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Serological investigation of brucellosis in one-humped camels (*Camelus dromedarius*) of Yazd province in Iran

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Abstract

Brucellosis is one of the important zoonotic diseases caused by gram-negative bacteria of the Brucella genus. Given that brucellosis in Iranian camels is not monitored, knowing the status of this disease in camels can be crucial from a health and economic point of view. The present study investigated the Brucella infection rate of camels in Yazd province. Blood samples were collected from the jugular vein of 86 healthy camels, and their serum was separated. Serum samples were analyzed using serological tests, including Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), 2-Mercaptoetanole (2-ME) test, and indirect ELISA (i-ELISA). In addition, the degree of agreement between RBPT and i-ELISA methods was evaluated using Cohen's kappa test. The relationship between cases of Brucella infection in studied camels and the variables of age, gender, and sampling location was investigated using the chi-square test and SPSS version 22 software. *P*-values less than 0.05 were considered significant. Brucella infection rate by RBPT, SAT, 2-ME, and i-ELISA methods was 4.7%, 3.5%, 4.7%, and 9.3%, respectively. A substantial agreement was also found between RBPT and i-ELISA. No significant relationship existed between Brucella infection and gender and age variables. However, this relationship was significant with sampling location ($P<0.05$). The current study revealed the presence of Brucella infection in one-humped camels of the Yazd province. Therefore, monitoring the disease status in camels is advisable to control brucellosis in the region.

Introduction

The Camelidae family comprises two distinct lineages of camels: the Old-World camelids and the New-World camelids. Old World camelids include one-humped camels (*Camelus dromedarius*) and two-humped camels (*Camelus bacterianus*), and camels of the New World include the two genera Lama and Vicugna with species *L. glama*, *L. guanicoe*, *V. pacos* and *V. vicugna* (1). According to the Food and Agriculture Organization of the United Nations (FAO), there are approximately 35 million camels in the world. According to this report, Iran is one of the countries where the camel population is unfortunately decreasing (2). Rainfalls have reduced in recent years, and long-

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term droughts resulted in decreased water resources and desertification of agricultural lands in low-rainfall areas in Iran. Therefore, it is crucial to focus on breeding and developing livestock like camels that are highly productive and resistant to drought (3). In addition, more attention should be paid to camel infectious diseases, especially brucellosis, as it can cause abortion and reproductive disorders in camels, disturbing the development of this animal in the country. Brucellosis is a major zoonotic disease that inflicts considerable economic loss in the livestock industry and has destructive effects on human public health. Despite significant efforts to control brucellosis, the disease continues to spread in many developing countries (4). In many countries where camels are raised, *B. abortus* and *B. melitensis* infections have been reported (5). Brucella species, which are gram-negative, non-sporing, non-motile, and aerobic coccobacilli belonging to the genus Brucella, can cause brucellosis in camels (6). In traditional breeding systems in Iran, domestic animals are raised concurrently, so pathogen transmission from other ruminants to camels is possible. Due to the lack of effective control measures against camel brucellosis in Iran, camel breeders and consumers of camel meat and dairy products are at risk of infection (7). In a study performed on raw camel milk samples in Isfahan and Semnan provinces, Brucella infection was revealed using culture and PCR methods. This study shows that people who consume raw camel milk can be at risk (8). This disease in camels can occur subclinically or with complications such as abortion, retained placenta, infertility, delayed sexual maturity in female camels, and orchitis, arthritis, and lameness in male camels (9). Various serological methods, including RBPT, SAT, 2-ME, i-ELISA, competitive ELISA (c-ELISA), and complement confirmation test (CFT), have been used to detect Brucella infection in camels (10-12). In countries where camel breeding is common, various studies have been conducted on the prevalence of brucellosis in camels using serological and molecular methods (10, 13). Likewise, some studies have been conducted in Iran to identify Brucella infection in camels in various regions using serological or molecular methods (7, 14-16). But, there is still a need for more studies in this field. Because this disease in camels is often subclinical, knowing the status of Brucella infection in the camel population is necessary to control it and to reduce the risk of transmission of infection to humans. Therefore, the present study aimed to investigate Brucella infection in camels of Yazd province using serological methods, RBPT, SAT, i-ELISA, and 2-ME.

Materials and methods

Sampling methods

This cross-sectional study was conducted between December 2021 and May 2022 in Yazd province, including the cities of Yazd, Bafq, and Saghand. Blood samples from jugular veins were taken from 86 healthy one-humped camels. They were obtained in tubes without anticoagulant (Mediplus, free additive tube, Sunphoria Co., Ltd, China) and immediately moved to the laboratory on ice. Then the samples were centrifuged at 3000 rpm for 5 min. The isolated sera were stored at -20 ℃ until serological tests were accomplished.

