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Sero-Epidemiological Survey of Brucellosis in Farm Animals in Khuzestan Province, Iran

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Abstract Brucellosis is one of the most important zoonotic diseases caused by different species of *Brucella* spp. This zoonotic disease has always been important from an economic and healthcare point of view, so many countries are trying to eradicate the disease from the farm animal population. The purpose of the present study was to investigate the sero-epidemiology of brucellosis in farm animals in Behbahan city, Khuzestan province, Iran. For this purpose, 330 serum samples were collected as the study population to track brucellosis prevalence. From March to June 2022, the samples were analyzed by Rose Bengal, Wright and 2-Mercaptoethanol tests. The results obtained in the present study indicated that 3.6% of the studied animals were infected with brucella bacteria. Examining the results of 2-Mercaptoethanol indicated that in general, 58.3% (7 samples) of the examined animals whose initial test results were positive had positive 2-Mercaptoethanol, which means chronic brucellosis was determined. There was no difference in the frequency of brucellosis between animal species ($p>0.05$). The contamination in female animals (2.6%) was significantly higher than that of male animals ($p<0.05$). The results of this study demonstrated that brucellosis was a prevalent disease among farm animals in Behbahan city. It is important to note that the widespread occurrence of brucellosis among farm animals could be distributed to the human population. This underscores the imperative for the implementation of an efficacious program to control, prevent and ultimately eradicate this infectious agent in this area.

Introduction

It is possible for the brucellosis disease (also known as Malta fever in humans), to be transmitted from farm animals to humans. This is achieved through the consumption of raw products derived from infected animals [1]. The etiological agent of this disease is a type of gram-negative bacterium, brucella, which is susceptible to destruction by pasteurization and boiling [2]. This disease is of significant public health importance in most regions of the world, particularly in developing countries. Its impact on the social status of societies is also noteworthy. In many developed countries, the control of this disease in animal communities has resulted in a notable reduction in human cases. However, it is a developing issue and a common occurrence, particularly in the Mediterranean, Western Asia, and parts of Africa and America [3]. The World Health Organization (WHO) has reported that 500,000 individuals are infected with brucellosis

annually. In countries with advanced healthcare systems, only 5-10% of actual cases are reported. The annual incidence of brucellosis in Iran from 1370 to 1387 was 43.24 per hundred thousand people. However, there is considerable variation in the incidence of the disease across different regions of the country. For instance, in Khuzestan province, the incidence has been reported to be around 11.05% within the human population [4].

The susceptibility and pathogenicity of brucellosis depend on the species of bacteria transmitted from animals. These species are typically transmitted via direct or indirect contact with infected sheep, goats, or their products, which represents the most prevalent mode of human brucellosis globally and in Iran [5].

There are two types of the disease, acute and chronic, with different clinical features, making it difficult for experts to diagnose. In general, the disease begins abruptly and is characterized by a persistent or regular fever with alternating periods, profuse sweating, fatigue, anorexia and weight loss, headache, muscle pain and general body pain. The aforementioned symptoms depend on the specific type of disease. The disease caused by brucella can manifest in four distinct forms, namely acute, sub-acute, chronic and local. The severity of the disease is determined by the form in which it presents [6 – 8].

The disease is observed in both genders, although there is a slight difference in prevalence. Females are more commonly affected (55.4%) than males (44.6%). It has been demonstrated that occupation is a significant risk factor in the contraction of the disease, with particular relevance to housewives, ranchers, and farmers. Furthermore, the disease is more prevalent during the spring and summer months, which coincides with the breeding and milking season of animals [7 – 9].

The continuous conduct of epidemiological studies in different regions of the country enables the evaluation of significant and crucial factors in the spread and control of brucellosis. This information can then be used to implement effective preventive strategies and to plan for the control of this important common disease. In recent years, a few epidemiological studies have been conducted on the epidemiology of brucellosis in different regions of Iran [4 – 8]. The city of Behbahan is the livestock breeding epicenter of Khuzestan province, with preeminence in both livestock breeding and dairy production across the province [10]. Therefore, the prevalence of the disease within the city should be subject to continuous monitoring. The objective of this study was to investigate the sero-epidemiology of brucellosis in farm animals in Behbahan city, Khuzestan province, Iran.

Materials and methods

Sample collection

A total of 330 serum samples were collected, including cattle (n=110), sheep (n=110) and goats (n=110). Of this total, 269 were females and 61 were males. The samples were taken from farm animals presenting symptoms such as reduced milk or meat production, infertility, stillbirth, abortion, retained placenta, as well as swelling of the testicles and epididymitis in male animals from all the livestock on farms located in Behbahan city, Khuzestan province, southwestern Iran. Initially, the Rose Bengal test was conducted on all samples, and subsequently, the Wright and 2-Mercaptoethanol (2ME) tests were performed on seropositive cases.

