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Research article

Bovine brucellosis, associated risk factors and preventive measures in industrial cattle farms

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ABSTRACT

Effective management of brucellosis in human populations is closely tied to controlling the disease in domestic livestock. This study focused on identifying determinants of brucellosis prevalence in mixed industrial dairy and beef cattle farms within Isfahan Province, Iran. Employing a case control design, we compared 32 ranches with documented brucellosis within the previous year (12 months) to 38 farms with no brucellosis during the same timeframe. The comparison examined the farms' adherence to health protocols for raising cattle. Utilizing multivariable logistic regression, the study identified several significant risk factors linked to brucellosis prevalence. These included cleaning milking accessories with a water and chlorine solution (OR 0.25, 95%CI 0.06 to 0.99), cleaning water troughs daily (OR 0.20, 95%CI 0.04 to 0.89), the cows' density per square meter (OR 0.14, 95%CI 0.03 to 0.75), being within 2000 m of another cattle ranch with brucellosis (OR 4.02, 95%CI 1.09 to 14.84) and ovine farms situated within a 500-m radius (OR 8.43, 95%CI 1.66 to 42.70). No meaningful difference was observed in vaccination frequency between infected and non-infected farms (P = 0.645). While vaccination is essential for preventing bovine brucellosis, robust biosecurity measures are crucial for effective disease management.

1. Introduction

Brucellosis is a zoonotic disease affecting domestic animals and wildlife, with its prevalence varying significantly by region. Brucella, a gram-negative bacterium with several species, causes brucellosis. The two main species prevalent among domestic ruminants in the Middle East, are *B. abortus* and *B.melitensis*. *B.suis* is also prevalent in countries that breed swine. The three mentioned species are highly virulent and, in addition to domestic animals such as cattle and sheep, cause severe febrile illness in humans. In areas with high prevalence, it is sometimes mistaken for febrile diseases like malaria and typhoid fever. Transmission of the disease to humans often occurs through unpasteurized dairy products and contact with an infected animal's body fluids and secretions (as an occupational disease) [1,2].

The latest estimates indicate approximately 1.6–2.1 million cases of human brucellosis in different regions of the world each year.

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Most of these cases are concentrated in high-risk areas such as the Middle East and sub-Saharan Africa, and most areas of Iran are classified as high-risk [1,2]. Based on statistics and data from the World Health Organization, it is estimated that in the Middle East, Iran's prevalence is more than 15,000 cases of brucellosis per year, ranking second after Yemen (with an annual report of more than 25, 000). Iraq is the third in the report, with over 10,000 cases annually [3]. In Iran, the prevalence of human brucellosis varies across different provinces, with a reported prevalence ranging from 10 to 20 per 100,000 (annually) within the last decade [3,4]. It is worth mentioning that underreporting is common in brucellosis surveillance, and the actual numbers are likely much higher [1,3].

The relationship between brucellosis prevalence in livestock and Maltese fever in humans is well-established. Effective control of this zoonotic disease in human populations hinges on robust management practices in livestock, ensuring the health of animal reservoirs [5,6]. The Mediterranean Zoonosis Control Program of the WHO underscores the importance of preventing animal brucellosis as a primary strategy for human health protection. Key interventions include implementing a 'test-and-slaughter' policy and establishing comprehensive vaccination programs to mitigate transmission risks [2,4,7].

Vaccination programs against brucellosis in Iran started in 1944. However, various social, economic, and political factors influenced their implementation. In Isfahan, the vaccination program did not begin until 1971, even though the national program for brucellosis control commenced about four years earlier, in 1967 [5]. RB51 is the vaccine used against brucellosis in Iran. The Pasteur Institute of Iran produces it. Since 2004, vaccination against brucellosis for heifers aged 4–6 months has been a mandatory protocol, necessitating a complete dosage of the vaccine. Furthermore, it is advisable for cows that have been vaccinated to receive a reduced dosage for revaccination biennially. The specific timing of vaccinations at each cattle ranch is customized according to the individual needs of the farms. It is important to highlight that brucellosis immunization services in Iran are provided at no cost.

