



Investigation of infection of pregnant dogs in the central parts of Mazandaran province with *Brucella Canis*

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Abstract

Brucellosis is one of the most common infectious diseases between humans and animals. It is caused by a gram-negative coccobacillus bacterium called *Brucella*. The disease is essential in two ways. It threatens public health and has many side effects, including abortion and reduced production in various livestock and, most importantly, infertility of infected animals, which causes significant economic losses. Since no research has been done on dogs in Mazandaran province in the field of prevalence and identification of infected foci of this disease, the present study was deemed necessary to determine the status of the disease in the region, control methods, prevention and appropriate treatment is applied. In this study, 20 pregnant dogs were sampled in dog kennels in Mazandaran province. Serum samples were sent to the laboratory for *Brucella* examination, and the steps were performed using Wright and Coombs Wright tests. Based on the results, all 20 models were negative. The results of the Wilcoxon test during the comparison of small and giant breed dogs in the two age groups of 1 to 3 years and 3 to 5 years show that there is no significant relationship between these two age groups in this breed in terms of stillbirth ($P > 0.05$). Therefore, this research could be a window for future research investigating the cause of stillbirth in dogs.

Introduction

Brucellosis is one of the most common infectious diseases between humans and animals. It is caused by a gram-negative coccobacillus bacterium called *Brucella*. *Brucella* has caused many diseases in animals and threatens public health. Dogs are the natural hosts of *Brucella canis*, but they can be infected with *Brucella abortus*, *Brucella melitensis*, and *Brucella suis* by eating infected placenta and infected aborted fetuses. Although human cases have been reported due to laboratory infection and contact with infected dogs, humans appear to be resistant to *Brucella canis*. Infertility in dogs is a growing problem in dog kennels. There are many bacteria, viruses and environmental conditions that must be considered to determine the cause of a decrease in the

number of puppies of a mother, abortions, weak puppies and non-pregnancy in dogs. Brucellosis is at the top of this differential diagnosis list. Dogs can be infected with 4 of the six species of Brucella, including Brucella canis, Brucella abortus, Brucella melitensis and Brucella suis [1]. Canine brucellosis has been a significant cause of economic loss to dog owners since Leland Carmichael first isolated Brucella canis in 1966. Even today, it is challenging to establish an accurate diagnosis of the disease and convince dog breeders that the normal functioning of their animal's reproductive system is over [1]. Brucella canis is a small, aerobic gram-negative coccobacillus. Brucella can multiply intracellularly and transmit to new hosts. Brucella is stable in the environment in low temperatures and organic residues for up to two months. However, the survival time of Brucella outside the body is very short and it is inactivated by common disinfectants such as iodophor, quaternary ammonium compounds and sunlight [2-4].

Brucella canis is more prone to steroid-producing tissues, including the testicles, epididymis, and prostate of male dogs and the uterus of female dogs, but is also found in the tissues of the eyes and spine, liver, spleen, and lymph nodes. Brucella canis infects sensitive host dogs by penetrating the mucous membrane, especially around the mouth, vagina and conjunctiva. Semen and vaginal discharge are considered the most likely source of infection due to the high concentration of organisms. Natural transmission of canine brucellosis is possible in several ways. Brucella canis spreads as much as possible in aborted material and vaginal discharge. Female Dogs transmit brucellosis-infected to canines during the estrous phase, mating, or after abortion through nasopharyngeal contact with vaginal secretions and aborted material. Brucella bacteria can also enter through skin lesions. Brucella canis was first isolated from the allantoic fluid of the Beagle aborted fetus in New Jersey, USA. Because dogs infected with their nonspecific brucellosis, in most cases, have no clinical symptoms and can transmit the disease to humans and other domestic animals. Therefore, timely detection is critical to prevent the rapid spread of this bacterium [5, 6].

Brucella canis has a limited host compared to other Brucella, and only dogs and canines are sensitive to it. Brucella canis is characterized by abortion in female dogs, testicular atrophy, swollen epididymis, and infertility in male dogs, and generalized lymphadenitis in both sexes. Due to the optional intracellular parasite of Brucella species, apparent treatment does not always correspond to the actual treatment, and in general, treatment is not considered in livestock due to the risks of transmitting the disease to humans, but controlling and eradicating the disease is essential. Definitive diagnosis of Brucella canis infection in dogs depends on the use of culture methods and serological tests. Using serological methods to find anti-Brucella antibodies in the serum of suspected animals is a more practical and practical method for diagnosis (Greene and Carmichael, 2006). Since no research has been done on dogs in Mazandaran province in the field of prevalence and identification of infected foci of this disease, the present study was deemed necessary to determine the status of the disease in the region, control methods, prevention and appropriate treatment is applied. In a study of 388 dogs in Finland, all tested negative for the Brucella canis antibody [7]. In another study, Alaskan wolves had high levels of anti-Brucella canis antibodies [8]. Also, in 1999, 1549 dogs were tested, which showed that

173 dogs (11.2%) were serologically positive; out of 148 positive dogs, 55 were isolated from *Brucella canis* [9].

