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The Sero-epidemiological Investigation of Brucellosis in Cows and Sheep in Firozabad city, Fars, Iran: A Cross-Sectional Study

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Abstract

Background and aim: Brucellosis is considered as one the bacterial zoonoses which is associated with abortions and infertility in cattle and sheep especially in developing countries. Among the different species of the genus Brucella, *B. abortus* is the most common species infects cattle globally, whereas *B. melitensis* mainly infects on sheep. The risk of brucellosis transmission (Malta fever) to consumers due to the existence of nomads and traditional livestock production and farming in Firozabad city, Fars province, Iran could be assumed high. Therefore, the recent study aims to determine the Sero-epidemiological investigation of brucellosis in cows and sheep in Firozabad city, Fars, Iran.

Materials and Methods: 200 blood samples were collected during 5 months, categorized based on species, sexuality, age, and type of maintenance. The samples were subjected to the Rose Bengal test, Wright, and 2-Mercaptoethanol (2-ME) tests. The results were statistically analyzed using SPSS software and the t-test statistical method.

Results: In the Rose Bengal test, out of the total of 200 animal serum samples, 10 samples (5%) were positive (cattle (4.16%)/sheep (6.25%)). The total of 8 cases (4%) were positive in the Wright test (cattle (3.33%)/ sheep (5%)) and in the 2ME test, out of 8 positive cases in the Wright test, the total of 7 cases (3.5%) were positive (cattle (2.5%)/ sheep (5%)). The prevalence of brucellosis is 4% (0.5% cattle+3.5% sheep) and the percentage of infection is 5.15% in female cattle, 0% in bulls, 7.40% in female sheep, and 3.84% in male sheep. In terms of age, infected cows and sheep under one-year are 2.38% and 0%, in over one year sheep and cows' cases are 8.33% and 5.12% respectively. Finally, the prevalence in traditional farms (7.75%) compared to industrial farms (1.19%) is significantly higher.

Conclusion: The statistical analysis shows that the prevalence of brucellosis in sheep was higher than cows and there was a significant association between brucellosis infection and the gender, age of animals and a significant relationship between the type of maintenance and the percentage of disease is observed. Also, the prevalence rate was higher in traditional farms than in industrial farms.

Keywords: *Brucellosis, livestock, Zoonotic diseases, Epidemiology*

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Introduction

Brucellosis is one of the most important bacterial zoonoses which is the major cause of abortions and infertility in cattle population and other livestock specially in developing countries. (Pappas *et al.,* 2006, Franco *et al.,* 2007, Omer *et al.,* 2000). Based on taxonomic distribution, Brucella is classified as α-proteobacteria, which is further divided into six species, each including several biovars. The causual agent of brucellosis is gram-negative coccobacilli which belongs to genus Brucella (Selim *et al.,* 2019). Among the different species of genus *Brucella*, *B. abortus* is the common species infecting cattle all over the world while *B. melitensis* is affecting mainly sheep, goats (O'Callaghan *et al.,* 2002). The bacteria infect reproductive tissues, lymph nodes, and the spleen, which can cause inflammation, edema, and necrosis (Wernery 2014; Narnaware *et al.,* 2017). Brucellosis gains public health importance when the bacteria are transmitted to human via unpasteurized milk, meat, and animal byproducts, from infected animals (Garcell *et al.,* 2016). The genome structure of Brucella is composed of two chromosomes, without plasmids, making it unique in Bacteriaceae.

