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Bovine Brucellosis Infection in Iranian Dairy Farms: A Herd-Level Case-Control Study

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Abstract

Background: Brucellosis is considered one of the most important diseases which is common among humans and animals with the great health and economic importance.

Objectives: This study aimed to investigate some risk factors of the brucellosis infection in Iranian dairy farms.

Methods: This study is a herd-level case-control study on dairy farms. Case dairy farms (95 dairy farms) included all registered cases of disease during 14 months of studying with at least one positive serum cow (Rose Bengal, Wright and 2-mercaptoethanol tests consecutively) and control dairy farms (95 dairy farms) in the condition of at least two disease-free years were selected and matched due to the capacity, and geographical area with case dairy farms. The data were analyzed by the multivariate conditional logistic regression test and Stata statistical software version 14.

Results: Due to the statistical relationship among studying independent variables and brucellosis infection in herd, it was found that the hygiene and disinfection of watering points (washing at least three times a week and using detergent or disinfectant) reduce the risk of brucellosis infection (OR = 0.04 , 95% CI = 0.003-0.499) and factors such as the history of abortion (OR = 7.01, 95% CI = 1.51-32.59), the replacement of livestock from outside (OR = 7.87, 95% CI = 1.07-58.07) and introducing new livestock during last 12 months (OR = 7.27, 95% CI = 1.20.43.90) increase the risk of brucellosis infection.

Conclusions: More serious attention to rancher training, the observance of hygienic principles, and legal restriction of livestock displacement are among the recommended strategies to prevent brucellosis infection on the farm.

Keywords: Brucellosis, Dairy Farms, Iran, Risk Factors

Introduction

Brucellosis is considered one of the most important and common diseases among humans and animals in the world and causes serious problems for health and economy specially in developing countries (Joseph, Oluwatoyin *et al*. 2015, Bagheri Nejad, Krecek *et al*. 2020, Tulu 2022, de Figueiredo, Ficht *et al*. 2015). In addition to the importance of the disease in humans, the economic loss of the disease in the livestock population is significant due to abortion, stillbirth, low calf birth, reduced milk production, delayed fertility, reduced calving, elimination of livestock due to infertility, loss of time for patients, and treatment costs (Boluki, Bahonar *et al*. 2017).

Infection occurs in the wild mammals such as deer, roe deer, and buffalo. Wild boar and dogs increase the risk of exposure to Brucella in cattle and the organism is isolated from these animals. Also, dogs may be the carriers of the organism (Davis 1990).

Brucella's main source in the epidemiology of brucellosis in cattle is uterine fluid, placenta, and aborted fetus (Anka, Hassan *et al.* 2014). The epidemiology of brucellosis in cattle is complex and characterized by various factors including individual predisposing factors and factors related to disease transmission and the risk factors of maintenance and the spread of infection among herds including the management factors (such as biosecurity, herd size and composition, population density, and herd safety status) and the environmental factors such as climate (Alhaji, Wungak *et al.* 2016).

Bovine brucellosis is widely distributed around the world but in recent decades in the most European countries, Japan, Canada, and the United States has been eradicated from the livestock populations due to forced pasteurization of dairy products and the strict control of dairy herds (Joseph, Oluwatoyin *et al.* 2015). Brucellosis is an endemic disease in Iran and Brucella abortus was firstly isolated from the bovine embryos in 1944 and since 1967, the National Livestock Brucellosis Control Plan has been implemented which was included in testing, slaughtering, and vaccinating adult cows and 3 to 8-month-old calves. (Leylabadlo, Bialvaei *et al.* 2015, Bahonar, Bahreinipur *et al.* 2019). Although the prevalence of brucellosis among cows in the industrial and semi-industrial dairy farms in recent years, on average, was estimated 3 in one thousand cows, but this figure is definitely higher than this estimation due to the non-consideration of other animals which are traditionally kept (Esmaeili, Tajik *et al.* 2012, Esmaeili 2014).

In total, in 2018, 3322 dairy farms are under the active surveillance of Brucellosis. Due to the results of active surveillance in these farms, a total of 759 cows in 131 units (including 6 dairy farms complexes, 104 dairy farms, 15 dairy, and beef cattle farms, 5 dairy cow and sheep farms and 1 beef cattle farm were recognized as positive due to the brucellosis (Bahonar, Bahreinipur *et al.* 2019).

