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Zinvestigation of Canine Brucellosis in Klang Valley Malaysia

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Abstract: One-hundred and twenty three (123) blood samples were collected from dogs in Klang Valley Malaysia to investigate the present of brucellosis. On culture, all blood samples were negative for *Brucella* sp. The serological tests carried out were Rose Bengal Plate test (RBPT) and 2-mercaptoethanol tube agglutination test (ME-TAT). The RBPT found that all serum samples were negative for *Brucella* infection. However, ME-TAT shown six serum samples were positive to *Brucella* infection with a high antibody titer (1/200).

Key words: Dog, brucellosis, serological tests, RBPT, ME-TAT

INTRODUCTION

Brucellosis is a disease mainly affecting the reproductive tract of domestic animals. Brucellosis in dogs is a relatively new disease. It is caused by *B.canis* and the clinical signs involved are abortion, epididymitis, periorchitis, and prostitis. It was first recognized in 1966 in the United States of America[1]. Ever since the finding, the disease has been reported from a number of countries in the world. This disease is of concern because it has been reported that canine brucellosis can infect human being[2]. Infection can occur through direct contact with the vaginal discharges of infected bitches.

An investigation of the disease was done by P.G. Joseph from the Veterinary Research Institute (VRI), Ipoh, Malaysia in 1981[3]. The study was started with serological testing of canine sera collected from stray dogs submitted to VRI and the Animal infirmary of Kinta Veterinary Department, Ipoh. Serum samples were also obtained from cases submitted by veterinarians stationed in Petaling Jaya, Selangor. The study discovered one positive case for canine brucellosis out of 55 dogs. The population of human in Klang Valley is among the highest in the country with a total population of 3329503 people or 12% of the total Malaysian population[4]. Large number of people may be in constant contact with potentially infected dogs. The information for brucella infection is still lacking.

Thus, the aim of this present study is to isolate and identify *Brucella sp.* from dogs and to determine the seroprevalence of brucellosis in dogs in Klang Valley, Malaysia.

MATERIALS AND METHODS

Animals and samples: One hundred and twenty three (123) dogs from two stations were selected from DBKL-*sp.*CA and PAWS in Klang Valley, Malaysia. Blood samples were collected from a mixture of both male and female dogs and all of them were adult mongrels.

Isolation of *Brucella sp.*: Approximately 100 µl of blood were inoculated into a *Brucella* transport medium. The inoculated medium was then incubated at 37°C for 24 hours. A drop of inoculated media was plated on *Brucella* agar and incubated at 37°C for 5 days. Modified Zield-Neilson stain was carried out to determine the cell morphology of *Brucella.* Suspected *Brucella* colony was inoculated into a new agar plate for further identification.

Serology

Rose Bengal Plate Test (RBPT) : Approximately 30 µl of serum sample and 30 µl of *Brucella* antigen in Rose Bengal stain were mixed together on a white plate and mixed thoroughly using a tooth pick. The mixture was then agitated for approximately three minutes. Positive sample will exhibit agglutination.

Mercaptoethanol Tube Agglutination Test (ME-TAT): One mL of formaldehyde was mixed with 9 mL of normal saline to make a 10% solution of FNS. A 3.5% NaCl solution was prepared by diluting 7 g of NaCl in 200 mL of distilled water. Antigen diluent was prepared by mixing 1.2 mL of 10% FNS and 199.8 mL of 3.5% NaCl solution. The ME solution were prepared by mixing 0.715 mL of ME to 99.285 mL of antigen diluent. In order to prepare the antigen, 2.2 mL of *B. canis* RM 6-66 P_2 antigen was added to 47.8 mL antigen diluent. The serum sample was diluted into a 1/25 dilution, 100 µl of serum was added into 2.4 mL of the diluent prepared previously. Diluted serum was then dispensed into 3 tubes labeled as tube 1, 2 and 3. Tube 1, 2, and 3 were filled with 1, 0.5, and 0.25 mL of the diluted serum, respectively. The volume in tubes 2 and 3