The recent introduction of genome sequence projects and genome information of *B. melitensis* (Gene Bank NC003317) and (NC003318), B. suis (Gene Bank NC002969), and *B. abortus* has opened up further gates towards the understanding of the disease pathogenicity and its mode of virulence (Sánchez *et al.,* 2001; DelVecchio *et al.,* 2002). The infection of Brucella species is commonly mediated by direct contact with the placenta, fetus, fetal fluids, and vaginal discharges or byproducts (e.g., milk, meat, and cheese) from infected animals (Köse *et al.,* 2014; Rossetti *et al.,* 2017). This explains why the typical route of infection is either direct ingestion or via mucous membranes, broken skin, and in rare cases intact skin. Brucellosis also spreads via vertical transmission, by infecting new-born calves and lambs in the uterus (Rossetti *et al.,* 2017). Brucellosis is a widespread reproductive disease, commonly causing abortion, stillbirth, retained placenta or birth of weak calves, delayed calving, male infertility, and marked reduction in milk yield. In bulls, the disease is characterized by fever, vesiculitis, orchitis, and epididymitis. In severe cases, it can also be the reason for testicular abscesses, metritis or orchitis that can lead to lifetime infertility (Arif *et al.,* 2017; Currò *et al.,* 2012; Çelebi *et al.,* 2007; Garofolo *et al.,* 2016). In animals, brucellosis symptoms can be varied from severe acute to sub-acute or chronic, depending upon the organ of infection and the type of animal (When a pregnant animal is infected by Brucella, a visible swelling of the mammary gland to the navel region and bleeding from the vagina is not uncommon, even if the cow does not abort. The enlarged udder size (appearance of the 9th month of a pregnant cow) could be used as an indication for the high stage of the disease, where animals shed bacteria in urine, milk, and vaginal discharges (Khan *et al.,* 2018).

Since the brucellosis control program in the livestock population has many challenges and problem in Iran, and considering the fact that Fars province is one of the sides of animal husbandry in the country and the risk of brucellosis transmission (Malta fever) to consumers due to the existence of nomads and traditional livestock production and farming in Firozabad city, Fars province, Iran could be assumed high. A recent study conducted in Firozabad city, Fars showed that the Prevalence of *Giardia duodenalis* as a parasitic zoonotic disease was significantly high in traditional dairy cattle farming system (Zarei *et al.,* 2024). Therefore, the recent study aims to determine the Sero-epidemiological investigation of brucellosis in cows and sheep in Firozabad city, Fars, Iran.

Materials and methods

This cross-sectional study was carried out from April 1, 2023 to the end of June 2023. According to the number of husbandries in the city, 200 blood samples were taken within five months from sheep and cows, which were categorized as follows: 1. Animal species: out of the 200 samples, 80 were from sheep and 120 from cattle. 2. Sexuality: out of the population of 80 sheep, 54 were female and 26 were male. Out of the 120 cattle, 97 were females and 23 were males. 3. Age: 80 sheep samples, 20 samples were under one-year-old and 60 samples were over one-year-old, and in the cows, 42 samples were sample under one-year-old and 78 samples were over one-year-old. 4. Type of Husbandry: The entire sheep population we studied were from traditional farms in Firozabad, but out of the cattle population, 84 animals from industrial cattle farms and 36 animals from traditional farms were sampled for the study. In order to accurately track and monitor brucellosis, the

livestock population of Firozabad city was considered as the study population in this study.

The information of all the villagers, farmers and ranchers who, due to the occurrence of symptoms such as reduced milk or meat production, infertility, stillbirth, abortion, retained placenta, as well as swelling of the testicles and epididymis in male animals, went to the private sector, or the veterinary networks of Firozabad city were referred to, was collected. Therefore, immediately after the report of the Infection, a team consisting of a veterinarian, an expert assistant and a sampler were sent to the place.

Sampling was conduct from the jugular vein of the diseased animals and other animals in contact with them in the husbandry and also randomly from the rest of the animals that were around the possible center of the disease. In this regard, by using vacuum tubes (Exvac brand produced in Iran), blood samples were taken and transferred to the Bitran laboratory. After blood clotting, according to Pakzad's method (Nielsen, 2000), the tubes were placed in a centrifuge (Behsan brand produced in Iran) for 5 minutes at 1500 rpm to separate the serum with a clear yellow color. Rose Bengal test was performed on all serum samples as a rapid initial diagnostic test. The rose bengal antigen used in all samples was the product of Razi Vaccine and Serum Research Institute, Iran with batch number 10491002 containing 8% of Brucella microbial mass, which was mixed with the same volume of serum in a microplate for 2 minutes, and the cases where the agglutinated seeds It was clearly seen in them, additional tests of Wright and 2-Mercaptoethanol (2ME) were performed on them.