Regarding the role of brucellosis in public health, and in dairy cow breeding industry that causes many economic damages, in this study, identification of factors associated with brucellosis such as fertilizer management, livestock fences, mare satins and, etc. is considered to provide effective guidelines for controlling the disease in the farms and preventing economic and health damages.

Material and Methods

This study is a case-control study in which the statistical population consists of the dairy farms across the country which were covered by the Brucellosis Test and Slaughter Plan of Iran Veterinary Organization.

• Selection of the case and control dairy farms:

Each dairy farm had at least one positive serum of cows (cases since the beginning of 2018) according to the serological tests of Rose Bengal, Wright, and 2-mercaptoethanol (cases since the beginning of 2018) was considered as an infected dairy farm as a case.

The control dairy farms were selected from serum negative dairy farms by the results of serological tests (negative serum at least in the last two years) which matched with dairy farms due to the capacity, and geographical area (table 1).

Table 1. Geographical distribution of case and control farms

Number	Province of Case Farms	Number of Farms	Province of Control Farms
1	Alborz	2	Alborz
2	Azerbaijan, East	2	Azerbaijan, East
3	Chahar Mahaal and Bakhtiari	1	Chahar Mahaal and Bakhtiari
4	Fars	9	Fars
5	Golestan	6	Golestan
6	Hamadan	2	1 from Hamadan, 1 from Kurdistan
7	Ilam	2	1 from Ilam, 1 from Kermanshah
8	Isfahan	8	Isfahan
9	Kerman	11	Kerman

10	Khorasan, Razavi	9	Khorasan, Razavi
11	Khorasan, South	2	Khorasan, South
12	Kurdistan	1	Kurdistan
13	Lorestan	1	Lorestan
14	Markazi	3	Markazi
15	Qazvin	4	Qazvin
16	Qom	5	Qom
17	Semnan	7	Semnan
18	Tehran	4	Tehran
19	Yazd	15	Yazd
20	Zanjan	1	Zanjan
Total		95 Case Herds and 95 Control Herds	

• Sample size

The sample size at the dairy farm level was obtained according to the sample size formula by considering 95% confidence level, 80% test power and a ratio of one for the number of controls to cases and OR = 2.5:

$$n=2(Z_{(1-\alpha/2)} + Z_{(1-\beta)})^2 \times P(1-p) / (P_0 - P_1)^2$$

$$P_1=P_0\ OR/[1+P_0(OR-1)]$$

New livestock introducing: the exposure rate in the control of 53.23%

Minimum required sample: 85 cases and 85 controls

Indirect Contact: Exposure Percentage in Control of 29.03%

Minimum required sample: 82 cases and 82 controls

Improper fertilizer management: exposure rate in the control of 30.65%

Minimum required sample: 80 cases and 80 controls

Improper flame treating: exposure percentage in control of 33.87%

Minimum required sample: 79 cases and 79 controls

Considering the four sample sizes, cases and the maximum sample size calculated, the number of dairy farms required for the study includes 170 dairy farms (85 case groups and 85 control groups).

• Data collection

Several experts of the Iran Veterinary Organization were trained to collect the required data in each studying province, and the data were collected from the case and control dairy farms using a questionnaire designed by the research team. At the next stage, the data was entered into SPSS software and then statistically analyzed.

• Data analysis

The data were analyzed using Stata statistical software version 14. Conditional logistic regression was used to determine the relationship between the disease's risk factors at the herd-level. The studying variables were firstly entered in the univariate conditional logistic regression model. Then those variables which had a p-value>0.2 were eliminated from the model, and the other variables were entered into the multivariate conditional logistic regression model. The model was simplified by the Backward Elimination method using Wald and Likelihood Ratio tests. After the simplification, significant variables were entered into the model, and using the Backward Elimination method, the model has simplified again so that all variables finally showed a significant relationship. At last, frequency distribution, odds ratio, and p-value of independent variables were calculated and estimated based on the multivariate conditional logistic regression model. Also, the interaction among variables was evaluated to ensure the presence or absence of effective interactions among variables in the final model.

Results

The mean ± standard deviation of the studied rancher or farm manager's age was 51.8 ±13.1 years in the case group and 52.3 ± 12.6 years in the control group. The highest and lowest level of education in the case group were Diploma (29.5%) and illiterate (1.1%), respectively, and in the control group, the highest frequency of education level was related to Diploma (32.6%), and the lowest frequency was related to three levels of education of illiterate, Associate Degree, and Master and higher with a relative frequency of 3.2%. Frequency of all studied variables have been showed in figure 1.