Rose Bengal Plate test

All serum samples were checked for the anti-Brucella antibodies presence using the RBPT. In brief, equal volumes (50 µL) of RBPT antigen (Pasteur Institute, Iran) and serum were mixed on a slide using an applicator. The slide was then shaken on a shaker for about 5 min and checked for agglutination reaction.

Serum agglutination test

A row of hemolysis tubes was provided from the sample's serum dilutions ranging from 1/20 to 1/2560. 900 µL of phosphate-buffered saline (PBS) was added to the first tube, followed by 500 µL into the subsequent tubes. To accomplish the test, 100 µL of serum was added to the first tube. The tube was then shaken well to ensure the reagents were thoroughly mixed. Next, 500 µL from the first tube was added to the second tube, and then 500 µL of the mixture from the second tube was added to the third tube. This process was repeated until the last tube was reached. Finally, 500 µL was discarded from the last tube. During each step of the process, the tubes were thoroughly shaken to ensure proper mixing of the reactants. Then, 500 µL of Wright tube antigen of *B. abortus* (purchased from the Razi

Vaccine and Serum Research Institute in Iran) was added to all tubes and, after covering the lids of the tubes with parafilm, were incubated at 37 °C for 24 hours. The results were then read. One tube was chosen as a negative control, for which the negative control serum was used instead of the sample serum with the same dilution. One row of tubes was selected as a positive control, for which the positive control serum was used instead of the sample serum with the same dilutions. The last tube, where the agglutination reaction was observed at the bottom of the tube, was considered the test titer.

2-Mercaptoethanol test

The 2-ME test was performed the same way as Wright's tube agglutination, with the difference that serum dilutions were prepared with 2-ME buffer (obtained from the Razi Vaccine and Serum Research Institute in Iran) instead of PBS. Of course, before performing the test, the sample serum, positive and negative control were incubated with 2-ME buffer at 37 °C for 1 hour, after closing the lid with parafilm. After serial dilution, like Wright's test, 2-ME antigen (obtained from the Razi Vaccine and Serum Research Institute in Iran) was added to all the tubes and was incubated at 37 °C for 24 hours.

Indirect ELISA

This test was performed using a commercial i-ELISA kit according to its manufacturer's instructions (ID Screen Brucellosis Serum Indirect Multi-species kit, ID-Vet, France). The optical density (O.D.) of each well in the plates was measured at a wavelength of 450 nm. According to the information in the kit, the average O.D. in the positive control ($O.D._{PC}$) should be more than 0.35 for accurate results, and the average O.D. ratio of the positive control to the negative control ($O.D._{PC}$ / $O.D._{NC}$) should be more than 3. The formula used to obtain i-ELISA test results in each serum sample is as follows:

$$S/P\% = \frac{OD\ sample - OD\ NC}{ODPC - ODNC} \times 100$$

In the samples with an S/P% less than 110, it was negative, 110-120 was suspicious, and a result over 120 was positive.

Statistical analysis

Data were analyzed using SPSS version 22 statistical software (IBM, SPSS Inc., Chicago, IL, USA). To investigate the relationship between seropositive camels (based on i-ELISA test results) and age (including three groups under four years, 4-6 years, and over six years), gender (male and female), and sampling location (Yazd, Bafq, and Saghand cities) chi-square test was used. In addition, Cohen's Kappa statistical test was used to check the agreement between i-ELISA and RBPT diagnostic methods. $P<0.05$ was considered significant.

Results

Out of 86 serum samples examined using RBPT, only four (4.7%) tested positive. The highest rate of infection was found in camels over six years old, with a rate of 3.5%. It was observed that all the positive cases were female camels, while no infection was detected in male camels. Among the positive cases, three were identified in Saghand City, only one positive case was found in Bafq City, and no positive sample was observed in Yazd City (Table 1). All the serum samples that showed positive results in RBPT were further tested to determine the titer in SAT and 2-ME tests. Out of four positive serum samples in RBPT, three samples were also positive in SAT. The titers obtained in this test ranged from 1/640 to 1/2560 (Table 2). Brucella infection rate was 3.5% using this method. Then, the 2-ME test was performed on all four positive samples, and titers from 1/80 to 1/2560 were obtained (Table 2). Given the minimum titer of 1/80, all four samples were considered positive and the Brucella infection rate was 4.7% using this method. As shown in Table 3, out of 86 serum samples examined in i-ELISA, eight cases (9.3%) were positive. In addition, all the samples that were positive in RBPT, SAT, and 2-ME also showed positive reactions in i-ELISA. The i-ELISA test showed the highest infection rate in camels older than six years, and only female camels tested positive. However, no significant relationship was found between age and gender and seropositive camels ($P<0.05$). Examining the

Table 1. Frequency and percentage of Brucella infection in one-humped camels of Yazd province using RBPT method.

