

Original Article

Bovine Brucellosis Infection in Iranian Dairy Farms:
A Herd-level Case-control Study

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**ABSTRACT**

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

Objectives: This study aimed to investigate some risk factors of 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 obtained data were analyzed by the multivariate conditional logistic regression test and SPSS software, version 20.

Results: According to the statistical relationship between studying independent variables and brucellosis infection in herd, 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.

Conclusion: 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

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1. Introduction

Brucellosis is one of the most important and common diseases among humans and animals in the world and causes serious problems for health and the economy, especially in developing countries (Joseph et al., 2015; Bagheri Nejad et al., 2020; Tulu, 2022; de Figueiredo et al., 2015). In addition to the prominence 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 et al., 2017a; Boluki et al., 2017b).

Infection occurs in 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 fetuses (Anka 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 et al., 2016).

Bovine brucellosis is widely distributed around the world, but in recent decades in most European countries, Japan, Canada, and the United States have been eradicated from the livestock populations due to forced pasteurization of dairy products and the strict control of dairy herds (Joseph et al., 2015). Brucellosis is an endemic disease in Iran, and *Brucella abortus* was first isolated from 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 et al., 2015; Bahonar 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 1000 cows; this figure is higher than this estimation due to the non-consideration of other animals which are traditionally kept (Esmacili et al., 2012; Esmacili, 2014).

In 2018, 3322 dairy farms were under 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 et al., 2019).

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

2. Materials and Methods

This is a case-control study in which the statistical population consists of dairy farms across the country covered by the brucellosis test and slaughter plan of the 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).

Sample size

The sample size at the dairy farm level was estimated according to the sample size Equations by considering a 95% CI, 80% test power, a ratio of 1 for the number of controls to cases, and OR=2.5 (Equation 1 and 2).

$$1. n = 2(Z_{(1-\alpha/2)} + Z_{(1-\beta)})^2 \times P(1-p) / (p_0 - p_1)^2$$

$$2. P_1 = P_0 OR / [1 + P_0(OR - 1)]$$

There are different results with different scenarios:

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

Table 1. Geographical distribution of case and control farms

No.	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	

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%, and minimum required sample: 79 cases and 79 controls. Considering the 4 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. In the next stage, the data were analyzed with SPSS software, Version 22.

Data analysis

Conditional logistic regression was used to determine the relationship between the disease's risk factors at the herd level. The studying variables were first entered into the univariate conditional logistic regression model. Then those variables which had a $P > 0.2$ were eliminated from the model, and the other variables were entered into the multivariate conditional logistic regression model. The backward elimination method simplified the model 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 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.

3. Results

The Mean±SD 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. In the control group, the highest education level was related to a diploma (32.6%), and the lowest to three levels of education of illiterate, associate degree, and master and higher, with a relative frequency of 3.2%. The frequencies of all studied variables are shown in Figure 1.

Regarding the relationship between the studying independent variables and brucellosis infection at the dairy farm level, it was found that the observance of hygiene and disinfection of watering points (at least 3 times a week and using the detergent or disinfectant) reduces the risk of infection, but 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 increases 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).

The 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 presented in Table 2.

4. Discussion

So far, several factors related to brucellosis have been reported in dairy farms worldwide. Some of these factors are the level of hygiene on the farm, the herd size, the age of the cattle, sex, the production system, the presence of wildlife, and multiple livestock species within the herd (Anka et al., 2014). In this study, the hygiene and disinfection of watering points (washing at least 3 times a week and using detergent or disinfectant) reduce the risk of brucellosis infection (OR=0.04). Factors such as a history of abortion (OR=7.01), replacement of livestock from outside (OR=7.87), and introducing of new livestock during the last 12 months (OR=7.27) increase the risk of brucellosis infection.

