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Original Article

Prevalence and risk factors of *Brucella* infection in sheep and goats in the Sistan region by PCR method

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Summary
Brucellosis is an anthropozoonotic disease. In addition to abortion and economic losses for livestock farmers, brucellosis is also important in terms of public health. In this study, the epidemiology and prevalence of *Brucella* infection in sheep and goats in the Sistan region were investigated by PCR method. A total of 150 animals, including 92 sheep and 58 goats, were selected from different regions of Sistan, including five counties. Blood was taken from the animals through the jugular vein. The variables of counties, age, sex, and species of livestock, and for female animals, history of abortion, and pregnancy status were recorded by a questionnaire. After the separation of serum from blood, the sera were stored at -20°C for further experiments. Then all sera were tested by PCR for detection of Brucella genus and then positive samples were tested for detection of Brucella melitensis. Out of 150 samples, infection with Brucella genus bacteria were detected in 17 (11%) samples. Also, all samples were positive for Brucella belonging to the *melitensis species*. In the present study, the prevalence of brucellosis in animals that had a history of abortion (57%) was significantly higher than that in animals which did not have such a history (8%) (P=0.003). It was also found that the prevalence of brucellosis in animals less than one year of age, in animals aged 1 to 2 years, and in animals over 2 years of age was 0%, 20% and 23%, respectively, which shows that the prevalence of brucellosis increases with age.
Keywords: *Brucella melitensis*, Epidemiology, Polymerase chain reaction, Sistan.

Introduction

Brucellosis is an anthropozoonotic disease caused by gram-negative coccobacilli of the genus *Brucella* and the infection occurs in a wide range of mammals, including cattle, goats, sheep, camels, pigs, dogs, etc. Brucellosis causes abortion and infertility in sheep and goats (Esmaeili, 2014; Tadjbakhsh et al., 2017). Humans usually get brucellosis by consuming unpasteurized milk, cheese, and other dairy products. Also, animal breeders and veterinarians can get brucellosis when they are in contact with the aborted tissues of infected animals (Bokaie et al., 2008). Brucellosis often causes a severe and

acute febrile illness in humans. This disease can affect different body systems. Splenomegaly and hepatomegaly are complications of this disease. This disease can also cause endocarditis, arthritis, epididymo-orchitis, and encephalitis (Saadati and Saadatjou, 2020). Although, the preference of this bacterium for some parts of the body such as genital organs, breast, and joints, isolation of the *brucella* from other organs such as the lung has also been reported. (Rezaie Saber, 2014).

Infection with this organism is endemic in some parts of Asia, especially in the Indian subcontinent, Mediterranean basin and Countries around the Persian Gulf such as Iran (Robert and Kemp, 2001). Sheep and goats are most of Iran's livestock population (Bokaie et al., 2008). But so far, the brucellosis control program has focused more on cattle than on sheep and goats. The prevalence of brucellosis among industrial and semi-industrial dairy cows in Iran is 0.3% (Esmaeili et al., 2012). Several studies show that the rate of brucellosis in the sheep and goat populations is higher than the rate of this disease in the cattle population in the country (Gharekhani et al., 2016; Mombeini et al., 2014; Sharifi et al., 2014). Therefore, it is necessary to pay more attention to the control of brucellosis in small ruminants.

Serological tests such as Rose Bengal, Wright, and 2-mercaptoethanol (2-ME) tests are commonly used to diagnose ruminant brucellosis in Iran. Serological methods can lead to false positive results due to cross-reaction with other bacterial antigens (Neha et al., 2017). A false negative result is also seen when the antibody titer in the animal serum is low. In addition, sometimes in agglutination tests, due to high amounts of antibodies, which makes the ratio of antigen to antibody not optimal, the Prozone effect occurs and the test may result in a falsely negative (Saxena et al., 2015). Isolation of *Brucella* is considered the gold standard test for the diagnosis of this disease. But the isolation of this bacterium is tedious, time-consuming and difficult due to its intracellular location and fastidious nature, and due to its zoonotic nature, it requires laboratories with special safety equipment (Kaynak-Onurdag et al., 2016). The use of polymerase chain reaction (PCR) to detect Brucella DNA in blood, serum, other body fluids and tissues of humans and animals is becoming increasingly prevalent. The use of PCR, on the one hand, leads to the rapid and accurate detection of *Brucella*, and on the other hand, it minimizes the risk of infection with this bacterium in the laboratory. PCR is more sensitive than the culture method and has more specificity than serological tests for the diagnosis of brucellosis (Doosti and Ghasemi Dehkordi, 2011).