The implementation of vaccination initiatives from 1990 to 2010 in Iran resulted in a substantial decrease in the incidence of brucellosis, with notable effects observed in Isfahan Province. This indicated that the disease was under control [4]. However, despite the ongoing vaccination programs, there has been a resurgence of brucellosis in recent years, and the number of infected cattle farms has been increasing [4,9–11]. While the 'test-and-slaughter' strategy and vaccination programs continue, it seems that factors other than vaccination influence the disease's prevalence.

This study aimed to investigate why new outbreaks of brucellosis continue to occur in the industrial livestock ranches in the Isfahan province despite the implementation of a regular vaccination program. Additionally, it seeks to identify the risk factors associated with the presence and prevalence of Brucella infection within these farms.

2. Material and methods

2.1. Study setting

Isfahan province, with a population of approximately 5.39 million residents and an area of about 107,000 square kilometers, is situated at the center of the Persian plateau. It has stretched in the east from the deserts of the central plains of Iran to the fertile foothills of the Zagros Mountains in the west. In terms of size, Isfahan ranks sixth among the provinces of Iran (Fig. 1). Isfahan is recognized as one of the key centers for the livestock industries in the country. The study was from March 21, 2021, to March 20, 2022, covering one year in the Iranian calendar.

2.2. Design and sample size

The study was designed as a retrospective case-control investigation. The primary sampling units were all industrial farms in the Isfahan province dedicated to breeding cattle for beef and milk production. All the farms included in this study were mixed dairy and beef farms, with no involvement in raising or keeping other animals, such as sheep and goats. In this study, a farm was considered a

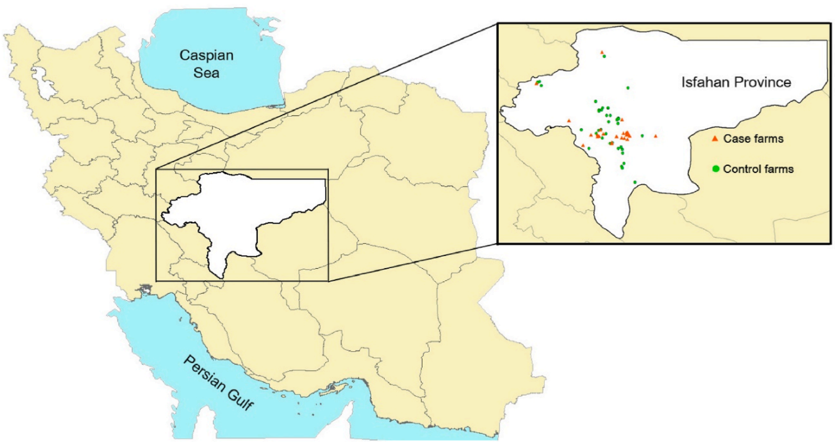

Fig. 1. Map of Iran showing the distribution of case and control cattle farms in the Isfahan province.

case farm if it was classified as positive for brucellosis by the VOIP (the Veterinary Organization of Isfahan Province). In calculating the sample size, the exposure rate in the control farms was assumed to be 25 %, and in the case farms, 60 %. With a type one error rate of 5 % and statistical power of 80 %, the sample size was determined to be 31 for each group of the infected (case) and the non-infected (control) farms (62 farms in total). The VOIP data was used to select infected and non-infected farms. From each list of 56 farms with brucellosis (cases) and 233 farms without brucellosis (controls) during the past 12 months (i.e., during the study as mentioned above), 40 farms were selected and invited to participate in the study, respectively as case and control farms (in total, 80 farms). A random numbers table was used to select the case and control farms. Finally, 32 case farms and 38 control farms accepted the invitation and cooperated to participate in the study.

2.3. Rose Bengal and Wright Agglutination tests

As a routine diagnostic procedure, the first test performed for diagnosing brucellosis in cows is the Rose Bengal Test. This test serves as a screening tool, and any animals that test positive will undergo the Wright Agglutination Test. The Wright Agglutination Test is considered the gold standard for diagnosing brucellosis; a test-positive animal is deemed infected, while a test-negative animal is considered non-infected. However, results can sometimes be reported as "borderline." In such cases, the test should be repeated up to three times, with three-week intervals between tests. If, after the third test, the result remains borderline, the decision on further action will be left to the veterinarian's judgment and expertise.

2.4. Data collection

Veterinarians from the VOIP utilized a 51-item questionnaire to gather data during field assessments. The methodology included direct observation of farm operations for certain queries and data retrieval from the VOIP's established database. Additionally, data were gathered through structured interviews with farm managers and personnel. An English translation of the questionnaire is available as a supplementary document.