Variable		Positive samples (%)	Negative samples (%)	Total (%)
Age	<4 years	0 (0.0%)	12 (14.0 %)	12 (14.0%)
	4-6 years	1 (1.2%)	14 (16.3%)	15 (17.4%)
	>6 years	3 (3.5%)	56 (65.1%)	59 (68.6%)
	Total	4 (4.7%)	82 (95.3%)	86 (100%)
Gender	Female	4 (4.7%)	76 (88.4%)	80 (93.0%)
	Male	0 (0.0%)	6 (7.0%)	6 (7.0%)
	Total	4 (4.7%)	82 (95.3%)	86 (100%)
Sampling location	Bafq	1 (1.2%)	32 (37.2%)	33 (38.4%)
	Saghand	3 (3.5%)	37 (43.0%)	40 (46.5%)
	Yazd	0 (0.0%)	13 (15.1%)	13 (15.1%)
	Total	4 (4.7%)	82 (95.3%)	86 (100%)

Table 2. The titer of SAT and 2-ME tests in positive samples in the RBPT test in the one-humped camels of Yazd province.

Test Method	The titer of SAT, and 2-ME tests			
	Sample 1	Sample 2	Sample 3	Sample 4
SAT	1/640	1/1280	1/2560	Negative
2-ME	1/160	1/640	1/2560	1/80

Table 3. Frequency and percentage of Brucella infection in one-humped camels of Yazd province using i-ELISA method.

Variable		Positive samples (%)	Negative samples (%)	Total (%)	*P*-value	Chi-square
Age	<4 years	0 (0.0%)	12 (14.0 %)	12 (14.0%)	*P*>0.05	1.81
	4-6 years	1 (1.2%)	14 (16.3%)	15 (17.4%)		
	>6 years	7 (8.1%)	52 (60.5%)	59 (68.6%)		
	Total	8 (9.3%)	78 (90.7%)	86 (100%)		
Gender	Female	8 (8.1%)	72 (83.7%)	80 (93.0%)	*P*>0.05	0.66
	Male	0 (0.0%)	6 (7.0%)	6 (7.0%)		
	Total	8 (8.1%)	78 (90.7%)	86 (100%)		
Sampling location	Bafq	1 (1.2%)	32 (37.2%)	33 (38.4%)	*P*<0.05	6.06
	Saghand	7 (8.1%)	33 (38.4%)	40 (46.5%)		
	Yazd	0 (0.0%)	13 (15.1%)	13 (15.1%)		
	Total	8 (9.3%)	78 (90.7%)	86 (100%)		

Table 4. The agreement rate of i-ELISA and RBPT methods in the diagnosis of brucellosis in the one-humped camels of Yazd province.

Test Method*		RBPT		Total
		Positive	Negative	
i-ELISA	Positive	4	4	8
	Negative	0	78	78
Total		4	82	86

* Cohen's Kappa coefficient (κ) = 0.645, *P*<0.001, there is a significant agreement between the two diagnostic methods (17).

relationship between the sampling location and Brucella infection showed a significant relationship (P<0.05). The highest infection rate was identified in Saghand City (7 cases), only one positive case was recognized in Bafq City, and no positive serum samples were observed in Yazd City (Table 3).

Cohen's Kappa statistical method was used to check the agreement between i-ELISA and RBPT methods, and the interpretation of the Kappa coefficient showed a significant agreement between these two methods (17). Cohen's Kappa coefficient (κ) = 0.645, 95% confidence interval = 0.33 - 0.96, P<0.001 (Table 4).