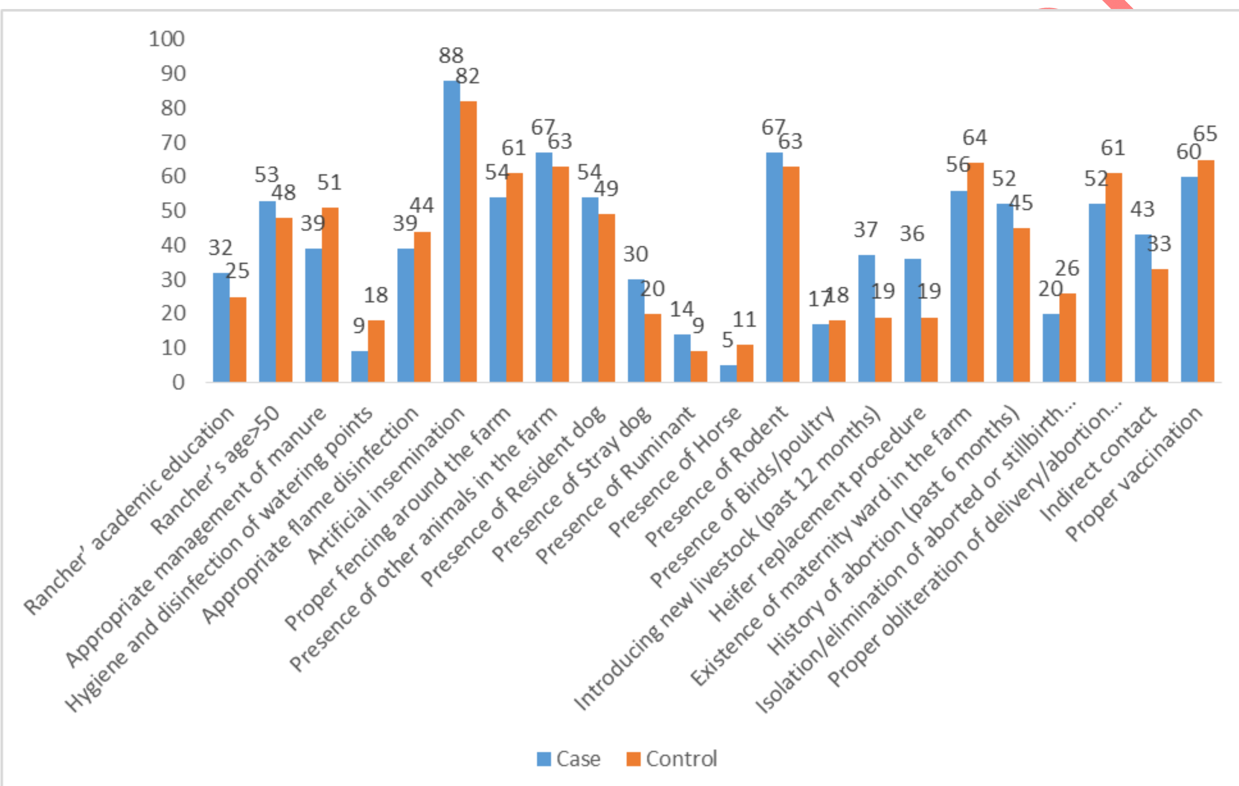

Figure 1. Frequency of studied variables in both case and control groups

Regarding the relationship between the studying independent variables and brucellosis infection in the dairy farm level, it was found that the observance of hygiene and disinfection of watering points (at least three times a week and using the detergent or disinfectant) reduces the risk of infection and the history of abortion, presence of stray dogs in the dairy farm, the replacement of livestock from outside and the introducing new livestock during the past 12 months increase the risk of brucellosis infection. The interaction between the hygiene status of watering points and the presence of stray dogs in the dairy farm was significant (table 2).

Odds Ratio and significance level of studying independent variables in the univariate conditional logistic regression model, and the multivariate conditional logistic regression model without and with the interaction are shown in table 2.