was topped-up to 1 mL by adding the diluent. One milliliters of antigen was mixed in each tube and agitated to ensure a proper mix of the diluent and serum sample. Positive and negative controls were set up for both tests using a high titer and negative sera. The mixture was then incubated at 37°C for 48 hours. The final dilutions of serum in the 3 tubes were 1/50 for tube 1, 1/100 for tube 2, and 1/200 for tube 3. Positive serum shows clearing of the mixture. The degree of clearing is scored from 0 to 4. A titer of 4 + at 1/200 dilution and above is considered positive. Titers below 4 + at 1/100 dilution are considered negative.

RESULTS AND DISCUSSION

All samples were negative for *Brucella sp.* on culture. All serum samples were negative for brucella infection on RBPT. Out of 123 serum samples, six samples were positive for *B. canis* infection on ME-TAT. All the positive samples had a titer of 4 + with the final dilutions of 1/200. This present study shows that 4.8% of dogs tested were serologically positive to *Brucella* infection. It is discovered that almost five dogs for every 100 dogs have brucellosis in Klang Valley Malaysia. This fact must not be overlooked. This is mainly because canine brucellosis constitutes a serious problem for dog breeders and pet owners. It is also zoonotic and may cause mild to moderate disease in humans. Moreover, there are people working closely with potentially infected dogs such as the dog catchers around Klang Valley.

Canine brucellosis is a relatively new disease and there have not been many reports of its prevalence here in Asia. Recent epidemiological studies have been carried out in Japan, Korea, Malaysia, China, and Sri Lanka. The prevalence of canine brucellosis in Japan for example ranges from 0.7-6.5%[5]. In Malaysia, there was only one positive case from 55 dogs used in the 1981 study of the disease[3]. The same range of prevalence was also reported in Korea and Sri Lanka. China has a range of 0.3-42.7%[6].

The serological methods used in this study include the Rose Bengal Plate Test (RBPT) and the 2-ME tube From National Veterinary Services Laboratory United States Department of Agriculture, Ames, Iowa, U.S.A. agglutination test (ME TAT). Both tests utilize the antibodies directed to surface antigens of the bacteria, mainly to the rough LPS[7]. In this study no serum samples were found positive for *Brucella* infection using the RBPT. It detects non-specific antibodies to surface antigen of the bacteria mainly the rough LPSs[7]. When the samples underwent the ME TAT, six samples were found to be positive for a high titer of antibodies against *B. canis*. 2-Mercaptoethanol tube agglutination test is able to differentiate active and inactive infection.

The mercaptoethanol removes the membrane immunoglobulins alone by destroying the disulfide bond linking the pentamer structure of the immunoglobulin, rendering it inactive, thus an initial high titer changing to a low titer with Mercaptoethanol suggests recent infection[8]. This inactivation of the immunoglobulins will ensure elimination of non-specific agglutination.

In bacteriological study, however no target organism was isolated. This may be due to the fact that the isolation of *B. canis* is lengthy and its sensitivity may be compromised by intermittent absence of bacteremia[9]. The organism is also a slow growing organism usually taking somewhere between 10-12 days to grow at temperature of 37°C without 10% CO_2. It also requires special media such as serum dextrose, tryptose, and brucella (Albimi) agars. To better eliminate contamination, isolation attempts should be made using media containing actidione, polymyxin B, and bacitracin. The factors influencing the prevalence may be the fact that the organism is readily transmitted to other dogs and also the increasing number of stray dogs. Dog to human transmission occurs mostly among people working with potentially infected dogs. These include veterinarians, animal caretakers, laboratory technicians, and even municipal workers. The result of this study may give some idea about the likelihood of encountering a dog with the disease. The people working close with potentially infected dogs should be more cautious at work because of its zoonotic potential.

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