

Seroepidemiology of canine brucellosis in Beijing, China

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Abstract: A seroepidemiological survey of canine brucellosis was carried out in Beijing, China. Serum samples were taken from dogs admitted to the Beijing Companion Animal Hospital for immigration and emigration inspection, including dogs from 4 breeding farms and stray dogs (n = 1200, 14 districts), between January 2008 and April 2009. All of the sera were first tested and preselected by Rose Bengal plate agglutination test (RBAT), and the positive samples by RBAT were determined by tube agglutination test (TAT) again. Of the 1200 dogs studied, 60 (5%) were positive by RBAT, 21 (51.7%) of which were confirmed by TAT. The seroprevalence of canine brucellosis was 1.75% (21/1200), and 5 samples were smooth-type, while 16 samples were rough-type. Rottweiler, Husky, Chihuahua, and Poodle are predisposed to brucellosis, and it occurred in all age groups. The prevalence of female dogs was higher than that of males. This suggests that canine brucellosis infection exists in Beijing.

Key words: Seroepidemiology, canine brucellosis, Rose Bengal plate agglutination test, tube agglutination test, China

1. Introduction

Canine brucellosis is a zoonotic disease caused by smooth-type and rough-type *Brucella* spp., which is distributed worldwide. The seroprevalence of canine brucellosis in different countries has been reported. There are geographic variations, such as in India (1), South Korea (2), Nigeria (3), Argentina (4), and Turkey (5).

Clinical manifestations of brucellosis in dogs are epididymitis, testicular atrophy, and sterility in male dogs, while the major symptom in females is abortion (6). Transmission to humans may be through contact with the semen, urine, and/or aborted fetuses of infected animals (4).

Dogs are popular companion animals in Beijing. Dog trade and entry and exit inspection are growing increasingly common. Most owners have close contact with their pets, providing favorable conditions for the transmission of zoonotic pathogens such as *Brucella canis*. The epidemiology of canine brucellosis in dogs in Beijing has not been previously studied. We undertook a seroepidemiological survey of canine brucellosis in dogs in Beijing over a 15-month period to assess the prevalence of canine brucellosis infection and provide a basis for management strategies and policies in preventing and controlling canine brucellosis in animals and humans.

2. Materials and methods

Blood samples were collected from the jugular or caudal vein of dogs by local veterinary practitioners between January 2008 and April 2009 in Beijing, China. Blood samples were centrifuged at 1000 × g for 10 min and the serum was frozen and stored at -20 °C until analysis.

The breed, age, sex, living environment, and food composition data were collected through a questionnaire by the local veterinarians at the time of blood collection. The owner's consent to the publication of the data was obtained.

A total of 34 dog breeds including large-, middle-, and small-type dogs were investigated in the survey. Blood samples were collected from 1200 dogs (648 males and 552 females). The ages ranged from 3 months to 15 years. Most dogs were kept indoors and fed commercial dog food. Shelter dogs were fed cornmeal and beef, and foster dogs housed in the animal hospital were provided with a commercial dog food.

Antibodies to *Brucella* spp. were determined using the Rose Bengal plate agglutination test (RBAT), and all of the RBAT-positive sera were analyzed using the tube agglutination test (TAT). The serological tests were performed at the Beijing Small Animal Hospital. The

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positive and negative controls and *Brucella* antigen (*Brucella canis*) of the smooth and rough types for RBAT and TAT were sourced from the Chinese Center of Disease Control and Prevention. Briefly, the *Brucella*-specific antigen (*Brucella canis*) was coated on a ceramic plate. Serum samples were added and the agglutination reaction was recorded after 4 min.

All of the positive sera were diluted in U-bottom 96-well microtiter plates. After incubation of the diluted serum sample (1:100) in the test well, 100 µL of antigen diluted by 0.5% phenol saline (1:20) was added before the 96-well plate was sealed, and the plate was then incubated at 37 °C for 22–24 h. Samples with more than 50% agglutination (titer of 1:80) were regarded as positive.

The tests were performed by the laboratory staff in animal hospitals.

The data were analyzed using SPSS 12.0 (SPSS Inc., Chicago, IL, USA). The results are given as mean ± standard deviation.

3. Results

Brucella antibodies were detected in 5% (60/1200) of the dogs by RBAT. Both smooth-type (17, 28.3%) and rough-type (43, 71.7%) were found. All of the detected positive sera were confirmed by TAT. Therefore, the seroprevalence

of canine brucellosis was 1.75% (21/1200) and the ratio of smooth-type to rough-type was 5:16. Detailed results are shown in Table 1.

Of the 21 seropositive dogs, 7 had reproductive abnormalities (pyometra, didymitis, metritis, hysteromyoma, abortion, or scrotitis) and 2 had skin disorders (dermatitis and molt).

Brucella antibodies were detected in all of the breeds, with the highest seroprevalence in Husky (6/71, 8.5%) and Chihuahua (1/17, 5.9%) and the lowest in Pekinese (2/299, 0.67%) and unidentified breeds (0/281) (Table 1). A serum sample from one Rottweiler also tested as positive. The seroprevalence between breeds was not significantly different.

