevalence of canine brucellosis in 30 stray dogs at 10.0% with ELISA.

Female dogs had a higher seropositives percentage (3.3%) than male dogs (2.2%). A major contributing factor to higher rates in females could be that a single male dog, if infected, is used in mating different females, it can transmit the infection through infected semen (9). However, (19) have shown that erythritol, a polyhydric acid found in higher concentration in the placentas and foetal fluids of females than in seminal vesicles and testis of males, can be responsible for females being more susceptible than males. This result was in agreement with the findings of (9) who reported a prevalence of 6.17% in females and 4.9% in males and (12) who reported prevalence of 29.3% in female dogs and 28.6% in male dogs. However, it disagrees with findings in a previous study where a slightly higher rate in males (29.6%) than in females (26.7%) (2).

Seropositivity was lower among the young animals screened as compared to the older ones (Fig. 2.). Usually young animals are protected by maternal immunity and thus they are less susceptible to infections. This shows that the infection increases with age. The high prevalence seen in older animals shows the chronic nature of brucellosis as it has been shown to increase with age, and most affected animals carry the infection throughout their lives (19).

The reason for the increase in prevalence as the animal age increases may be due to the fact that the bacteria localizes mainly in the reproductive tracts, especially in gravid animals. There is also evidence that the mammary gland may be even a more probable area of localization than the reproductive tract (1). Age- wise prevalence studied by (1) and (12) showed that the incidence is higher in sexually mature animals. Therefore, the increase in age, increases probability of exposure to infection in dogs. However, the results in this study do not agree with previous study by (9), as they reported more prevalence in dogs below one year old than in adult dogs.

Pedeegre dogs showed high seroposivitity with 4.40% and on examination of breed involvement, Great Dane showed a higher per cent of overall positivity (2.2%) . The detection of canine brucellosis in exotic dogs may indicates a new source of infection from abroad as these dogs may be imported from countries and regions where the disease is endemic (8). The higher prevalence among the exotic breeds is in agreement with the findings of (8); they recorded a prevalence of 19.35% in exotic breeds. It is also in agreement with the findings of (9) who got 50.55% in Alsatian breeds of dogs.

• Conclusions

The overall positive case rates detected by RBPT and Canine Brucellosis Ab Rapid Test (GENOMIX®) were 4.40 and 1.10 % respectively, showing that there is a conspicuous presence of Brucella antibodies in the dogs population in the study area, indicating the presence of Brucella infections in the population and justifying the need for continued screening and confirmatory programs in a wider region of the disease in the study area which in turn might be useful for strategic planning to establish appropriate control measures and prevent further spread of an infection. The study also supported the evidence of cross reaction of brucella species in different animal species, as *Brucella abortus* antibodies was detected from dogs sera in this study.