The Sistan region is located in the north of Sistan and Baluchistan province. Sistan and Baluchistan, the largest province of Iran, borders Afghanistan and Pakistan from the east. The outbreak of brucellosis in the livestock population in the eastern neighboring countries can cause the disease to be transmitted to this province. The present study was conducted with the aim of determining the prevalence of brucellosis and its risk factors in small ruminants of the Sistan region using the PCR method.

Materials and methods

Sampling

Sampling was done in February and March of 2015. In total, 150 sheep (92 cases) and goats (58 cases) were randomly selected from 5 counties of the Sistan region. Blood samples were collected from the jugular vein of all animals in tubes without anticoagulants. A questionnaire was used to collect information about species, age, sex, the status of pregnancy, and history of abortion of the animals. Sera were separated from clotted blood samples and stored at -20°C for molecular tests.

Molecular method

Sera were tested by PCR. The boiling method was used to extract DNA from serum samples (Zamanian et al., 2015). After extraction, the quality of the extracted DNA and its concentration were checked. For this purpose, the spectrophotometric method was used. The extracted DNA was kept at -20°C until PCR.

Molecular detection of *Brucella* genus was done by amplification of *bcsp31* gene and *melitensis* species by amplification of *is711* gene. The primers were synthesized by Pishgam co., Iran (Table 1), then transferred to the microbiology laboratory of the veterinary faculty of Zabol University.

Table 1. Primer sequence designed for *Brucella* genus and *Brucella melitensis* species

Gene	Primer Type	Primer Sequence	Product length	Pathogen	Source
bcsp31	forward	5'- TGGCTCGGTTGCCAATATCAA - 3'	223 bp	*Brucella.* Spp.	Baily et al., 1992
	Reverse	5'- CGCGCTTGCCTTTCAGGTCTG - 3'			
is711	forward	5'-AAATCGCGTCCTTGCTGGTCTGA- 3'	731bp	*Brucella. melitensis*	Bricker and Halling, 1994
	Reverse	5'-TGCCGATCACTTAAGGGCCTTCAT- 3'			

PCR reactions were performed using 15 µl of PCR mixture solution (including 1 µl of each forward and reverse primer, 3 µl of distilled deionized water, 8 µl of Mastermix (Pishgam Biotechnology Company ©, Iran) and 2 µl of DNA extract). The initial denaturation step was performed at 94°C for 5 minutes. After that, 30 cycles including (denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute and elongation at 72°C for 1 minute) were performed. And the final amplification stage was set at 72°C for 7 minutes. PCR products were loaded on a 1.5% agarose electrophoresis gel. Then the gel was stained with ethidium bromide and the bands were visualized by the Gel Doc system. A positive control (*Brucella abortus* strain S19) and negative control (sterile water) were included in all reactions (Figure 1).

Statistical analysis to determine risk factors

The independent variables were species, age, sex, location (counties), pregnancy status and history of abortion of animals. The brucellosis infection was considered the dependent variable. Due to the dependent variable was a dichotomous nominal variable, a multivariate logistic regression test was used to analysis of data. In the first stage, the relationship between the independent variables and the dependent variable was determined using the chi-square test and Fisher's exact test, the variables whose p-value was higher than 0.25 were entered into the logistic regression model, Any variable that had the highest p-value was removed from the model, this continued until the p-value for all variables in the model was less than 0.10 .SPSS version 23 software was used for data analysis. A $P<0.05$ was considered significant.

Results

Prevalence of brucellosis

In the present study, out of 150 samples, 17 samples (11.3%) (95% confidence: 6.7% -17.5%) were infected with *Brucella*. Figure 1 shows the specific bands of the *Brucella* gene with the size of 223 bp on 1.5% electrophoresis gel.