Method

Animals studied

In this study, 20 pregnant dogs were sampled in a dog breeding shelter located in the central cities of Mazandaran province (Sari, Babol, Babolsar, Fereydunkenar, Ghaemshahr and Behshahr). For the study, dogs were placed in two age groups of 1-3 years and 3-5 years and also, the history of abortion during pregnancy was examined. Of these 20 dogs, nine belonged to small breeds, and 11 belonged to large species. The exact history of the studied animals was recorded, and blood samples were taken randomly on the 30th, 40th and 50th days of pregnancy. The number of stillbirths and poor puppies were also recorded.

Preparation of serum sample

Blood samples were collected from dogs. For this purpose, about five cc of blood was taken from dogs' cephalic vein or external saphenous vein. After transfer to the laboratory using a centrifuge at 1000 rpm, the serum was separated for 5 minutes. The prepared serums were named and stored in a freezer for testing. Culture and serological methods are used to diagnose this disease, which is more common and critical serological tests. Serological tests are performed on the patient's blood serum to detect antibodies against *Brucella*, the most important of which are: Wright, 2-ME and Coombs Wright. The disease is clinically seen in three forms: acute, subacute and chronic. Early in the second week of the acute phase, IgM antibodies in the patient's serum gradually rise to a maximum on days 13 to 21. At the beginning of this stage, 2-ME was negative, but the rest of the tests were positive. Then the production of IgG against *Brucella* begins, and after a few weeks, its amount (titer) exceeds IgM. At the end of this stage, all serological tests for *Brucella* are positive. In the fourth to eighth week of the acute phase of the disease, the IgG level reaches its maximum, and with proper treatment, the level of IgG decreases. Still, the level of IgM may last from 6 months to two years, and usually, a dilution reduces every three months. If a decrease in the number of antibodies is observed, it indicates a positive effect of treatment and remission of the disease. Now, if the treatment is not done or is incomplete, the infection enters the chronic course.

Data Analysis

To analyze the data, the results were first entered into SPSS software version 22. Then, the frequency distribution table of positive and negative cases of brucellosis was drawn by age and body size. For

statistical analysis, the Wilcoxon test was used to evaluate the relationship between variables, and for this purpose, a significance level of 0.05 was considered.

Results

Rose Bengal test

Based on the results obtained in the rapid test of Rose Bengal, no agglutination samples were seen and reported negative.

Wright test

Based on the results obtained in the Wright test, no agglutination samples were observed in the tubes.

Coombs Wright test

Based on the results obtained in the Coombs Wright test, all samples were negative.

Cases of brucellosis

According to the present study results, no case of brucellosis was seen among the 20 dogs that were sampled.

The stillbirth rate in small breed dogs

No stillbirths were observed in small breed dogs 1 to 3 years old out of a total of 5 dogs (table1).

Table 1 Frequency distribution of stillbirth in small breed dogs one to 3 years

	Compression frequency	Frequency	Abundance
have not been seen	100	100	5
Stillbirth	0	0	0
Total		100	5

In small breed dogs 3 to 5 years old, out of 4 dogs, 2 cases of stillbirth were observed (table2). As the results of the Wilcoxon test during comparison of small breed dogs in two age groups of 1 to 3 years and 3 to 5 years show, there is no significant relationship between these two age groups in this breed in terms of stillbirth ($P > 0.05$).

Table 2 Frequency distribution of stillbirth in small breed dogs 3 to 5 years old

	Compression frequency	Frequency	Abundance
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Have Not Been Seen	2	50	50
Stillbirth	2	50	100
Total	4	100	

The stillbirth rate in large breed dogs

In large breed dogs 1 to 3 years old, out of a total of 6 dogs, 1 case of stillbirth was observed (table3).

Table 3 Frequency distribution of stillbirth rate in large breed dogs one to 3 years

	Compression frequency	Frequency	Abundance
Have Not Been Seen	5	83.3	83.3
Stillbirth	1	16.67	100
Total	6	100	

In large breed dogs 3 to 5 years old, out of 5 dogs, two stillbirths were observed (table4). As the results of the Wilcoxon test showed when comparing large breed dogs in two age groups of 1 to 3 years and 3 to 5 years, there is no significant relationship between these two age groups in this breed in terms of stillbirth ($P > 0.05$).