Wright's seroagglutination antigen used was the product of Institut pourquier-Montpellier, France with batch number 200110 207. In order to perform the tube test, a dilution of 1.10 to 1.80 was prepared separately from each positive Rose Bengal serum sample, 0.5 ml of 10% antigen was added to each of them and kept in a water bath at 37 Celsius degrees for 20 to 24 hours. According to the amount of agglutination and the clarity of the supernatant in the last agglutinated dilution, the answer was read from negative to +4. Then, in order to detect the type of antibody and determine the active or inactive state of the disease, 2-mercaptoethanol tests were performed on the said sera. In this experiment, before preparing the dilution, after pouring physiological serum and suspected serum and 0.2 moles of mercaptoethanol (DNAbiotec (Pty) Ltd, South Africa) per liter into the first tube, the tubes were placed in a 37-degree water bath for one hour. After the mentioned time, as before, dilutions of 1.10 to 1.80 were prepared from the sera and placed again in a 37 Celsius degree water bath for 18 to 24 hours. The result of the test was also read based on the transparency of the supernatant liquid in the last agglutination dilution.

Results

The obtained data were statistically analyzed using SPSS software (version 26.0) and T-test statistical method and calculation of mean and standard deviation. In the Rose Bengal test, out of a total of 200 animal serum samples, 10 were positive (5%) and the rest were negative. Out of 10 positive cases, according to the type of animal, 5 cases were related to cattle (4.16%) and the other 5 cases were related to sheep (6.25%). The samples that were positive in the Rose Bengal test were subjected to a tube burn test for a definitive test. A total of 8 cases (4%) were positive in the Wright test, of which 4 cases (3.33%) were positive in cattle and 4 cases (5%) were positive in sheep. 2 cases of Rose Bengal test were also false positive. According to the Rose Bengal test, brucellosis is more in sheep (6.25%) and less in cattle (4.16%), and it is 5% of the total animals under investigation (Table 1). In the 2ME test, out of 8 positive cases in the Wright tube test, a total of 7 cases (3.5%) were positive, of which 3 cases were from cattle (2.5%) and 4 cases were from sheep (5%), in terms of IgG production are positive. 1 other case, which was negative in the 2ME test, is one of the IgM positive cases, the rate of which is 0.5%. According to the above results, the prevalence of brucellosis is 4% (0.5+3.5) (Table 1). In this study, it was found that the percentage of infected females (5.96%) is more than that of males (2.04%). In total, the percentage of infection in female cattle is 5.15%, in bulls it is 0%, and in female sheep it is 7.40%. It is 3.84% in male sheep (Chart 1).

In the statistical tests performed, a significant relationship between gender and disease percentage was observed and this observation is that the amount of infection in females is more than males ($p<0.05$). In terms of age, in the sheep population, the number of brucellosis patients is 0 out of 20 sheep under one year and 5 out of 60 sheep over one year (8.33%), while in the cattle population, this amount is in cattle

under one year, among the total number of 42 cases, 1 case (2.38%) was positive, and in cattle over one-year-old, 4 cases (5.12%) were positive out of 78 cases sampled. Thus, in the statistical tests performed, a significant relationship between age and disease percentage was observed. Indeed, the amount of infection in the elderly population (6.52%) is more than the young population (1.61%) ($p<0.05$) (Chart 2).

In terms of the type of maintenance, since we had taken all the samples from traditional livestock farming in the sheep population, after the total number of 80 sheep, all of which were kept in a traditional way, 5 cases (6.25%) were found in the population (Chart 3). In Cows, out of the total number of 84 industrial farming cases, only 1 case (1.19%) was positive and 4 cases (11.11%) out of the total number of 36 traditional farming cases were positive. In this study, it was found that the percentage of infection among the animals that were kept in a traditional farm (7.75%) was more than the animals that were kept in an industrial farm (1.19%) (Chart 4). In the statistical tests performed, a significant relationship between the type of maintenance and the percentage of disease was observed and this observation is that the amount of infection in the traditional maintenance method is more than the industrial type ($p<0.05$).