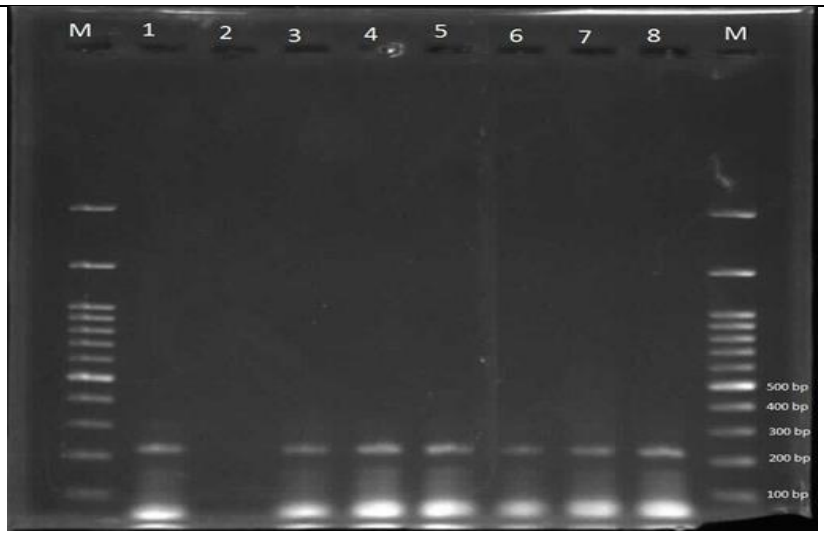

Fig. 1. Electrophoresis of bcsp31 gene amplification products. Lane M: 100 bp DNA ladder marker; Lane 1: Positive control; Lane 2: Negative control; Lanes 3-8: Samples with positive reactions.

All the animals that were positive in the PCR test using by *Brucella* genus primer (*bcsp31*) were also positive using by *Brucella melitensis* primer (*is711*), So the prevalence of *Brucella melitensis* was 11.3% (95% confidence Interval: 6.7% - 17.5%) too. Figure 2 shows the specific bands of the *Brucella melitensis* gene with the size of 731bp.

Univariate analysis of risk factors

Table 2 shows the prevalence of brucellosis according to the status of independent variables. The relationship between independent variables and *Brucella* infection was determined using the Chi-square test and Fisher's exact test.

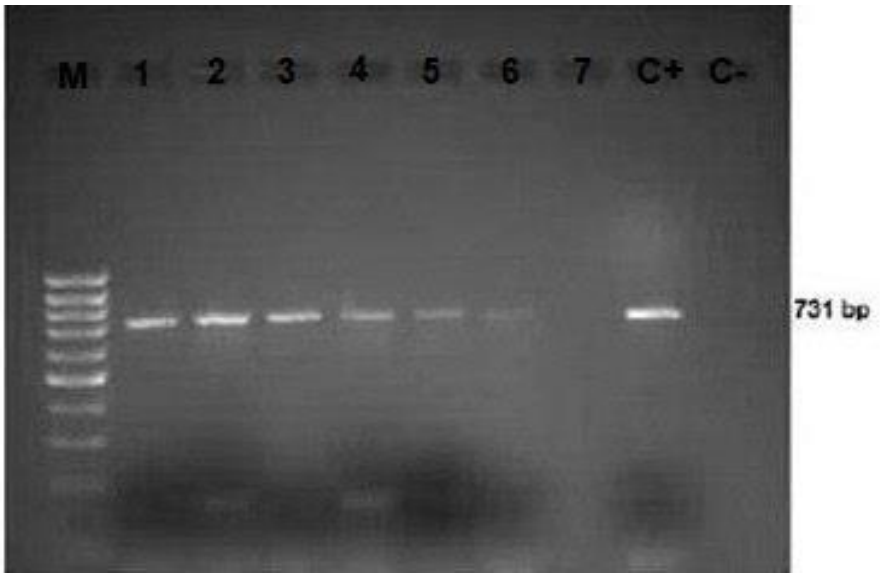

Fig. 2. Electrophoresis of is711 gene amplification products, Lane M: 100 bp DNA ladder marker; Lane C+: Positive Control; Lane C-: Negative control; Lane 1-8: PCR samples were poured.