Table 4 Frequency distribution of stillbirth rate in large breed dogs 3 to 5 years

	Compression frequency	Frequency	Abundance
Have Not Been Seen	3	60	60
Stillbirth	2	40	100
Total	5	100	

Discussion

A study investigated the prevalence of *Brucella canis* infection in urban and rural dogs in the Ahvaz region. In this study, which was performed by immunochromatography, it was shown that 4.90% (5 cases out of 102 points) of urban dogs and 10.34% (12 cases out of 116 patients) of rural dogs in the Ahvaz region have anti-antibody *Brucella canis* antibodies. It is essential to spread *Brucella canis* infection in urban and rural dogs in the Ahvaz region because it is contagious. And there are many dogs in rural and urban areas that can play a role in transmitting the disease to other dogs and even humans because this bacterium is intracellular and can remain in the body for a long time. The results showed that several dogs without clinical symptoms carry antibodies that indicate previous infections [10]. The percentage of brucellosis in the present study is zero.

In 1966, the prevalence in the United States was reported to be 1.5 per cent [11] (Greene and Carmichael, 2006). In Japan, Kimura et al. (2008) showed that 12 out of 485 dogs studied (2.5%) were infected with *Brucella canis* by the MAT method. In the researchers' study, the MAT method was compared to the TAT method, and it was shown that the MAT method was more sensitive and more straightforward and allowed us to test more samples at a time. The results showed that *Brucella canis* infection was still present in Japan [12]. In a study conducted in China, the rate of infection was higher in dogs over one year old (compared to young dogs under one year old), in females and in southern regions (compared to northern China), which shows that with increasing with age, the risk of infection increases in the dog population [13].

All dog breeds are sensitive to *Brucella canis*, and no racial aptitude has been reported. However, a report from the southern United States said a higher prevalence of the disease in Beagle and Labrador Retriever breeds [11].

Although dogs are not the primary hosts of *Brucella melitensis*, *Brucella suis* and *Brucella abortus*, if severely infected, they can play a role in transmitting objects to other animals and even humans. In addition to dogs and humans. anti-*Brucella canis* antibodies have been found in the serum of other animals. In a study conducted in Florida, USA, it was found that in the serum of 38% of horses that were referred to the Florida Veterinary School Hospital, there was an anti-*Brucella canis* antibody [10]

In the study of Zoghi et al. (2006), blood samples were taken from 112 dogs in the suburbs of Tabriz, and they were tested for brucellosis. In this study, serum samples were tested by Rose Bengal, Wright and 2-ME methods, and 4 cases (3.57%) showed laboratory evidence of *Brucella* infection that all four dogs were killed and necropsied. It has been isolated from the culture of lymph nodes and their internal organs by *Brucella melitensis* [14].

In the study on 50 herding dogs in Neishabour city and performing Rose Bengal and Wright tests, only two dogs had a positive serum grade that the infected dogs were in the age range of 4-6 years. In the present study, the infection rate was zero, and there was no concern in the canals. *Brucella canis* has been reported from most different parts of the world [10]

The only areas that appear to be free of the disease are New Zealand and Australia. The bacterium is widely isolated, especially in Central and South America and in the US states and among dogs kept in crowded places for sale or research [11].

Another study on 12949 serum samples from 23 provinces of China showed that the prevalence of infection varies from 42.7 to 0.3%. Eight serological methods have been used in their research. R-SAT and R-RBPT methods were more sensitive than others [13] (Liu et al., 2020).

In another study conducted in 1979 on 341 dogs using the 2-mercaptoethanol method from different parts of Quebec province in Canada, 6 cases (1.6%) had significant titers [15].

Also, a study of 200 serum samples from southwestern Ontario by rapid agglutination method showed that 31 cases had suspicious titers [16].

Because infected dogs can have bacteria in their blood for long periods, the risk is always high for humans. Approximately 30 human cases of *Brucella canis* have been reported since 1960.

If you follow the above tips, prevention of brucellosis in dogs is relatively easy. Some infected people are asymptomatic and in some cases recurrence of the disease has been observed. The mortality rate in untreated people is reported to be 2-5%. In most cases, death is due to endocarditis or meningitis (Greene and Carmichael, 2006). Therefore, it is emphasized that the isolation of infected dogs is very important in preventing the transmission of the disease to healthy dogs and humans. Infected animals should not be used for mating and slaughter, and if kept in a cage, they should be removed immediately before infecting other animals. Otherwise, the risks involved can be threatening [17].

Conclusion

Based on the present study results, no cases of brucellosis were observed in dogs in the central regions of Mazandaran province. Therefore, some concerns about the disease are alleviated, but to identify the cause of stillbirth in dogs, it is recommended that more extensive and complementary studies be performed.

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