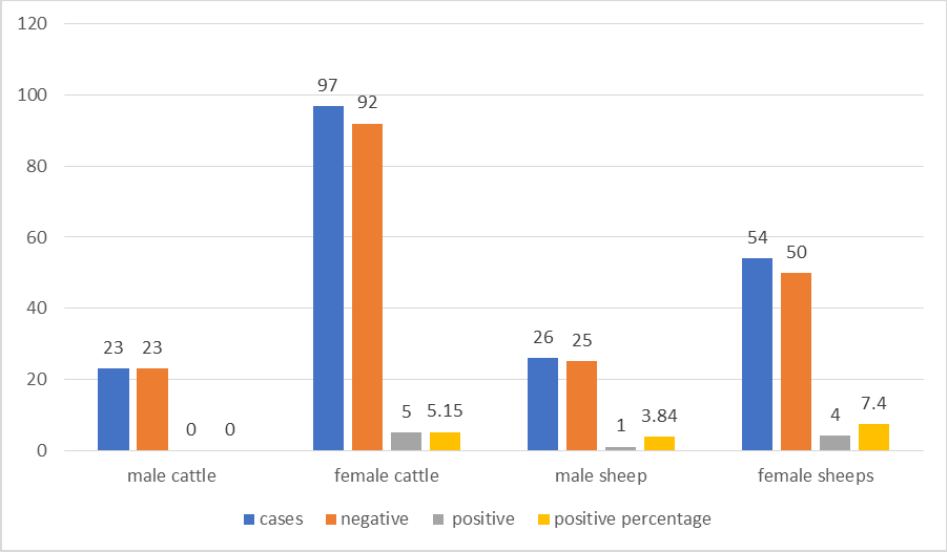


Chart 1. The prevalence of brucellosis by sexuality.