Table 2. Brucellosis prevalence according to independent variables

Variable	Levels	Number of tested animals	Number of positive animals	Prevalence	Statistical test	*P-value*
Species	Sheep	92	8	9%	Pearson chi Square	0.199
	Goat	58	9	16%		
Age	Two years and less	120	10	8%	Fisher's exact test	0.046
	More than two years	30	7	23%		
Sex	Male	35	4	11%	Fisher's exact test	0.596
	Female	115	13	11%		
Pregnancy status (in females)	pregnant	16	3	19%	Fisher's exact test	0.269
	non-pregnant	99	10	10%		
History of abortion (in females)	has	7	4	57%	Fisher's exact test	0.003
	does not have	108	9	8%		
Location (counties)	Zabol	38	0	0%	Likelihood ratio chi Square	<0.001
	Zahak	20	0	0%		
	Nimrooz	17	4	24%		
	Hamoon	68	9	13%		
	Hirmand	7	4	57%		

Multivariate logistic regression

The variables of species, age, and location were selected for multivariate logistic regression analysis, since the variables of pregnancy status and history of abortion can be measured only in female animals, so these variables were not considered for the logistic regression model. In the next step, the species was removed from this model due to the high p-value and multivariable logistic regression was performed again, in which all the p-values were below 0.10 and only the p-value for the variable of age was significant (less than 0.05) (Table 3). The independent variables included in the model express 0.353 of the variation of the dependent variable (Nagelkerke R Square = 0.353).

Table 3. Multivariate logistic regression results, RC= Reference for Comparison

Variable	Levels	B	Odds Ratio	95% CI for Odds Ratio	*P-value*
Age					
	Two years and less	-1.468	0.230	0.060-0.878	0.031
	More than two years (RC)	0	1	-	-
Location					0.071
	Zabol	-21.582	0.000	0.000	0.997
	Zahak	-21.57	0.000	0.000	0.998
	Nimrooz	-1.403	0.246	0.036-1.659	0.150
	Hamoon	-2.65	0.071	0.011-0.434	0.004
	Hirmand (RC)	0	1	-	-

In addition to age, that was significant in the logistic regression model, the variable of history of abortion, which was not entered in the logistic regression, was significant in Fisher's exact test,

so in general, two variables including age and history of abortion were significantly related to brucellosis.

Discussion

In the present study, the prevalence of *Brucella* infection among sheep and goats in the Sistan region by PCR method was 9% and 16%, respectively, and the difference in the prevalence of *Brucella* infection between sheep and goats was not statistically significant. In the study of Shakerian et al., in Isfahan and Shahrekord 9.6% and 18% of sheep and goats milk samples were *brucella* positive, respectively, by PCR. Similar to the recent research, no significant difference was observed between the prevalence of *Brucella* infection in sheep and goats (Shakerian et al., 2016). In addition to molecular studies, serological studies have also compared the prevalence of brucellosis in goats and sheep. In a study that was conducted in slaughterhouses of East Azerbaijan province, the seroprevalence of brucellosis in goats and sheep was 5.33% and 4.53%, respectively, and there was no statistically significant difference in the prevalence of brucellosis between sheep and goats (Javadi et al., 2007), which was consistent with the results of the present study. In another study conducted by Kaboutari et al. in the south of Kerman, a statistical significant relationship was observed between the seroprevalence of brucellosis in sheep and goats, so that the odds of brucellosis in sheep were 2.12 times more than the odds of brucellosis in goats (Kaboutari et al., 2015), which was not consistent with the results of the present study. In the study of Kobutari et al., this difference was due to the different breeding systems of these two animals and it was mentioned that in that study area, goats usually have free grazing, while sheep are usually kept in a pen environment. While in the Sistan region, the breeding system is often traditional and sheep and goats are kept together.

In the present study, all the animals that were positive for the genus of *Brucella* in the PCR test were also positive using the *Brucella melitensis* primer. In Hashemifar et al.'s study, 60 blood and cerebrospinal fluid samples from people suspected of having brucellosis and 60 blood, spleen, and liver samples from sheep and cattle that were positive in Routine Serological Tests, were collected from three regions of Iran (Hashemifar et al., 2017). Among the 120 samples tested in this study, 68 samples were positive in the PCR test, of which 60 samples (88.2%) were *Brucella melitensis* and 8 samples (11.8%) were *Brucella abortus*. this study confirms the high prevalence of *melitensis* species in sheep and goats compared to other *Brucella* species in Iran. In a meta-analysis that was conducted during 1970-2020 on the country's livestock population (Dadar et al., 2021). It was found that on sheep and goats respectively, 29% and 28% of the *Brucella* isolates were *melitensis*, also respectively, 6% and 2% were *Brucella abortus*, 2% and 2% were a mixture of *Brucella abortus* and *Brucella melitensis*, and 3% and 2% were Rev1 vaccine strain. In other cases, *Brucella* species was not determined. The results of the present study show that in the Sistan region among brucellosis cases, the percentage of infection with *Brucella melitensis* is high compared to the other regions of Iran.