As shown in Table 2, seropositive dogs were distributed in all age groups, with no differences among the age groups. The seroprevalence of canine brucellosis in the female dogs (2.35%) was higher than in the male dogs (1.23%) (Table 3).

Dogs fed dining table food had the highest prevalence (3/12, 25%) of brucellosis, while dogs fed commercial dog food had the lowest prevalence (2/1050, 0.19%). Detailed results are shown in Table 4. The highest occurrence of canine brucellosis was also seen in stray dogs (28.6%), while no foster dog was positive for *Brucella* (Table 5).

Table 1. Seroprevalence of canine brucellosis by dog breed.

Breed	Number of dogs (n)	TAT		
		Rough-type (number of positive samples)	Smooth-type (number of positive samples)	Seroprevalence (%)
Rottweiler	1	1	0	100
Chow Chow	53	1	0	1.89
Pekinese	299	0	2	0.67
German Shepherd	97	0	1	1.03
Poodle	219	4	2	2.73
Husky	71	6	0	8.45
Chihuahua	17	1	0	5.88
Golden Retriever	109	2	0	1.83
Labrador Retriever	53	1	0	1.89
Other	281	0	0	0
Total	1200	16	5	1.75

Chi-square = 0.800, P = 0.999.

Table 2. Prevalence of canine brucellosis by age group.

Age (years)	Number of samples tested (n)	TAT		Seroprevalence (%)
		Rough-type (number of positive samples)	Smooth-type (number of positive samples)	
0-1	282	3	0	1.06
1-2	340	4	2	1.77
2-3	177	4	0	2.26
3-4	107	1	0	0.39
4-5	121	3	1	3.31
5-6	94	0	1	1.06
>6	79	1	1	2.54
Total	1200	16	5	1.75

Chi-square = 0.714, P = 0.982.

Table 3. Prevalence of canine brucellosis by sex.

Sex	Numbers of sample (n)	TAT		Seroprevalence (%)
		Rough-type (number of positive sera)	Smooth-type (number of positive sera)	
Male	648	5	3	1.23
Female	552	12	1	2.35
Total	1200	17	4	1.75

Table 4. Seroprevalence of canine brucellosis by food composition.

Daily food composition	Numbers of sample (n)	TAT		Seroprevalence (%)
		Rough-type (number of positive sera)	Smooth-type (number of positive sera)	
Dining table food	12	1	2	25.0
Mixed food*	50	3	3	12.0
Commercial dog food	1050	2	0	0.19
Self-made dog food	88	10	0	11.4
Total	1200	16	5	1.75

*Mixed food: dining table food and commercial dog food.

Table 5. Seroprevalence of canine brucellosis by living environment.

Living environment	Numbers of sample (n)	TAT		Seroprevalence (%)
		Rough-type (number of positive sera)	Smooth-type (number of positive sera)	
Indoor	1083	5	2	0.64
Shelter	88	10	0	11.4
Foster	15	0	0	0
Stray	14	1	3	28.6
Total	1200	16	5	1.75

4. Discussion

Since the discovery of *Brucella* spp. in 1885 by Bruce (7), a number of serological assays including the RBAT, standard TAT, and immunofluorescence (8) have been developed for the detection of antibodies to *Brucella* spp. The RBAT and the standard TAT are routinely used to detect brucellosis in China.

The seroprevalence of canine brucellosis in dogs has been studied in some countries. One Argentinean study showed a 10.7% prevalence of canine *Brucella canis* infections (9), and a Japanese study revealed a prevalence of 2.5% (10). The seroprevalence of *Brucellosis canis* in China varied in different regions, ranging from 0.3% to 42.7% (11). Our study detected a prevalence of 1.75% (21/1200) in Beijing, and the positive rate in female dogs was approximately twice the rate in males, similar to the findings by Shang (11).

We observed that all dog breeds could be infected by *Brucella* spp., and Husky and Chihuahua appeared to be more prone to *Brucella* infection than other breeds. However, infection is most likely influenced by other factors such as the local dog population or owners than by dog breed.

Brucella antibodies were detected in dogs of all age groups. This is not surprising, since brucellosis could be

transmitted vertically from pregnant bitches to puppies (transplacental transmission) and horizontally between dogs (postnatal transmission) (12).

Most of the dogs seropositive for canine brucellosis were fed dining table food and self-made dog food. Most of the indoor dogs were fed commercial dog food, such as Royal Canin (Aimargues, France) and Pedigree (Mars Inc., USA) dog food. Generally speaking, the commercial dog foods were of high quality and contained balanced nutrition. Therefore, the incidence rate of canine brucellosis was the lowest. Dining table and self-made dog foods might be not disinfected and contained low nutrient value. The self-made dog foods were made from corn flour and bone or viscera from domestic animals. They might have high risks of infection with canine brucellosis. Malnutrition, insanitation, and complex environmental factors might be the main reasons for high occurrence of canine brucellosis.

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