The questionnaire items were meticulously formulated to encompass all fundamental sanitary protocols relevant to a cattle farm. Key areas addressed include the following: The vaccination schedule tailored for the herd; livestock density and overall capacity of the farm; the details about both the quantity and educational background of personnel and their experiential qualifications; the structural integrity and conditions of fencing perimeters surrounding the livestock; proximity considerations of livestock to adjacent agricultural entities; specifications regarding the number and square footage of barn facilities; evaluations of light absorption characteristics of barn flooring; the establishment of disinfectant basins at barn entrances for biosecurity; comprehensive manure management strategies, including the effluent discharge timetable from barn environments; designation of calving areas (for heifers) and protocols for housing newborn calves; rigorous schedules for sanitation of drinking troughs and milking barns; timeframes and protocols for the thorough cleaning of milking equipment; hygienic measures enforced in the event of abortion or stillbirth incidents; health management practices initiated upon procuring new livestock; protocols for the acquisition and storage of feed within the livestock rearing context.

2.5. Statistical analysis

Data analysis was conducted utilizing Stata (StataCorp, 2015, Stata Statistical Software: Release 14). Continuous variables were

Table 1
Risk factors associated with brucellosis in infected and non-infected farms.

	Control (non-infected) farms Median (25–75 percentiles) (N = 38)	Case (infected) farms Median (25–75 percentiles) (N = 32)	P-value[a]
Frequency of execution of the immunization program.	2 (2–3)	2 (1–3)	0.645
Total count of cows at the time of assessment.	1169.5 (500–2605)	665 (375–1200)	**0.029**
Count of dairy cows at the time of assessment.	505 (200–1100)	300 (142.5–575)	**0.023**
Total number of employees	30.5 (12–50)	11.5 (5.5–30)	**0.016**
Number of employees who have completed high school.	10 (3–25)	3 (0.5–9)	**0.005**
The surface area of barns (meter^2).	16850 (6420–34100)	10385 (4924–29135)	0.191
The density of cattle in barns (number of cows/meter^2)	0.09 (0.06–0.11)	0.06 (0.04–0.1)	0.101
Proximity to the closest ovine farm	500 (500–2000)	500 (155–1500)	0.064
Count of ovine farms within a 500-m radius	1 (0–2)	2 (0–4)	**0.050**
Count of ovine farms within a 2000-m radius	2.5 (0–10)	5 (2–15)	**0.037**
Proximity to the primary road	550 (300–2000)	637.5 (225–1375)	0.570
Proximity to the closest cattle farm with confirmed cases of infected livestock	5500 (1500–30000)	1000 (275–3050)	**0.005**
The count of specialized barns designated for housing neonate calves.	1 (1–1)	1 (0.5–1)	0.605
Count of dry housing facilities designated for full-term heifers	1 (1–1)	1 (1–1)	0.052
Count of conventional barns	2.5 (2–3)	2 (1–2.5)	0.102
Total count of barns	13.5 (11–17)	11 (6.5–15.5)	0.083
Duration of professional experience of the employees (in years)	17.5 (14–23)	14.5 (9.5–20.5)	0.062

[a] The reported P-values from the test of Mann-Whitney.

summarized using median values along with interquartile ranges (first and third quartiles), while categorical variables were expressed in terms of frequency and percentage. The normality of continuous variables was assessed using the Shapiro-Wilk test. The Mann-Whitney *U* test was used to compare non-normally distributed continuous variables between two groups. Simple and multivariable logistic regression models were used to measure the association of potential predictors influencing the prevalence of brucellosis. The multivariable logistic regression included predictors that showed significant association with brucellosis ($P < 0.05$) in univariate analysis. A backward elimination procedure was used to select a model, and the asymptotic standard error was used to determine the confidence intervals for the model parameters. The findings from the logistic regression analysis are reported as odds ratios (OR) along with their corresponding 95 % confidence intervals.