Discussion

The present study was conducted to investigate the Brucella infection in the one-humped camels of Yazd province. The results of serological tests showed that Brucella infection using RBPT, SAT, 2-ME, and i-ELISA serological methods were 4.7%, 3.5%, 4.7%, and 9.3%, respectively. Overall, the serological methods used in this study revealed an infection rate of less than 10% in the camels examined. Culture and isolation of Brucella are the most definitive methods for diagnosing brucellosis. However, this technique is time-consuming, may expose the examiner to Brucella infection, and may produce false negative results (18). Several serological methods have been proposed to diagnose brucellosis, each of which has advantages and disadvantages. Therefore, it is advisable to use multiple serological methods in conjunction for a more accurate diagnosis. Considering that vaccination is not performed in Iran to prevent brucellosis in camels, RBPT can be considered a suitable and cost-effective screening test. However, it should be noted that this test might produce false negative results (19). Then, SAT, and 2-ME serological methods were used to confirm the positive cases of this test. In the SAT, three samples tested positive while one tested negative. In contrast, all four samples tested positive in the 2-ME test, indicating that IgG is the dominant antibody in the serum samples. The i-ELISA method revealed a more positive reaction in the serum samples when compared to other serological methods used in this study. The higher level of Brucella infection in this method can indicate the higher sensitivity of i-ELISA compared to other serological methods and the presence of blocking antibodies in serum samples (20). In the present study, Cohen's Kappa statistical method was utilized to investigate the agreement between RBPT and i-ELISA methods in diagnosing Brucella infection. The results revealed a significant agreement between these two methods. In general, it can be stated that the i-ELISA method is deemed a preferable method for screening tests due to its higher sensitivity in detecting Brucella-infected cases. However, when the budget and facilities for conducting screening tests using the i-ELISA method are limited, the RBPT method, which has shown significant agreement with i-ELISA, can be used as an alternative.

The prevalence of camel brucellosis was investigated in different regions of Iran. For instance, in an abattoir study, out of 310 blood and lymph node samples collected from camels slaughtered at the Najaf Abad slaughterhouse in Isfahan, the Brucella infection rate was 1.94% using different serological methods, which was less than the present study (21). In a study conducted at the Najaf Abad slaughterhouse in Isfahan from 2012 to 2013, 150 camels were examined. The Brucella infection rate was determined to be 12%, 8%, and 6% using RBPT, SAT, and 2-ME serological methods, respectively. However, when the PCR method was used, the prevalence rate was 1.3% (16). Furthermore, the Brucella infection rate in a molecular study conducted on 100 camels at the Qom slaughterhouse was 3% (14). In a serological study conducted in 2016, anti-Brucella antibodies were found in 5 out of 248 camel serum samples (1.94%) in Bushehr province. Subsequently, *B. melitensis* biotype one was isolated from two cultures of lymph nodes from the positive samples (22). In South Khorasan Province, the infection rate of Brucella in 151 camels was determined to be 27.8% (42 samples) using the RBPT method. Subsequently, using the Wright method nine samples tested positive at a 1/80 level (7). In

Semnan Province, a slaughterhouse study was conducted on 150 camels in 2013, and the rate of Brucella infection was 9.3% using RBPT, SAT, and 2-ME serological methods (18). In neighboring countries of Iran that breed camels, such as Qatar, Iraq, and Pakistan, Brucella infection in camels in different studies has been confirmed using serological and molecular methods (10, 23, 24).

The present study revealed that camels over six had the highest rate of Brucella infection. However, no significant relationship was found between age and Brucella infection. It is understood that animals that have reached sexual maturity are more susceptible to brucellosis than younger animals (25). Sexual maturity appears to have a greater influence on susceptibility to brucellosis than age (26). In this study, Brucella infection was predominantly observed in animals at the age of sexual maturity and reproduction. However, likely due to the small sample size, no significant relationship was found between age and Brucella infection. All Brucella-infected camels in this study were female. However, no significant relationship was observed between gender and Brucella infection. Studies have shown that the infection rate of Brucella in female camels could be twice that of male camels (27). Nevertheless, some studies have found no significant difference between male and female camels in the occurrence of brucellosis (11). In this study, Saghand City exhibited the highest rate of Brucella infection, with seven confirmed cases (P<0.05). The findings suggest that camels could potentially serve as a source of Brucella infection in Saghand City, facilitating transmission to humans. The results underscore the importance of further investigation into this potential health risk.

Conclusion

The study's findings indicate a relatively high Brucella infection rate among the one-humped camels of Yazd province. This issue could potentially lead to significant economic losses for breeders of this resilient and productive animal through abortion, infertility, and reduced milk production. Furthermore, the high infection rate in the camel population of this region could pose a significant risk for the transmission of Brucella infection to humans.

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Ethical approval

This study was conducted following the guidelines and standards of the Animal Research Ethics Committee at the University of Tabriz.

Conflict of interest

There is no conflict of interest in conducting this research.

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