In the present study, the number of cases infected with brucellosis in animals under one year of age was zero (0%), in animals aged 1 to 2 years was 10 cases (20%) and in animals over 2 years of age was 7 cases (23%), which shows that the prevalence of brucellosis increases with age. Because in logistic regression, the prevalence of infection should not be zero in any group, so age group of under 1 year and age group of 1-2 years were merged into under the 2 years' age group. In the logistic regression test, the relationship between animal age and brucellosis infection was significant. In a study conducted by Ebrazah et al. on slaughterhouse animals in Amol city, it was found that the age group of 3-5 years old has more infection in sheep compared to other age groups, and as the age of the animal increases, the probability of animals being infected with brucella increases significantly. (Ebrazeh et al.,

2011). In a study in Ethiopia, 2030 male sheep were selected from export sheep farms and tested for *Brucella* infection with Rose Bengal and complement fixation test. In this study, the prevalence of brucellosis in the age group below two years was zero, the age group of 2 to 3 years was 0.38%, and the age group above three years was 2.25%, and the relationship between age and the prevalence of brucellosis infection was significant (Girmay et al., 2013). The results of these two mentioned studies were consistent with the present study. Also, similar results regarding the relationship between age and brucellosis infection have been reported in other researchers' studies (Ocholi et al., 2005; Suryawanshi et al., 2016; Gul et al., 2014). Walker states that animals become increasingly susceptible to *Brucella* infection when their age increases and they reach reproductive age (Walker, 1999).

In the present study, 115 blood samples were taken from female animals and 35 blood samples were taken from male ones, of which 13 cases from females and 4 cases from males were infected with *Brucella*, and the prevalence of brucellosis in both male and female animals was 11%. In the study conducted by Ebrazeh et al. the prevalence of brucellosis in slaughterhouse animals of Amol city in female animals was significantly higher than that in male animals. Female animals usually, are kept in the herd for a longer period of time, therefore, in slaughterhouses, female animals are usually older than male animals. Considering that the prevalence of brucellosis in sheep and goats increases with age, the difference observed in Ebrazeh's study may be due to the difference in the average age of male and female animals (Ebrazeh et al., 2011).

In this study, out of 115 female animals, 16 were pregnant, of which 3 were positive (19%) and 99 were non-pregnant, of which 10 were positive in the PCR test (10%). The difference in infection prevalence between pregnant and non-pregnant animals was not statistically significant. In this study, the vaccination history of livestock was also collected, but because many livestock farmers did not have accurate information about the type and the time of vaccination. Livestock vaccination was not analyzed statistically.

In the present study, the prevalence of brucellosis in animals with a history of abortion was significantly higher than in animals without such a history. In a study in the Mymensingh districts of Bangladesh, the prevalence of brucellosis and the risk factors of the disease were investigated on 200 sheep and goats (Rahman et al., 2011). In this research, in both sheep and goats, the history of abortion in animals infected with brucellosis was significantly ($p < 0.01$) higher than that of non-infected animals, the results of this study are consistent with the results of the present research. In another study that was conducted in the Sistan region, 78 sheep and goat embryos f were randomly collected and tested by PCR method, and *Brucella melitensis* was detected in 15 embryos (19.2%) (Mahdavi-Roshan et al., 2018). The most important symptoms of brucellosis in sheep and goats are abortion. And retained placenta and metritis may occur after abortion. Sheep and goats with brucellosis usually abort only one time, but uterine secretions in subsequent parturitions may be contaminated with brucellosis. (Saadati and Saadatjou, 2020; Saxena et al., 2018).

It is recommended that brucellosis vaccination coverage in sheep and goats must be increased in the Sistan region and the people, especially the livestock breeders in the region must be notified about methods for preventing brucellosis.