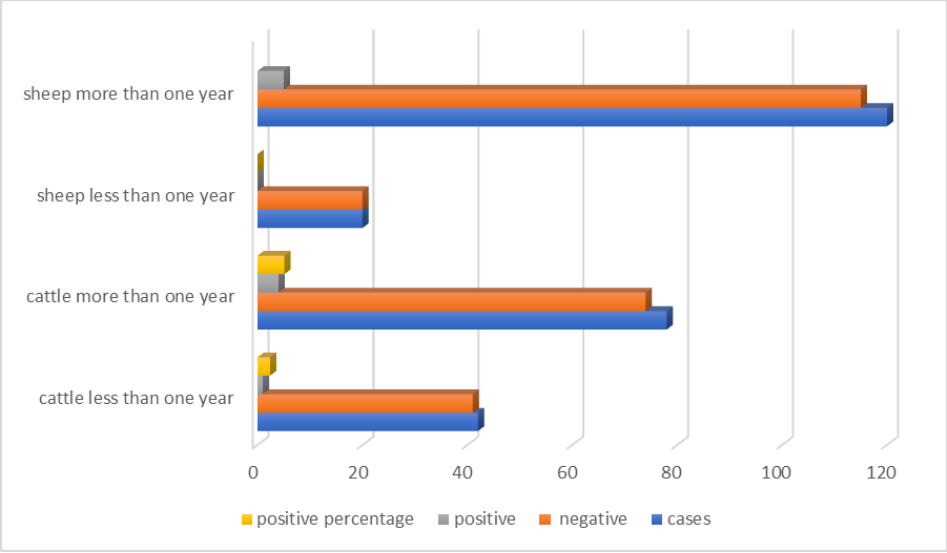
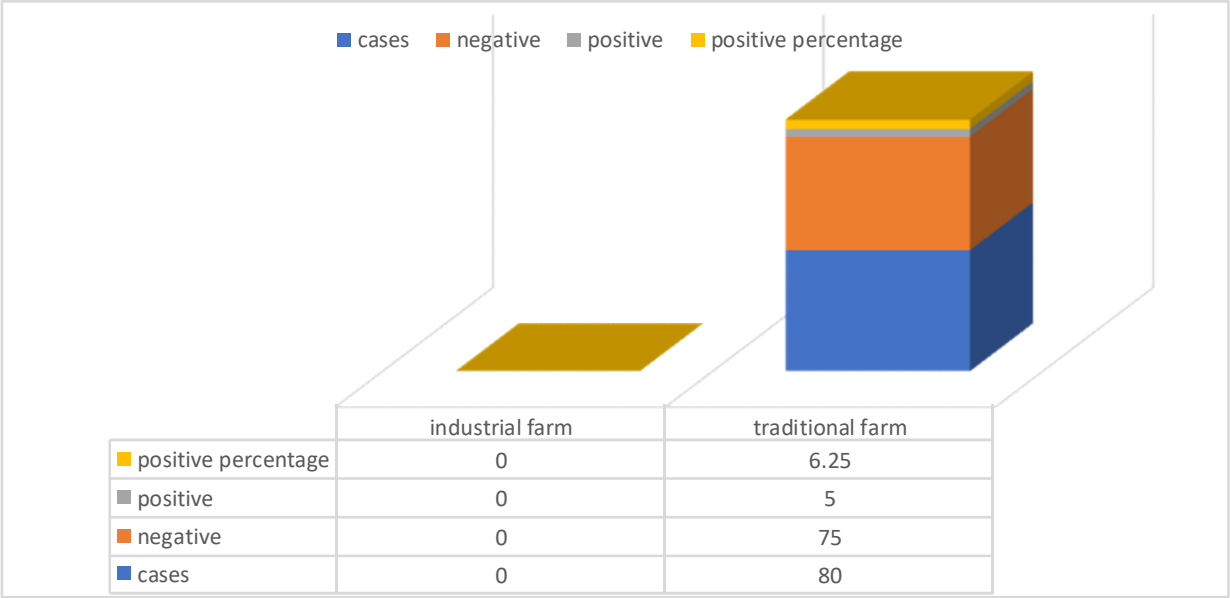
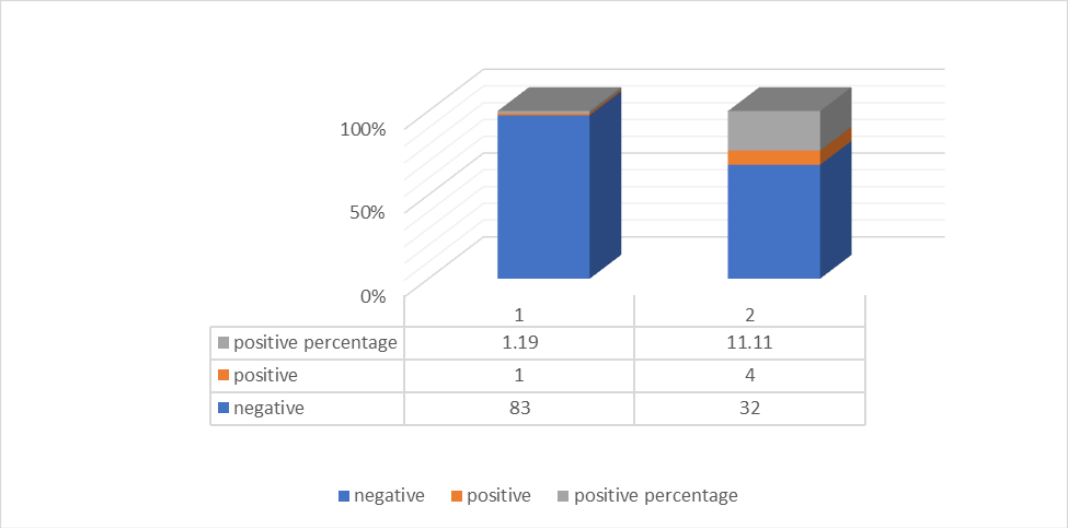


Chart 2. The prevalence of brucellosis by age.



	industrial farm	traditional farm
positive percentage	0	6.25
positive	0	5
negative	0	75
cases	0	80

Chart 3. The prevalence of brucellosis in sheep by the type of maintenance.