Table 2. Point and interval estimation of odds ratio based on univariate and multivariate conditional logistic regression model with and without considering interaction between variables

Variable		univariate conditional logistic regression model		multivariate conditional logistic regression model		multivariate conditional logistic regression model considering interaction between variables	
		OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Hygiene and disinfection of watering points	Yes	0.25 (0.07-0.88)	0.03	0.065 (0.007-0.59)	0.015	0.04 (0.003-0.499)	0.012
	No	1 (Ref.)		1 (Ref.)		1 (Ref.)	
Heifer replacement procedure	Outside the farm	6.67 (1.98-22.43)	0.002	9.83 (1.27-75.89)	0.028	7.87 (1.07-58.07)	0.043
	Inside the farm	1 (Ref.)		1 (Ref.)			

Introducing new livestock (during the past 12 months)	Yes	10.50 (2.46-44.78)	0.001	7.23 (1.12-46.65)	0.037	7.27 (1.20-43.90)	0.031
	No	1 (Ref.)		1 (Ref.)		1 (Ref.)	
History of abortion (during the past 6 months)	Yes	1.87 (0.79-4.42)	0.15	5.49 (1.37-22.01)	0.016	7.01 (1.51-32.59)	0.013
	No	1 (Ref.)		1 (Ref.)		1 (Ref.)	
Presence of stray dog	Yes	6.00 (1.34-26.81)	0.02	13.91 (1.34-144.04)	0.027	---	---
	No	1 (Ref.)		1 (Ref.)		---	
Rancher' academic education	Yes	1.54 (0.76-3.10)	0.23	---	---	---	---
	No	1 (Ref.)		---	---	---	---
Rancher's age	50<	1.43 (0.72-2.83)	0.31	---	---	---	---
	50≥	1 (Ref.)		---	---	---	---
Appropriate management of manure	Yes	0.33 (0.13-0.84	0.02	---	---	---	---
	No	1 (Ref.)		---	---	---	---
Appropriate flame disinfection	Yes	0.57 (0.24-1.36)	0.21	---	---	---	---
	No	1 (Ref.)		---	---	---	---

Artificial insemination	Yes	2.50 (0.78-7.97)	0.12	---	---	---	---
	No	1 (Ref.)		---	---	---	---
Proper fencing around the farm	Yes	0.53 (0.23-1.26)	0.15	---	---	---	---
	No	1 (Ref.)		---	---	---	---
Presence of other animals in the farm	Yes	1.36 (0.63-2.97)	0.43	---	---	---	---
	No	1 (Ref.)		---	---	---	---
Presence of resident dog	Yes	1.50 (0.67-3.33)	0.32	---	---	---	---
	No	1 (Ref.)		---	---	---	---
Presence of sheep and goat	Yes	2.67 (0.71-10.05)	0.15	---	---	---	---
	No	1 (Ref.)		---	---	---	---
Presence of horse	Yes	0.25 (0.05-1.18)	0.08	---	---	---	---
	No	1 (Ref.)		---	---	---	---
Presence of rodents	Yes	2.23 (0.60-9.02)	0.22	---	---	---	---

	No	1 (Ref.)		---	---	---	---
Presence of birds/poultry	Yes	0.83 (0.25-2.73)	0.76	---	---	---	---
	No	1 (Ref.)		---	---	---	---
Existence of maternity ward in the farm	Yes	0.33 (0.11-1.03)	0.06	---	---	---	---
	No	1 (Ref.)		---	---	---	---
Isolation/elimination of aborted or stillbirth cow	Yes	0.50 (0.15-1.66)	0.26	---	---	---	---
	No	1 (Ref.)		---	---	---	---
Proper obliteration of delivery/abortion detritus	Yes	0.28 (0.09-0.87)	0.03	---	---	---	---
	No	1 (Ref.)		---	---	---	---
Indirect contact*	Yes	2.25 (0.98-5.17)	0.06	---	---	---	---
	No	1 (Ref.)		---	---	---	---
Proper vaccination (received both full and reduced vaccine doses)	Yes	0.50 (0.15-1.66)	0.26	---	---	---	---
	No	1 (Ref.)		---	---	---	---

Discussion

So far, several factors related to brucellosis have been reported in dairy farms around the world. Some of these factors are the level of hygiene on the farm, the herd size, age of the cattle, sex, system of production, the presence of wildlife, and multiple livestock species within the herd (Anka, Hassan et al. 2014). In this study, the hygiene and disinfection of watering points (washing at least three times a week and using detergent or disinfectant) reduce the risk of brucellosis infection (OR = 0.04) and factors such as history of abortion (OR = 7.01), replacement of livestock from outside (OR = 7.87) and introducing new livestock during last 12 months (OR = 7.27) increase the risk of brucellosis infection.