Conclusion

The findings of the present study showed that there is a high prevalence of brucellosis in the population of sheep and goats in the Sistan region and this disease is one of the causes of abortion in livestock in this region. It was also found that the risk of brucellosis in sheep and goats increases with increasing the age of animals.

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Ethical approval

The protocol of the present research was reviewed and approved by vice chancellor for academic affairs of the veterinary Faculty of Zabol University (registration number: 25/20451).

Conflict of interest

The authors declare that there was no conflict of interest in the present research.

References

Baily G., Krahn J., Drasar B. & Stoker N. Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. *Tropical Medicine and International Health*, 1992, 95(4), 271-5.

Bokaie S., Sharifi L. & Alizadeh H. Epidemiological survey of brucellosis in humans and animals in Birjand, east of Iran. *Journal of Animal Veterinary Advances*, 2008, 7(4), 460-3.

Bricker B.J. & Halling S.M. Differentiation of *Brucella abortus* bv. 1, 2, and 4, *Brucella melitensis*, *Brucella ovis*, and *Brucella Suis* bv. 1 by PCR. *Journal of clinical microbiology*, 1994, 32(11), 2660-6.

Dadar M., Shahali Y. & Fakhri Y. Brucellosis in Iranian livestock: A meta-epidemiological study. *Microbial Pathogenesis*, 2021, 155, 104921.

Doosti A. & Ghasemi Dehkordi P. Application of real-time PCR for identification and differentiation of *Brucella abortus* and *Brucella melitensis* in cattle. *Bulgarian Journal of Veterinary Medicine*, 2011, 14(2), 109-15.

Ebrazeh N., Asmar M., Amir Mozafari N. & Esfandiari B. Investigation of prevalence and incidence of brucellosis in Amol of slaughterhouse livestock. *Journal of Biological Science*, 2011, 5(2), 1-9.

Esmaeili H. Brucellosis in Islamic republic of Iran. *Journal of medical bacteriology*, 2014, 3(3-4), 47-57.

Esmaeili H., Tajik P., Ekhtiyarzadeh H., Bolourchi M., Hamedi M., Khalaj M. & Amiri K. Control and eradication program for bovine brucellosis in Iran: an epidemiological survey. *Journal of veterinary research*, 2012, 67(3), 211-21.

Gharekhani J., Rasouli M., Abbasi-Doulatshahi E., Bahrami M., Hemati Z., Rezaei A. & Shahreiari A. Sero-epidemiological survey of brucellosis in small ruminants in Hamedan province, Iran. *Journal of Advanced Veterinary and Animal Research*, 2016, 3(4), 399-405.

Girmay A., Hussien D. & Afera B. Seroprevalence of ovine brucellosis in a sheep export farm, Ethiopia. *Global Veterinaria*, 2013, 11(3), 325-8.

Gul S.T., Khan A., Rizvi F. & Hussain I. Sero-prevalence of brucellosis in food animals in the Punjab, Pakistan. *Pakistan Veterinary Journal*, 2014, 34(4), 454-8.

Hashemifar I., Yadegar A., Jazi F.M. & Amirmozafari N. Molecular prevalence of putative virulence-associated genes in *Brucella melitensis* and *Brucella abortus* isolates from human and livestock specimens in Iran. *Microbial pathogenesis*, 2017, 105, 334-9.

Javadi A., Akrami Nojadeh G., Javadi M.R. & Ahmad Khanli M. Serological study of sheep and goat brucellosis in slaughterhouses of East Azerbaijan province in 2004-2005. *Veterinary Clinical Pathology The Quarterly Scientific Journal*, 2007, 1(1), 15-9.

Kaboutari J., Sharifi H., Yousefzade A., Mashayekhi K., Khoshkam M. & Afsharipour N. Eroprevalence of ovine and caprine anti-*Brucella* antibodies in south of Kerman province (Iran, 2012). *Journal of Veterinary Research*, 2015, 70(4), 371-5.

Kaynak-Onurdag F., Okten S. & Sen B. Screening *Brucella* spp. in bovine raw milk by real-time quantitative PCR and conventional methods in a pilot region of vaccination, Edirne, Turkey. *Journal of dairy science*, 2016, 99(5), 3351-7.