	1	2
positive percentage	1.19	11.11
positive	1	4
negative	83	32

Chart 4. The prevalence of brucellosis in cattle by the type of maintenance.

Discussion

The Prevalence of brucellosis in sheep and cows in a region is different and depends on several factors such as type of animals, animals farming system, sexuality and age.

In the present study, the prevalence brucellosis in sheep was higher compared to cattle, and this issue was confirmed in all three tests. Moreover, some other research gains same result. For example, Akbarmehr *et al.,* examined a total of 1500 animals (600 cattle, 740 sheep and 160 goats) from Sarab city, East Azarbayjan province, Iran. Out of 1500 blood samples 61 (4.06%) were positive for brucellosis. The prevalence of brucellosis in cows, sheep and goats was 3.66, 4.18 and 5%, respectively (Akbarmehr *et al.,* 2011). Mombeni *et al.,* investigated the 18 districts of Khuzestan Province in Southwest Iran, and serum samples of 87798 cattle and 119020 sheep were tested. The seroprevalence derived from total samples was 0.72% for cattle and 3.01% for sheep, revealing that though brucellosis is present among livestock populations in Khuzestan and that infection among sheep is significantly higher than among cattle ($p<0.05$) (Mombeni *et al.,* 2014). In other survey conducted in Hamedan, Iran, A total of 3,250 blood samples from 2,550 sheep and 700 goats were

collected randomly and they found that the seroprevalence rate of brucellosis in animals and flock level were found in 4.6% and 13.6% of goats and 3% and 27.9% of sheep, respectively (Gharekhani *et al.,* 2016). In the survey conducted in southeastern Iran by Sharifi *et al.,* in 2015, serum samples were randomly collected from 1767 sheep and 1233 goats, older than 18 months, from 300 flocks, sixty-three flocks (21.00%; 95% CI: 16.80-26.60) had at least one seropositive animal (Sharifi *et al.,* 2015). In other survey conducted in Famenin, Iran, Blood samples of 1758 animals (1470 sheep, 190 goats, and 98 cattle) were obtained in different rural regions of Famenin. The highest rate of brucellosis (6.25%) was detected in Emamzadeh-Pirnahan region (22.2% goats and 5.6% sheep) (Adabi *et al.,* 2022). Study conducted in Kurdistan, Iran, serum sampling was performed from 51 slaughterhouse workers, veterinarians, and husbandry workers, along with 260 livestock (80 cattle, 120 sheep). The seroprevalence (based on ELISA assay) in sheep and cattle was 5.8%, and 1.2%, respectively (Aghamohammad *et al.,* 2023). Semironi *et al.,* investigated the prevalence of brucellosis in rural livestock of Bushehr province during 2011-2013. In rural cattle population, 24 villages out of 118 villages, 20.34 percent and 30 head of cattle out of 891 head of cattle, 3.36 percent and in sheep and goat population, 89 villages out of 202 villages, 44.06 percent, 142 herds out of 526 Herd, 27% and 335 sheep and goats out of 10,246 sheep and goats, 3.27% were seropositive (Semironi *et al.,* 2018). Shahbazi *et al.,* examined 3,570 cases of sheep, 66,077 cases of cattle, for brucellosis, which were infected 39% and 26% respectively (Shahbazi *et al.,* 2015).Boroujeni *et al.,* investigated a total of 21950 cases of cattle, 35000 sheep and 1300 goats for brucellosis during 2010-2016, which 161 (0.73%), 2 (0.0006 %) and 2 (0.15 %) of the samples were infected (Boroujeni *et al.,* 2019). In a study, Soleimanzadeh *et al.,* investigated the prevalence of Brucella abortus in Holstein cattle in northwestern Iran. Thus, it can be concluded that the prevalence of brucellosis in cattle (Soleimanzadeh *et al.,* 2017). Hajibemani *et al.,* examined a total of 200 serum samples which were obtained from sheep and brucellosis were detected using the Rose Bengal test. The overall prevalence of brucellosis was 18.5% in sheep (Hajibemani *et al.,* 2023). Therefore, the problem that the infection in the sheep population is more than that of cattle, may be due to veterinary supervision, vaccination and more investigations in cattle than in sheep. Or another reason can be the individual breeding of cattle or the fixed location of their storage, but sheep are usually raised in herds for grazing and are taken to wider environments. It should be noted that this result is contrary to the result of the research conducted by Omer *et al.,* in Eritrea because they found that the percentage of infection in cattle (8.20%) was higher than that of sheep (1.40%). Also in Saudi Arabia, Al-Majali *et al.,* they declared 18.70% infection in cattle and 6.5% in sheep (Omer *et al.,* 2000; Al-Majali *et al.,* 2009).