The main route of Brucella entry is oral by eating the food or water infected with the secretions or remains of aborted fetuses from infected cows or by licking vaginal secretions, aborted fetuses or newborn calves from infected cows (Aparicio 2013). So observing the hygiene and disinfection of watering points can reduce the risk of brucellosis by reducing the number of the pathogens in the environment. Like our study, in a study in Jordan, using the disinfectants was identified as a protective agent against the disease (Al-Majali, Talafha *et al.* 2009). In a case-control study to identify risk factors of brucellosis in small ruminants in Portugal on 255 herds including 123 cases (herds with a serum prevalence above 5%) and 132 controls (negative serum herds) not cleaning watering points (OR = 3.05) was introduced as a risk factor for the disease which can be interpreted by the possibility of water infection with urine or feces and better growth of bacteria in water containing mud (Coelho, Coelho *et al.* 2007).

Another point that can be discussed in this topic is the interaction between the hygiene status of watering points and the presence of stray dogs in the dairy farms. It means that the effect of the health status of watering points depends on the presence of stray dogs in the dairy farm and vice versa. Unlike Stray dog, Presence of resident dog in the farm has no significant effect on the infection because of low probability of disease transmission. In other words, the hygiene level of the farm is directly related to its management, as a result of which the entry of stray dogs (which can play a role on transmission of the disease from other farms) is prevented. Unlike resident dog. Stray dog can be a risk factor for the herd to be seropositive for brucellosis.

Our results showed that the replacement of livestock from other herds/farms significantly increases the chance of serum positivity for brucellosis by 7.87 times. Also, the introducing new livestock during last 12 months with an odds ratio of 7.27 had a significant relationship with the infection. Purchasing the infected animals for large-scale replacement was reported as a major factor responsible for brucellosis in disease-free herds. The results of a case-control study on 98 case dairy farms and 93 control dairy farms matched for capacity, and geographic area showed a significant chance of developing brucellosis (4.84 times) in dairy farms by buying heifers from unknown places compared to dairy farms that are replaced from their own farm or from the herds free of disease (Cárdenas, Peña *et al*. 2019). The results of several other studies in this regard are in line with our study. In a study in Uganda, the arrival of new livestock in the last two years with an odds ratio of 4.4 was reported as a risk factor for brucellosis (Mugizi, Boqvist *et al.*

2015). In a cross-sectional study in Jordan, the most important risk factor for the seroprevalence of Brucella in cattle herds was the introducing new animals to the herd (OR = 11.7, CI: 2.8-49.4) (Musallam, Abo-Shehada *et al.* 2015). In another study on 113 herds in northern Nigeria, the introduction of new cattle bought at livestock market (OR = 15.27, CI: 4.77-48.92) was significantly associated to the occurrence of herd-level brucellosis (Alhaji, Wungak *et al.* 2016). Also, a study on the identification of risk factors of herd-level bovine brucellosis in Brazil, the purchase of alternative livestock from other farms (OR = 1.19, CI: 1.07-1.32) or from livestock brokers (OR = 1.27 CI: 1.08-1.47) were identified as the risk factors of the disease (de Alencar Mota, Ferreira *et al.* 2016). Lithg-Pereira, Rojo-Vázquez et al. (2004), Coelho, Coelho et al. (2007), and B Lopes, Nicolino et al. (2010) have reported similar results in this matter, Although Some studies such as a research about the risk factors associated to the bovine brucellosis in Italy (Calistri, Iannetti *et al.* 2013), and a research in India (Pathak, Dubal *et al.* 2016) didn't report this variable as a risk factor for the disease.

As mentioned before, the history of abortion in livestock had a significant difference between the case and control groups. In a study on 113 herds in three regions of northern Nigeria, a history of herd-level abortion with an odds ratio of 13.43 was introduced as a risk factor for disease (Alhaji, Wungak *et al.* 2016). According to a review paper on risk factors of bovine brucellosis in Brazilian states, a history of abortion in Goiás with an odds ratio of 5.83, in Mato Grosso with an odds ratio of 1.7, in Minas Gerais with an odds ratio of 1.81, in the Rio Grande do Sul with an odds ratio of 3.27 and in Rondônia with an odds ratio of 1.42 was introduced as a risk factor for brucellosis (B Lopes, Nicolino *et al.* 2010). Also In a case-control study in four Malaysian states (Anka, Hassan *et al.* 2014), in a study in Nigeria (Boukary, Saegerman *et al.* 2013) and another researches in India (Shome, Padmashree *et al.* 2014) and Uganda (Makita, Fèvre et al. 2011) history of abortion have been reported as a risk factor for brucellosis in. Ali showed that a history of abortion in the herd in the last trimester of pregnancy increases the chance of seroposivity by 17.4 times (Ali, Akhter *et al.* 2017). However some studies in Uganda (Mugizi, Boqvist *et al.* 2015) and India (Pathak, Dubal *et al.* 2016), no significant relationship was observed between the history of abortion and seropositivity of the herds.