It should be noted that all sampling protocols were based on the Animal Research Ethics Committee of Behbahan University and were conducted in accordance with its guidelines and standards.

Sample preparation

Whole blood tubes were subjected to centrifugation for five minutes at 3000 g, during which time the serum separated into a clear yellow layer.

Diagnostic tests

A rapid initial diagnostic test was conducted on all serum samples using the Rose Bengal test. The

Rose Bengal antigen used in all samples was produced by Razi Vaccine and Serum Research Institute (batch number: 10491002), containing 8% of brucella microbial mass. This was mixed with the same volume of serum in a micro plate for 2 minutes. In cases where agglutinated clots were clearly visible, additional tests of Wright and 2ME were performed as the procedure was conducted in accordance with the guidelines established by the Razi Vaccine and Serum Research Institute (Wright antigen batch number.: 200110 207).

In order to ascertain the type of antibody present and the active or inactive (vaccinated) state of the disease, a two-mercaptoethanol (2ME) test was conducted on the serum samples. To identify the carrier animal in the non-vaccinated sheep and goat population and those that have been vaccinated for more than one year, the Wright test should be 1/80 or above with any amount of antibody titer in 2ME. Furthermore, the results of the Wright test should range from 1/20 to 1/640, with a seropositive result indicated by a titer of 1/80 or above. In the case of 2ME, a result of 1/40 or above is regarded as indicative of a seropositive outcome [11].

Statistical analysis

The data was entered into the relevant table, the distribution of the data was determined, and the percentage of prevalence across different areas was calculated using SPSS version 24 statistical software. The chi-square and fisher exact tests were employed to compare categorical variables. A p-value of less than 0.05 was deemed to be statistically significant. Categorical variables were presented in the form of numbers and percentages.

Results

A total of 330 samples were examined. The mean age of the examined sample was 3.66 ± 1.84 years, with a range of 6 months to 10 years. The average age of the examined sample, separated by animal species, was 2.35 ± 4.71 years (range 0.5 to 10) for cows, 3.4 ± 1.19 years (range 1 to 7) for sheep, and 2.88 ± 1.207 years (range 0.5 to 5) for goats. The results of the Kruskal-Wallis test indicated a significant difference between the three groups in terms of the age factor ($Z=44.68$, $p<0.005$).

According to Table 1, a total of 269 cases (81.5%) of the examined animals were female, while 18.5% (61 cases) were male. The results of the chi-square test indicated that there was no significant difference between the three groups in terms of gender ($Z=0.52$, $p>0.05$).

The initial brucellosis test, which was the Rose Bengal test yielded seropositive results in 3.6% (12 samples) of the examined animals and negative results in 96.4% (318 samples). The test results for the three study groups indicated that 2.7%, 4.5%, and 3.6% of cows, sheep, and goats, respectively (Table 2).

The results of the Wright test for the Rose Bengal test-seropositive samples indicated that 83.3% (10 samples) had a seropositive Wright test, while 16.7% (2 samples) had a negative Wright test. The evaluation of the results of the Wright test for the diagnosis of brucellosis in three groups revealed that only two of the cows with a seropositive primary brucellosis test had a negative Wright test. In both the sheep and goat groups, all animals with a seropositive primary brucellosis test exhibited a seropositive Wright test result (Table 3).

The results of the 2ME test revealed that 58.3% (7 samples) of the animals in which the Rose Bengal test results were seropositive exhibited positive 2ME, while 41.7% (5 samples) exhibited negative 2ME. The evaluation of 2ME results in three groups revealed that all cows with a seropositive primary brucellosis test had a negative 2ME result. Among sheep samples, 80% (4 cases) of the animals with a seropositive primary brucellosis test had positive 2ME, while 20% (1 case) had negative 2ME. In the goat samples, 75% (3 cases) of the animals with a seropositive primary brucellosis test were positive 2ME, while 25% (1 case) were negative 2ME (Table 4). It is worth mentioning that to identify the carrier animal in the non-vaccinated sheep and goat population and those that have been vaccinated for more than one year, the presence of 2ME in the buffer removes IgM, while IgG remains active.