Meanwhile, the percentage of infection in both types of livestock is higher in females, and a significant relationship between gender and infection is observed ($p<0.05$), and the reason for this may be the weaker physical condition of the females. While Some studies confirm this results such as a survey conducted in Famenin, Iran, 5.94% of males and 1.11% of females were detected positive for brucellosis ($p<0.001$). The chance of brucellosis in rams was 5.6 folds higher than in others (odds ratio = 5.64) and no significant statistical correlation ($p > 0.05$) was observed between prevalence of brucellosis and risk factors (Adabi *et al.,* 2022), Or Akbarmehr *et al.,* study which show the prevalence rates of the disease in male and female animals were determined as follows: male cattle, 1.53%, female cattle, 3.92%, male sheep, 2.8%, female sheep, 4.89% (Akbarmehr *et al.,* 2011). Hajibemani *et al.,* also show that the prevalence of brucellosis was 2% in male and 16.5% in female (Hajibemani *et al.,* 2023). However, in other survey conducted in Hamedan, Iran show no evidence of correlation between gender and Brucella infection rate were found in animals ($P>0.05$) (Gharekhani *et al.,* 2016).

In relation to age, we concluded that the percentage of infection can increase with age. So it can be concluded that with increasing age due to the decrease in the physiological activities of the body, infection rate can be increased. Two other studies gain same result like survey conducted in Famenin, Iran which shows in sheep, most cases of the disease were in 3-4 age-old group (1.92%) (Adabi *et al.,* 2022). Also, Hajibemani *et al.,* detected that the odds of brucellosis exposure in > 1 Year sheep (adult) were about 2 times higher than that of <1 Year sheep (Hajibemani *et al.,* 2023), But Gharekhani *et al.,*

resulted that statistical significant differences were seen between age groups and infection rate in goats (P=0.033, OR=2.1); unlike to sheep (P=0.373) (Gharekhani *et al.,* 2016), which this result is contrary to the result of our study.

Regarding to the type of animal maintenance system, it can be concluded that because of health issues in industrial maintenance, thus, the percentage of infection is lower than in traditional maintenance, where sanitary issues are less observed. These results are as same as Gharekhani *et al.,* Bahonar *et al.,* and Alamian *et al.* (Gharekhani *et al.,* 2016; Bahonar *et al.,* 2018; Alamian *et al.,* 2021).

The present study shows the importance of brucellosis in both veterinary and public health aspects especially in nomads of Firozabad city, Iran which have a traditional type of maintenance system and have more female animals in their flocks, but unfortunately, due to some restricted conditions for taking sample from sheep and cows on traditional husbandries, our samples mostly was taken at industrial husbandries. It is obvious that the incidence of brucellosis is much higher in traditional husbandries, thus, we highly recommend that for future studies more samples should be collected from traditional husbandries and it seems necessary to investigate brucellosis prevalence and incidence in all regions of the country.