3. Results

A total of 70 farms took part in the study: 32 farms with brucella infection (case farms) and 38 farms without brucella infection (control farms) over the study period (March 21, 2021, to March 20, 2022). Fig. 1 displays the map of Isfahan province in Iran, showing the geographical distribution of the participating farms, categorized by cases and controls. In the infected and non-infected farms, the median total count of cows was 665 (IQR: 373–1200) and 1169 (IQR: 500–2605), respectively. The median number of milk (dairy) cows in the case farms was 300 (IQR: 142.5–575) and in the control farms was 505 (IQR: 200–110). It is worth mentioning that All the farms in this study were mixed dairy and beef cattle farms and none of them were breeding or keeping any other animals, such as sheep or goats.

Table 1 displays the median, 25th, and 75th percentiles of potential risk factors for brucellosis in case and control farms. Table 1 shows no significant differences between the two groups studied regarding the frequency of the vaccination program in the farms ($P = 0.645$, Mann-Whitney test).

Table 2 displays the outcomes of multivariate logistic regression analyses examining the relationships between various risk factors and the prevalence of the disease. The logistic regression model incorporated six of the previously identified variables. The presence of brucellosis on a cattle farm was positively correlated with the proximity of a case farm within a 2-km range ($P = 0.037$; OR 4.02, 95 % CI 1.09–14.84), as well as the existence of two or more sheep farms within a 500-m radius ($P = 0.10$; OR 8.43, 95%CI 1.66–42.70). In contrast, factors such as the routine cleaning of water troughs ($P = 0.035$; OR 0.20, 95 % CI: 0.04–0.89), the use of water and chlorine for cleaning milking equipment after each session ($P = 0.048$; OR 0.25, 95 % CI: 0.06–0.99), a higher population of milk cows ($P = 0.021$; OR 0.19, 95%CI: 0.05–0.78), and a lower density of cows per square meter within the barn environment ($P = 0.021$; OR 0.14, 95 % CI: 0.03–0.75) exhibited a negative correlation with brucellosis presence.

In the present retrospective study, we utilized laboratory test results recorded over the past 12 months (specified above). Based on these records provided by the VOIP, the date of diagnosis of the first infection on each case farm during the study period was obvious. In addition, the exact date of implementation of the vaccination program in each farm was also available from the records. Table 3 displays the results of subtracting the month of the farm's first vaccination from the month of diagnosis of the first infection for each case farm.

4. Discussion

This study aimed to identify the specific measures, practices, and characteristics that differentiate bovine farms with brucellosis from those that are non-infected. A significant contribution of this research lies in its emphasis on factors underexplored in the literature. The findings indicate that implementing daily cleaning of water troughs and utilizing chlorine solutions for sanitizing

Table 2
Logistic regression modeling on factors related to brucellosis prevalence in industrial bovine ranches of Isfahan province, Iran.

Variables	Case (infected) farms N (%)	Control (non-infected) farms N (%)	Adjusted odds ratio (95 % CI)	P
Proximity to the closest infected bovine farm				
≤2000 m	23 (71.88)	12 (31.58)	4.02 (1.09–14.84)	0.037
>2000 m	9 (28.13)	26 (68.42)		
Cleaning of water troughs				
Less than once per day	9 (23.68)	19 (59.38)	0.20 (0.04–0.89)	0.035
Once per day	29 (76.32)	13 (40.63)		
Cleaning milking equipment after each session				
Utilizing a water and chlorine solution	10 (31.25)	27 (71.05)	0.25 (0.06–0.99)	0.048
Utilizing water (only)	22 (68.75)	11 (28.95)		
The density of cows per square meter within the barn environment				
≥0.05	18 (56.25)	30 (78.95)	0.14 (0.03–0.75)	0.021
<0.05	14 (43.75)	8 (21.05)		
Population of dairy cows				
>360	10 (31.25)	25 (65.79)	0.19 (0.05–0.78)	0.021
≤360	22 (68.75)	13 (34.21)		
Sheep farms situated within a 500-m radius.				
≥2	17 (53.13)	11 (28.95)	8.43 (1.66–42.70)	0.010
1	15 (46.88)	27 (71.05)		

Table 3
The interval between the month of vaccination implementation and the month of detection of the first brucella infection in the *case farms* (negative numbers indicate the occurrence of infection before vaccination).