Table 1. The distribution and frequency of brucellosis cases by gender in cattle, sheep, and goats

Species	Male		Female		Z	p-value
	No.	Percent	No.	Percent		
Cow	22	20	88	80	0.52	0.77
Sheep	18	16.4	92	83.6		
Goat	21	19.1	89	80.9		

Table 2. The results of positive and negative tests for brucellosis using the Rose Bengal test

Species	Initial positive test		Initial negative test	
	No.	Percent	No.	Percent
Cow	3	2.7	107	97.3
Sheep	5	4.5	105	95.5
Goat	4	3.6	106	96.4

Table 3. The results of positive and negative tests for brucellosis using the Wright test

Species	Positive test		Negative test	
	No.	Percent	No.	Percent
Cow	1	33.3	2	66.7
Sheep	5	100	0	0
Goat	4	100	0	0

Table 4. The results of positive and negative tests for brucellosis using 2 -Mercaptoethanol test

Species	Positive test		Negative test	
	No.	Percent	No.	Percent
Cow	0	0	3	100
Sheep	4	80	1	20
Goat	3	75	1	25

Table 5. The distribution and frequency of brucellosis cases by species in all examined samples

Species	Positive test		Negative test		X^2	p-value
	No.	Percent	No.	Percent		
Cow	0	0	110	100	3.79	0.15
Sheep	4	3.6	106	96.4		
Goat	3	2.7	107	97.3		

Table 6: The distribution and frequency of brucellosis cases by gender in all examined samples

Gender	Positive test		Negative test		X^2	p-value
	No.	Percent	No.	Percent		
Male	0	0	61	100	1.62	0.203
Female	7	2.6	262	97.4		

Table 7: The distribution and frequency of brucellosis cases by age in all examined samples

Samples	Average age		Z	p-value
	Mean	SD		
Positive	4.28	0.75	-1.56	0.11
Negative	3.65	1.85		

The results of the brucellosis test indicated that 2.1% (7 samples) of the examined animals were seropositive for brucellosis, while 97.9% (323 samples) were negative. A total of 3.6% and 2.7% of sheep and goats, respectively, showed a seropositive result on the final brucellosis test, while none of the cows were found to be positive. The effect of the species of animal on the prevalence of brucellosis was examined, and it was found that there is no significant difference between the three groups ($\chi 2 = 3.79$, $p>0.05$) (Table 5).

The results showed that 2.6% (7 samples) of female samples were seropositive for brucellosis, while 97.4% (262 samples) were negative. Notably, none of the male samples tested positive for brucellosis. The evaluation of the effect of gender on the prevalence of brucellosis indicated that there was no significant difference between male and female samples in terms of the incidence of brucellosis ($\chi 2 = 1.62$, $p>0.05$) (Table 6).

The results showed that the mean age of animals with positive and negative brucellosis tests was 4.28 ± 0.75 (range 3 to 5) and 3.65 ± 1.85 (range 0.5 to 10) years, respectively. The analysis of the impact of age on the prevalence of brucellosis revealed no significant difference between the age profiles of animals with positive and negative brucellosis ($Z = 1.62$, $p>0.05$) (Table 7).

Discussion

Brucellosis is a significant zoonotic disease caused by different species of *Brucella* spp. This disease has always been important from economic and healthcare point of view, so many countries are trying to eradicate the disease from the livestock population. In 1943, *Brucella melitensis* was isolated from the blood of individuals suffering from brucellosis at the Pasteur Institute, France. Subsequently, in 1948, *Brucella. melitensis* was isolated from sheep. In 1958, *Brucella melitensis* was isolated from humans, sheep and goats. In 2001, the Veterinary Organization reported that 1.8% of all sheep in Iran were infected with brucella (without species identification). Investigations conducted in 1982 revealed that *Brucella abortus* is endemic in Iran. The disease known as brucellosis in humans is also referred to as Malta fever, Wave fever or Mediterranean fever. In countries where this disease has been controlled in animals, its transmission to humans occurs frequently and remains an important human disease. Due to the duration of the illness and recovery period in humans, the disability and loss of the patient's normal activities and the long treatment time, this disease is considered an important health and economic problem in societies. With regard to the risk of human infection, the priority brucella species are *melitensis*, *suis*, *abortus* and *canis*, respectively. To date, no *Brucella ovis* has been reported in humans [4].

It is crucial to note that the primary objective of this article is to examine the sero-prevalence of a significant zoonotic disease, capable of transmission to humans via small ruminants. The preponderance of zoonoses diagnosed in ovine and caprine species is attributable to direct human contact with these animals, constituting occupational diseases that predominantly affect veterinarians and slaughterhouse personnel. It is imperative to implement continuous monitoring procedures for this disease in animals, particularly livestock, with a view to averting its propagation and the potential for zoonotic transmission.