Conclusion

The statistical analysis shows the prevalence of brucellosis in sheep with 3.5% is higher than cows with 0.5% and there is a significant association of brucellosis infection with the gender ($p<0.05$) (females is more than males), age of animals ($p<0.05$) (elderly population with (6.52%) is more than the young population). A significant relationship between the type of maintenance and the percentage of disease was observed ($p<0.05$) (traditional farms are more than the industrial farms).

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Conflict of interest

The authors declare no conflict of interest.

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بررسی سرواپیدمیولوژیک بروسلوز در گاوها و گوسفندان شهرستان فیروزآباد، فارس، ایران: یک مطالعه مقطعی

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چکیده

زمینه و هدف: بروسلوز یکی از بیماری‌های مشترک باکتریایی است که با سقط جنین و ناباروری در گاو و گوسفند، به ویژه در کشورهای در حال توسعه، مرتبط است. در میان گونه‌های مختلف جنس بروسلا، *بروسلا آبورتوس* شایع‌ترین گونه آلوده‌کننده گاو در سطح جهان است، در حالی که *بروسلا ملیتنسیس* عمدتاً گوسفندان را آلوده می‌کند. به دلیل وجود عشایر و دامداری سنتی در شهرستان فیروزآباد، استان فارس، ایران، می‌توان خطر انتقال بروسلوز (تب مالت) به مصرف‌کنندگان را بالا فرض کرد. بنابراین، مطالعه اخیر با هدف بررسی سرواپیدمیولوژیک بروسلوز در گاو و گوسفند در شهرستان فیروزآباد، فارس، ایران انجام شد.

مواد و روش‌ها: ۲۰۰ نمونه خون در طول ۵ ماه جمع‌آوری شد که بر اساس گونه، جنسیت، سن و نوع نگهداری طبقه‌بندی شدند. نمونه‌ها تحت آزمایش رز بنگال، رایت و ۲-مرکاپتواتانول (2-ME) قرار گرفتند. نتایج با استفاده از نرم‌افزار SPSS و روش آماری t-test مورد تجزیه و تحلیل آماری قرار گرفت.

یافته ها: در آزمایش رزبنگال، از مجموع ۲۰۰ نمونه سرم حیوانی، ۱۰ نمونه (۵٪) مثبت بودند (گاو (۴/۱۶٪)/گوسفند (۲۵/۶٪)). در آزمایش رایت، ۸ مورد (۴٪) مثبت بود ((گاو (۳/۳۳٪)/گوسفند (۵٪)) و در آزمایش 2-ME، از ۸ مورد مثبت در آزمایش رایت، ۷ مورد (۳/۵٪) مثبت بود (گاو (۵/۲٪)/گوسفند (۵٪)). شیوع بروسلوز ۴٪ (۰/۵٪ گاو + ۵/۳٪ گوسفند) و درصد آلودگی در گاوهای ماده ۵/۱۵٪، در گاوهای نر ۰٪، در گوسفندان ماده ۴۰/۷٪ و در گوسفندان نر ۸۴/۳٪ بود. از نظر سن، گاوها و گوسفندان آلوده زیر یک سال به ترتیب ۳۸/۲٪ و ۰٪، در گوسفندان و گاوهای بالای یک سال به ترتیب ۳۳/۸٪ و ۱۲/۵٪ بود. در نهایت، شیوع بروسلوز در دامداری‌های سنتی (۷/۷۵٪) در مقایسه با دامداری‌های صنعتی (۱/۱۹٪) به طور قابل توجهی بالاتر بود.

نتیجه‌گیری: تجزیه و تحلیل آماری نشان داد که شیوع بروسلوز در گوسفندان بیشتر از گاوها بود و ارتباط معنی‌داری بین عفونت بروسلوز و جنسیت، سن حیوانات و ارتباط معنی‌داری بین نوع نگهداری و درصد بیماری مشاهده شد. همچنین میزان شیوع در دامداری‌های سنتی بیشتر از دامداری‌های صنعتی بود.

واژه‌های کلیدی: *بروسلوز، دام، بیماری‌های مشترک بین انسان و دام، اپیدمیولوژی*

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