Mahdavi-Roshan H., Saadati D. & Najimi M. Molecular detection of *Brucella melitensis*, *Coxiella burnetii* and *Salmonella abortusovis* in aborted fetuses of Baluchi sheep in Sistan

region, south-eastern Iran. *Iranian Journal of Veterinary Research*, 2018, 19(2), 128-32.

Mombeini E., Mombeini M., Khalaj M., Asadi R., Rezaei A., Amiri K., Bromand S., Kenarkohi M. & Mombeini A. Seroprevalence of brucellosis in livestock in Khuzestan province, southwest of Iran, 2008-2012. *İstanbul Üniversitesi Veteriner Fakültesi Dergisi*, 2014, 40(2), 139-46.

Neha A., Kumar A. & Ahmed I. Comparative efficacy of serological diagnostic methods and evaluation of polymerase chain reaction for diagnosis of bovine brucellosis. *Iranian journal of veterinary research*, 2017, 18(4), 279.

Ocholi R.A., Kwaga J.K.P., Ajogi I. & Bale J.O.O. Abortion due to *Brucella abortus* in sheep in Nigeria. *Revue scientifique et technique-Office international des epizooties*, 2005, 24(3), 973-9.

Rahman M.S., Hahsin M.F.A., Ahasan M.S., Her M., Kim J.Y., Kang S.I. & Jung S.C. Brucellosis in sheep and goat of Bogra and Mymensingh districts of Bangladesh. *Korean Journal of Veterinary Research*, 2011, 51(4), 277-80.

Rezaie Saber A. P. Isolation of different bacteria from pneumonic lungs of cows slaughtered in Tabriz (IRAN) abattoir. *Veterinary Clinical Pathology the Quarterly Scientific Journal*, 2014, 8(2), 481-91.

Roberts A. & Kemp C. Brucellosis (Mediterranean fever, Gibralter fever, Malta fever, Cyprus fever, undulant fever, typhomalarial fever), *Journal of the American Academy of Nurse Practitioners*, 2001, 13(3), 106-07.

Saadati D. & Saadatjou S. 2020, *Introduction and Prevention of Zoonotic Diseases*, 1st ed. Sistan & Baluchestan: University of Zabol Press, Zabol.

Saxena H.M., Chothe S. & Kaur P. Simple solutions to false results with plate/slide agglutination tests in diagnosis of infectious diseases of man and animals. *MethodsX*, 2015, 2, 345-52.

Saxena N., Singh B.B. & Saxena H.M. Brucellosis in sheep and goats and its serodiagnosis and epidemiology. *International Journal of Current Microbiology and Applied Sciences*, 2018, 7(1), 1848-77.

Shakerian A., Deo P., Rahimi E., Shahjavan A.R. & Khamesipour F. J. Molecular detection of *Brucella melitensis* in sheep and goat milk in Iran. *Tropical Journal of Pharmaceutical Research*, 2016, 15(5), 913-8.

Sharifi H., Tabatabaei S., Rashidi H., Kazeminia S., Sabbagh F., Khajooei P., Karamouzian M., Nekouei O., Sardooei M.A. & Leontides L. A cross-sectional study of the seroprevalence and flock-level factors associated with ovine and caprine brucellosis in southeastern Iran. *Iranian journal of veterinary research*, 2014, 15(4), 370.

Suryawanshi S., Tembhurne P., Gohain S. & Ingle V. Prevalence of *Brucella* antibodies in sheep and goats in Maharashtra. *Indian Research Journal of Extension Education*, 2016, 14(4), 75-7.

Tadjbakhsh H., Mokhber Dezfouli M. & Akbarein H. A review of the most important zoonoses with a special vision towards emerging & re-emerging diseases and its status in Iran Part (1), Bacterial zoonoses. *Veterinary Clinical Pathology the Quarterly Scientific Journal*, 2017, 11(3), 197-223.

Walker R.L. 1999, *Veterinary Microbiology*, Massachusetts: Science e Blackwell Publishing, Cambridge.

Zamanian M., Hashemitabar G., Rad M. & Haghparast A. Evaluation of different primers for detection of *Brucella* in human and animal serum samples by using PCR method. *Archives of Iranian Medicine*, 2015, 18(1), 44-50.