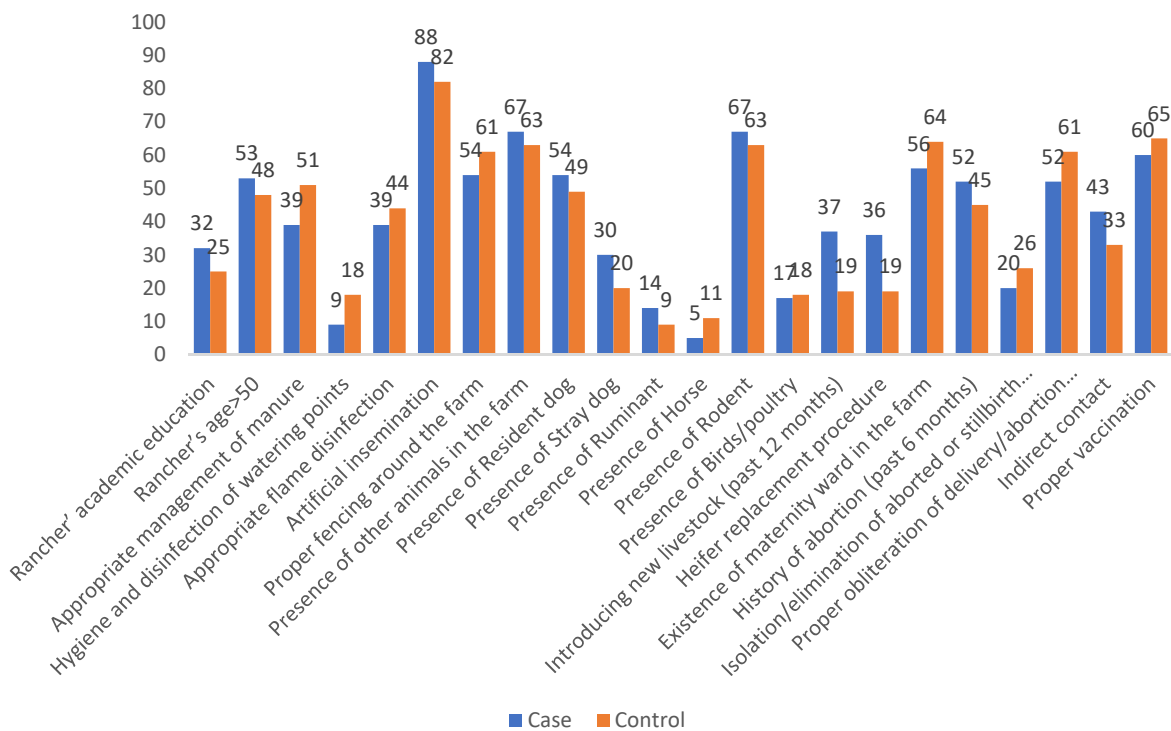


Figure 1. Frequency of studied variables in both case and control groups

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

Variables		Conditional Logistic Regression Model					
		Univariate		Multivariate		Multivariate Between Variables	
		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
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)	
	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)	
	Inside the farm	1 (Ref.)		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)	
	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)	
	No	1 (Ref.)		1 (Ref.)		1 (Ref.)	
Presence of stray dogs	Yes	6.00 (1.34-26.81)	0.02	13.91 (1.34-144.04)		---	---
	No	1 (Ref.)		1 (Ref.)	---	---	
Rancher's 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 the resident dogs	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.)		---	---	---	---

Variables		Conditional Logistic Regression Model					
		Univariate		Multivariate		Multivariate Between Variables	
		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
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 a mater- nity 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 debris	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.)		---	---	---	---

*Entry of workers or staff, equipment, machinery, fodder, or concentrate from another farm.

The main route of *Brucella* entry is eating the food or water infected with the secretions or remains of aborted fetuses from infected cows or licking vaginal secretions, aborted fetuses, or newborn calves from infected cows (Díaz Aparicio, 2013). Thus, observing the hygiene and disinfection of watering points can reduce the risk of brucellosis by reducing the number of pathogens in the environment. Like our study, in a study in Jordan, using disinfectants was identified as a protective agent against the disease (Al-Majali 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 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 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 dogs, resident dogs in the farm have no significant effect on the infection because of the 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 in the transmission of the disease from other farms) is prevented. Unlike resident dogs, stray dogs can be a risk factor for the herd to be seropositive for brucellosis.