Conclusion

Controlling brucellosis in ruminants is important to prevent the diseases in humans which can be achieved by vaccinating livestock, slaughtering infected animals, and improving health measures which minimize the risk of infection to disease-free herds / dairy farms. Besides the maximum coverage of vaccination in livestock which strengthens the immune system of livestock and their resistance to disease facing an insufficient number of pathogens, as well as the test and slaughter operations, the attempts should be made to provide the awareness and attitude in farmers. It makes farmers aware of requirement to take the preventive measures such as biosecurity (such as reducing the replacement and entry of livestock from other farms and minimizing the relationship between the indoor environment and the outside environment) as well as observing health principles inside the dairy farm (by reducing the number of pathogens and separating suspicious or infected livestock from other livestock, etc.) In other words, in the implementation of sustainable control plans, awareness, and behavior of livestock owners should be considered. Lack of knowledge about the disease and high-risk transmission methods and lack of effective prevention and management strategies lead to herd-level continuous disease. Also, controlling this disease in all domestic animals should be considered, and the necessary human and financial resources should be provided to successfully eradicate it.

Conflict of Interest

The authors have no conflicts of interest to declare.

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آلودگی گاوهای شیری به بروسلوز در دامداری‌های ایران: مطالعه مورد شاهدی در سطح گله

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چکیده

زمینه مطالعه: بروسلوز یکی از بیماری‌های مهم مشترک بین انسان و دام است که از لحاظ بهداشتی و اقتصادی دارای اهمیت بسیاری می‌باشد. اهداف: پژوهش حاضر با هدف بررسی برخی عوامل موثر بر آلودگی گاوداری‌های شیری ایران به بروسلوز انجام پذیرفت.

روش کار: این پژوهش، یک مطالعه مورد - شاهدی در سطح گاوداری‌های شیری است. گاوداری‌های مورد (95 گاوداری) شامل تمام موارد بروز ثبت شده بیماری در طی 14 ماه مطالعه با حداقل یک راس گاو سرم مثبت (آزمایش رزبنگال و آزمایشات رایت و

2- مرکاپتواتانول به صورت متوالی) و گاوداری‌های شاهد (95 گاوداری) با شرط حداقل دو سال عاری بودن از بیماری انتخاب و از نظر ظرفیت و منطقه جغرافیایی با گاوداری‌های مورد همسان شدند. تجزیه و تحلیل داده‌ها با آزمون رگرسیون لجستیک شرطی چند متغیره و نرم افزار آماری Stata نسخه 14 انجام پذیرفت.

نتایج: به لحاظ ارتباط آماری بین متغیرهای مستقل تحت مطالعه با ابتلا به بروسلوز در گله، مشخص گردید رعایت بهداشت و ضدعفونی آبشخورها (حداقل هفته‌ای سه بار شستشو و استفاده از مواد شوینده یا ضدعفونی‌کننده) باعث کاهش خطر آلودگی دامداری به بروسلوز (OR=0.04, 95% CI=0.003-0.499) می‌گردد و عواملی چون سابقه سقط (OR=7.01, 95% CI=1.51-32.59)، جایگزینی دام از بیرون (OR=7.87, 95% CI=1.07-58.07) و ورود دام جدید در 12 ماه اخیر به دامداری (OR=7.27, 95% CI=1.20.43.90) سبب افزایش خطر آلودگی به بروسلوز می‌شود.

نتیجه‌گیری نهایی: توجه جدی‌تر به آموزش دامداران، رعایت اصول بهداشتی توسط ایشان و محدود نمودن قانون‌مند جابجایی دام‌ها از راهکارهایی است که برای پیشگیری از ابتلای دامداری به بروسلوز توصیه می‌گردد.

کلمات کلیدی: بروسلوز، عوامل خطر، گاوداری شیری، ایران