The results obtained from the present study indicated that 3.6% of the studied animals were infected with brucella bacteria. The Rose Bengal test result was a screening test (12 samples; 3.6% seropositive). Further testing (Wright and 2ME tests) revealed that seven of these 12

samples (58.3%) were infected with chronic brucellosis. There was no discernible difference in the incidence of brucellosis between the investigated animals in Behbahan city. Furthermore, the age of the animals did not appear to affect the incidence of this disease. It can therefore be concluded that brucellosis affects all young and old animals in a similar manner, with no discernible effect of age on the level of infection. However, the current study revealed that the disease was more prevalent in female cattle. This could be attributed to the longer period of time spent by female cattle in the breeding cycle, which increases their exposure to contamination. A main risk factor analysis of farm animal brucellosis was conducted, revealing that gender and the insemination method significantly impacted prevalence. Higher risk was observed in females and in animals inseminated naturally. Furthermore, female animals are typically kept in husbandry for a greater duration, which leads to increased infection rates over time. As a result, the prevalence of brucellosis infection in female animals is higher than in male animals. To date, comprehensive research has been conducted in the country to ascertain the prevalence and contamination of brucella bacteria in farm animals and humans. In a study conducted by HajiBamani et al. (2023) in Tabriz, the serological prevalence of brucellosis in sheep was investigated. The results indicated that the prevalence of brucellosis was 18.5%, with 2% in males and 5.5% in females. The probability of contracting brucellosis in sheep over one year old was twice that of sheep under one year old. The results of the present study demonstrated that the prevalence of brucellosis serology is high in the Tabriz, Iran. Factors such as gender and age of sheep can influence the prevalence of serology for this disease. Consequently, the implementation of control and preventive measures for this disease is crucial in this city [12].

In a study conducted in 2022, Sadeghi et al. reported a serological prevalence in Saanen goats in the Alborz province, Iran. They found that in the blood serology test, eight samples tested positive for brucella antibodies, and one sample was suspected to be positive. All nine samples (7.4%) were confirmed to be seropositive [13]. In another study on the caprine sero-prevalence of brucellosis in Bangladesh, Yeasmin et al. (2024) reported that 2.6% of goats were seropositive [14]. This percentage is similar to the seropositivity rate reported in our study (2.7%).

In a study conducted in 2018, Dumari investigated the serology of brucellosis in cattle in Jiroft city, Kerman, Iran. The author determined that Sarodayeh was the center of infection and an important factor in the spread of the disease in the district. This district also received infected cattle from Baft city, Kerman, Iran. Sarodayeh is located in the first row, with an infection level of 37.11%. The central, Anbrabad, and Jabalbarz sections are situated in the second to fourth rows, with infection levels of 33.5%, 4.2%, and 0.38%, respectively. Furthermore, it appears that the practice of cattle grazing in the Sarodayeh district, Jiroft, Kerman province may be a contributing factor in the spread of infection in tropical areas [15].

In a study conducted by Kaboli Borujeni et al. (2010) on the epidemiological evaluation of brucellosis disease in Borujen city, Iran, during 2015-2016, it was reported that 2195 cows, 35000 sheep, and 1300 goats were seropositive for brucellosis. The investigation of brucellosis yielded 161 (0.73%) positive samples, 2 (0.0006%) in 2015, and 2 (0.15%) in 2016. The highest prevalence of brucellosis was observed in 8,362 calves (2.75%), 388,659 sheep and goats (22.87%), and 306,500 lambs and goats (4.88%). This study recorded a significantly higher prevalence than our report (Sheep: 3.6% and goat: 2.7%). The highest rate of vaccination against brucellosis (67.96%) was observed in cows, while the lowest rate (14.5%) was observed in calves in 2013. In general, the highest percentage of vaccination was 17.92 in 1393, while the lowest (5.73) was in 1392 [16].

The investigation yielded results indicating a decreasing prevalence of brucellosis in livestock populations of cattle, sheep, and goats between 2009 and 2015. It seemed the lowest brucella prevalence in cattle in our report, related to the fact that cattle vaccination is prioritized over small ruminants such as sheep and goats.

Concurrently, there was an observed increase in the number of vaccinated animals against brucellosis. These findings suggest that a greater focus on the vaccination program may be beneficial in controlling this disease.

Conclusion

The findings of this study indicated that the sero-prevalence of brucellosis was considerable (3.6%) in the Behbahan city, Khuzestan, Iran. The findings of this study have the potential to inform the implementation of suitable control program and revised guidelines. Furthermore, the observed heterogeneity in the prevalence of seropositive brucellosis across various regions of Iran may be attributable to the inadequacy of control and eradication program in certain areas, as well as the unregulated movement of animals. It is therefore vital that continuous monitoring is implemented in this district. It is important to note that the absence of adequate preventive measures for brucellosis can result in its widespread occurrence among livestock and human populations. This necessitates the implementation of an appropriate program to control, prevent and ultimately eradicate this infectious agent in this area.