The time interval (in month): (detection – vaccination)	Number of farms with brucella infection (case farms)[a]
−8	1
−3	1
−2	4
−1	6
0	5
1	3
2	3
3	2
4	1
5	3
6	1
8	1

[a] The table does not mention one farm that abstained from the immunization programs.

milking equipment—particularly between individual cows—each notably reduces brucellosis prevalence on farms by approximately fivefold (Table 2). Unlike previous studies, which primarily concentrated on the overall impact of disinfectant use, this research provides a more granular examination of individual health practices and their efficacy [12,13]. However, the results of this study need to be interpreted with the understanding that it is a case-control study, where the main focus is on the presence or absence of brucellosis in cattle farms. It is essential to clarify that this study does not examine the risk of disease transmission but focuses on the factors associated with the prevalence of brucellosis in farms. The study's concern is with evaluating the effect of variables associated with the presence of brucellosis, and it did not claim to assess the factors influencing the risk of transmission.

It is essential to consider that the main factor studied, "strict compliance with biosecurity measures in cattle farms," is not a single variable but rather a combination of behaviors and actions. These behaviors and actions determine the risk of infection transmission. Additionally, most variables in the study linked to brucellosis in cattle farms are likely not direct causes of disease transmission. Instead, they are intermediary factors in the chain of transmission or represent other decisive factors. For example, daily cleaning of water troughs and milking hygiene are not directly responsible for increasing or decreasing the risk of brucellosis transmission in farms. Instead, they are associated with compliance with other biosecurity measures, such as timely vaccinations on the farm.

The population of milk cows on a cattle farm is correlated with a decreased disease risk, suggesting that larger ranches have better resources to adopt measures that mitigate financial losses. This correlation likely serves as a proxy for financial status, where more extensive operations can enforce health regulations more rigorously. Conversely, research on smaller, predominantly non-industrial ranches indicates that infection transmission risk escalates with increasing herd size. This trend can be attributed to the confined spaces that facilitate the spread of the disease among cattle [14–16]. To differentiate the effects of herd size from those of cow density on infection prevalence, a variable representing the density of cows per square meter within the barn environment was included in the regression model. The analysis revealed a robust inverse relationship between low density and disease risk, showing a risk reduction factor exceeding seven (Table 2). This underscores the critical role of stocking density in influencing infection prevalence in cattle populations. Notably, some studies have failed to establish a consistent link between herd size and disease risk, indicating variability in these dynamics across different farm settings [17].

The findings in the present study indicate that cattle farms infected with brucellosis within a 2-km radius are associated with a 4-fold rise in the prevalence of the disease (OR 4.02, 95%CI 1.09–14.84). In previous sections, it was mentioned that none of the farms involved in this study raised animals other than cows (such as sheep, goats, or camels). However, the study did explore the impact of the vicinity of sheep farm**s** to cattle farms. The presence of two or more sheep breeding farms within a 500-m radius of the cattle farms heightens the odds of brucellosis prevalence by more than 8 (Table 2). It is essential to acknowledge that our analyses and estimates considered the mere presence of a sheep farm near cattle farms as a risk factor without investigating whether the sheep farms were positive. This limitation highlights the need for further research.

Various studies have highlighted the elevated risk of brucellosis transmission associated with the proximity of ovine farming operations, mainly when sheep and cattle are grazed on the same pastures [7,12,18–20].

The mechanisms underlying disease transmission from contaminated cattle and sheep farms to adjacent properties vary across studies. Several investigations indicate that factors such as shared pastures and water resources, inter-farm cattle exchanges for breeding purposes, and the sale of infected livestock to mitigate economic losses contribute to this transmission dynamic [2,18–20]. Additionally, the potential role of avian vectors, particularly crows that hydrate their food in water sources—possibly including water troughs—merits attention. In Isfahan, some veterinary professionals point out the heightened threat posed by proximity to sheep farms and brucella-contaminated locales, which is often attributed to these mechanisms, although substantiating this correlation can be challenging.

In comparing case and control farms, the data revealed no statistically significant difference in the frequency of vaccination program implementation. This indicates that both case and control farms exhibited comparable quantitative measures in executing their vaccination strategies.

Table 3 provides a different perspective on implementing the immunization program in the case farms. This table presents the temporal intervals, represented by positive, negative, and zero values, between the initiation of the vaccination program on the farm and the diagnosis of the first brucellosis case within the same farm. For instance, if the first case of infection was detected two months after the implementation of the vaccination program, it is represented as +2 for that farm. Conversely, if the diagnosis of the first case of infection occurred two months before the implementation of the first round of vaccination in that farm, −2 is assigned to that farm. A zero indicates that the detection of the first case of brucella infection on the farm occurred in the same month as the implementation of the vaccination program.