Our results showed that replacing livestock from other herds/farms significantly increases the chance of serum positivity for brucellosis by 7.87 times. Also, introducing new livestock during the last 12 months with an odds ratio of 7.27 had a significant relationship with the in-

fection. Purchasing the infected animals for large-scale replacement was reported as a major factor 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 farm or the herds free of disease (Cárdenas et al., 2019). The results of several other studies in this regard align with our research. 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 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 (O=11.7; 95%CI, 2.8%-49.4%) (Musallam et al., 2015). In another study on 113 herds in northern Nigeria, the introduction of new cattle bought at the livestock market (OR=15.27; 95% CI, 4.77%-48.92%) was significantly associated with the occurrence of herd-level brucellosis (Alhaji et al., 2016). Also, in 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; 95% CI, 1.07%-1.32%) or livestock brokers (OR=1.27; 95% CI, 1.08%-1.47%) was identified as the risk factors of the disease (de Alencar Mota et al., 2016). Lithg-Pereira et al., (2004), Coelho et al., (2007), and B Lopes et al., (2010) have reported similar results in this matter, Although some studies such as research about the risk factors associated to the bovine brucellosis in Italy (Calistri et al., 2013) and research in India (Pathak et al., 2016) do not 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 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 et al., 2010). Also, in a case-control study in 4 Malaysian states (Anka et al., 2014), a study in Nigeria (Boukary et al., 2013), and another research in India (Shome et al., 2014) and Uganda (Makita et al., 2011), a 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 seropositivity by 17.4 times (Ali et al., 2017). However, in some studies in Uganda (Mugizi et al., 2015) and India (Pathak et al., 2016), no significant relationship was observed between the history of abortion and seropositivity of the herds.

5. Conclusion

Controlling brucellosis in ruminants is important to prevent human diseases, which can be achieved by vaccinating livestock, slaughtering infected animals, and improving health measures that 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, attempts should be made to provide awareness and attitude in farmers. It makes farmers aware of the requirement to take 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, livestock owners' awareness and behavior should be considered in implementing sustainable control plans. 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.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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مقاله پژوهشی

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

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چکیده



زمینه مطالعه: بروسلوز یکی از بیماری‌های مهم مشترک بین انسان و دام است که از نظر بهداشتی و اقتصادی دارای اهمیت بسیاری است.

هدف: پژوهش حاضر با هدف بررسی برخی عوامل مؤثر بر آلودگی گاوهای شیری ایران به بروسلوز انجام شد.

روش کار: این پژوهش، یک مطالعه مورد-شاهد در سطح گاوهای شیری است. گاوهای مورد (۹۵ گاو) شامل تمام موارد بروز ثبت‌شده بیماری در طی ۱۴ ماه مطالعه با حداقل ۱ راس گاو سرم مثبت (۱). آزمایش رزینگال و آزمایشات رایت و ۲. مرکاپتواتانول به صورت متوالی) و گاوهای شاهد (۹۵ گاو) با شرط حداقل ۲ سال عاری بودن از بیماری انتخاب و از نظر ظرفیت و منطقه جغرافیایی با گاوهای مورد همسان شدند. تجزیه و تحلیل داده‌ها با آزمون رگرسیون لجستیک شرطی چند متغیره و نرم‌افزار آماری SPSS نسخه ۲۰ انجام شد.

نتایج: از نظر ارتباط آماری بین متغیرهای مستقل تحت مطالعه با ابتلا به بروسلوز در گله، مشخص شد رعایت بهداشت و ضدعفونی آبشخورها (حداقل هفته‌ای ۳ بار شست‌وشو و استفاده از مواد شوینده یا ضدعفونی‌کننده) باعث کاهش خطر آلودگی دامداری به بروسلوز

(درصد شانس=۰/۰۴، فاصله اطمینان ۹۵ درصد=۰/۰۳-۰/۴۹۹) می‌شود و عواملی چون سابقه سقط (درصد شانس=۰/۰۱، فاصله اطمینان ۹۵ درصد=۰/۰۷-۰/۰۷) و ورود دام جدید در ۱۲ ماه اخیر به دامداری (درصد شانس=۰/۲۲۷، فاصله اطمینان ۹۵ درصد=۰/۲۰-۰/۲۲۷) سبب افزایش خطر آلودگی به بروسلوز می‌شود.

نتیجه‌گیری نهایی: توجه جدی‌تر به آموزش دامداران، رعایت اصول بهداشتی از سوی دامداران و محدود کردن جابه‌جایی دام‌ها به‌طور قانون‌مند از راهکارهایی است که برای پیشگیری از ابتلای دامداری به بروسلوز توصیه می‌شود.

کلیدواژه‌ها: بروسلوز، عوامل خطر، گاوهای شیری، ایران

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