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Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

Our research protocol was approved by the Animal Research Ethics Committee of the University of Behbahan, and the research was conducted according to their guidelines and standards.

References

1. Saeed U, Ali S, Khan TM, El-Adawy H, Melzer F, Khan AU, et al. Seroepidemiology and the molecular detection of animal brucellosis in Punjab, Pakistan. Microorganisms. 2019;7(10):449.
2. Abdelbaset AE, Abushahba MF, Hamed MI, Rawy MS. Sero-diagnosis of brucellosis in sheep and humans in Assiut and El-Minya governorates, Egypt. International journal of veterinary science and medicine. 2018;6:S63-S7.
3. Lakew A, Hiko A, Abraha A, Hailu SM. Sero-prevalence and community awareness on the risks associated with Livestock and Human brucellosis in selected districts of Fafan Zone of Ethiopian-Somali National Regional State. Veterinary and animal science. 2019;7:100047.
4. Mostafavi E, Asmand M. Trend of brucellosis in Iran from 1991 to 2008. 2012.
5. Seleem MN, Boyle SM, Sriranganathan N. brucellosis: a re-emerging zoonosis. Veterinary microbiology. 2010;140(3-4):392-8.
6. Soleimani A, Alizadeh S, Farshad MS, Kusha A, Mohamdzadeh M, Haghiri L, et al. Descriptive epidemiology of human brucellosis in east Azerbaijan, 2001-2009. Medical Journal of Tabriz University of Medical Sciences. 2012;34(1):63-9.
7. Mombenı E, Mombeını M, Khalaj M, Asadı R, Rezaeı A, Amırı K, et al. Seroprevalence of brucellosis in livestock in Khuzestan province, southwest of Iran, 2008-2012. İstanbul Üniversitesi Veteriner Fakültesi Dergisi. 2014;40(2):139-46.
8. Mambini G, Maravand M, Rezaie A, Mashkuh M, Kenarkuhi M, Abdolahi D, et al. Survey on immunogenicity effcacy of a dual vaccine, combined Rev. 1 and Sheep Pox vaccine.

journal of veterinary clinical research. 2014;5(1):43-50.
9. Zeainali M, Shirzadi M, Hajrasouliha H. National manual for brucellosis control. Iran, Tehran: Raz Nahan. 2013;34(1):63-9.
10. Rahimi D, Kazemi A, Baratyan A. Identify areas at risk of Common zoonotic diseases in Khuzestan province. Spatial Planning. 2013;3(1):117-28.
11. Mahzounieh M, Golestanfar A, Salimi M, Torkabad M. Detection of bluetongue virus in aborted lamb fetuses in Chaharmahal va Bakhtiari and Isfahan provinces, by RT-PCR method. Veterinary Research & Biological Products. 2014;27(3):17-21.
12. Hajibemani A. Seroprevalence of brucellosis in sheep in Tabriz, Iran. New Findings in Veterinary Microbiology. 2023;5(2):1-10.
13. Sadeghi H, Ashrafi Tamai I, Vodjgani M, Gharagozlou F, Zahraei Salehi T. Isolation and Identification of Brucella Melitensis Biovar 1 using Bacteriological, Serological, and Molecular Tools from Saanen Goats (Capra aegagrus hircus) in Alborz, Iran. Journal of Veterinary Research/ Majallah-i Taḥqīqāt-i Dāmpizishkī University. 2022 June 22; 77 (2):107-115.
14. Yeasmin F, Sharmy ST, Siddique FI, Hossain MA, Hasan MM, Rabbani G, Nuruzzaman M, Rahman AKMA. Mamun, AA and Rahman MS (2024). Serological investigation of caprine brucellosis associated with abortion history in Bangladesh Agricultural University goat farm with a brief review. J. Vet. Med. OH Res. 6 (1-2): 109 - 121 [doi: 10.36111/jvmohr.2024.6(1-2).0039]
15. Kaboutari J, Sharifi H, Yousefzade A, Mashayekhi K, Khoshkam M, Afsharipour N. Seroprevalence of ovine and caprine anti-brucella antibodies in south of Kerman province (Iran, 2012). Journal of Veterinary Research/ Majallah-i Taḥqīqāt-i Dāmpizishkī University. 2015 Dec 25; 70 (4):371-375.
16. Kaboli Boroujeni H, Abutalebi M, Kheirollahi N, Haghighat S. Epidemiological surveillance of livestock brucellosis in Borujen county during 2010-2016. 2020.

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