Based on this, from the records in Tables 3 and it might be suggested that a negative or zero assignment indicates vaccine hesitancy or a delay in implementing vaccination. Conversely, a positive result suggests that the timing of vaccination was appropriate. Out of the 32 cattle farms in the case group, 14 farms were vaccinated before the first infected cow appeared, while 17 were vaccinated in the same month or the months after the first case was diagnosed on the farm. Remarkably, one farm did not vaccinate at all, bringing the number of farms with vaccine hesitancy to 18. This data highlights the crucial role of timing in vaccination, emphasizing that timely vaccination is as important as vaccination itself. It also underscores the difference between cases and the controls regarding vaccination. It is impossible to perform such an analysis in the control group, as there have been no cases of infected cattle among them. Nearly all relevant literature in this domain underscores the significance of vaccination strategies in the management and prevention of brucellosis in cattle [3,8,9,13,21]. However, none have considered and analyzed the vaccination delay and vaccine hesitancy as a crucial factor in the program's success or failure. This finding underscores the urgency of timely vaccination to control the prevalence of disease.

One of this study's limitations is its retrospective design. Specifically, the data pertaining to adherence to health regulatory standards were derived from observations made during one or, at most, two visits to the cattle farms. This approach raises concerns about the generalizability of the findings, as it may not adequately reflect the compliance status over the entirety of the year. Although to reduce variability and also due to their higher financial importance, only industrial cattle farms were investigated in this study; however, this choice could limit its findings' applicability to non-industrial and traditional cattle farms and should be considered a limitation. Additionally, the study was constrained by the small number of participating farms. In addition to these points, it should be acknowledged that the study lacks detailed information regarding the overall health status of the cattle in the participating farms. It's crucial for future research to overcome these limitations to obtain more reliable and valid results.

As explained in the materials and methods section, the routine tests performed in the brucellosis surveillance system of the cattle farms in Iran are the Rose Bengal and the Wright Agglutination tests. None of these tests is perfect, and the possibility of false positives is not low [22]. To increase the validity of the lab results, especially where borderline results are reported, it would be better to have a more accurate test after Wright's test, such as a CFT (Complement Fixation Test), ELISA (Enzyme-Linked Immunosorbent Assay), or 2-ME (2-Mercaptoethanol Test) [23]. Since only the results of the routine tests of the brucellosis surveillance system could be used in this retrospective study, there was no place for adding these complementary tests to the laboratory methods. These points should be considered when designing laboratory methods in future studies, especially if they are performed prospectively. In addition, equipping the laboratories of the brucellosis surveillance system with the necessary facilities for the routine implementation of these tests will increase the quality of care for this disease in cattle farms.

5. Conclusions

The systematic deployment of vaccination protocols stands as one of the most efficacious interventions for controlling and eventually eliminating brucellosis. However, other measures can be taken to prevent the disease until its elimination. Previous studies have focused on external factors contributing to the prevalence of brucellosis among cows, such as 'social and political instability,' 'insufficient resources and manpower,' 'diverse pasture management practices,' and 'traditional (often incorrect) management methods.' [1–3] In contrast, factors related to the internal management of cattle farms have been given less attention. The research emphasized that basic biosecurity practices, like daily cleaning of water troughs and utilizing chlorine solutions for sanitizing milking equipment between individual cows, are closely linked to the prevalence of infection in cow ranches. Although these factors may not have a causative role in transmitting brucellosis to cattle farms, they can help identify risky situations and areas needing intervention. The key conclusion drawn from this study is that, in addition to timely vaccination programs, strict compliance with biosecurity measures is essential in preventing brucellosis transmission to cattle farms.

CRediT authorship contribution statement

Shahrokh Izadi: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Vahid Moghaddas:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Awat Feizi:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Akram Bahreinipour:** Writing – review & editing, Conceptualization. **Zahra Barati:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Ethics declarations

The research protocol for this case-control study has undergone review at Isfahan University of Medical Sciences and received approval from the Ethics Committee in Medical Research. It has been assigned the ethics code IR.MUI.RESEARCH.REC.1400.558.

Data availability statement

The data associated with the present study have not been deposited into a publicly available repository; however, they will be available upon a reasonable request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40180.

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