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Seroprevalence of bovine besnoitiosis in Mosul city, Iraq

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Abstract

Besnoitia besnoiti (Bb)has become an important cause of severe commercial losses in endemic zones globally. The seroprevalence of this organism in Iraq is underreported. The purpose of this study was to investigate the prevalence and risk factors of bovine besnoitiosis (BB) in Mosul city, Iraq. A cross-sectional study was done from April 2024 to February 2025 on 20 farms. A total of 300 sera were collected from animals aged 1 to 7 years and examined using a commercially available indirect enzyme immune sorbent assay (ELISA). The probable risk factors investigated were age, sex, breed origin, herd size, and season. The overall prevalence of BB was 23% (69/300; 95% Confidence interval (CI)= 17.9 to 29.1). During spring, older animals (≥ 5 years old) and herds with imported breeds showed greater seroprevalences of antibodies against Bb, whereas a lower prevalence rate in animals with ≤ 1 year old. Univariable analysis showed that cattle aged ≥5 years, imported breeds, and those sampled in spring were significantly more likely to be seropositive. In a multivariable regression analysis, the adjusted odds ratio (AOR) of seropositivity was 6.0 times higher for animals ≥ 5 years old and 4.0 times higher for spring compared to younger animals and other times of year. Imported animals were 3.1 times more likely to be seropositive than native breeds. This study provides the first seroepidemiological studies on *B. besnoiti* in Mosul city, Iraq. Older animals, imported breeds, and spring represented considerable risk factors for infection. These findings are crucial in guiding future surveillance and control actions for BB in the region.

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Introduction

Bovine besnoitiosis, also named globidiosis, elephant skin disease, and bovine anasarque, is an acute or chronic debilitating protozoal disease caused by cyst-forming coccidian organism *B. besnoiti* in the family *Sarcocystidae*, subfamily *Toxoplasmatidae*, and phylum (Apicomplexa: *Sarcocystidae*) of which cattle are the significant intermediate host due to their possess tachyzoites and cyst-forming bradyzoites and felids final hosts (1,2). According to Alvarez-Garcia (3) and Kuraa *et al.* (4), the disease can cause significant economic losses and health issues, such as weight loss, infertility in bulls, abortion and reduced milk production in cows, and decreased hide value with systemic effects that can lead to mortality. In prior research, the prevalence of BB is alarming; it is crucial to consider that not all infected animals exhibit clinical signs (asymptomatic carriers) within herds, which may lead to underreporting and mismanagement of the disease (5). The clinical manifestations often involve anasarca, hyperkeratosis, tissue cysts in the epidermis, vulva, and sclera (6). Direct contact between animals is the predominant mode of transmission (7). Distribution can occur by insect bites (Stomoxys or Tabanus species) or by using shared needles between animals (8,9). The presence of these flies in cattle herds can exacerbate the spread of the disease, particularly in regions with high fly populations (10). Environmental factors and herd management practices also have an impact on the spread of the disease, with endemic areas showing persistently high prevalence rates (11). The most effective diagnostic method for detecting besnoitiosis in livestock combines molecular and serological techniques. Among

these, nested PCR has emerged as a highly sensitive method, while serological tests like ELISA and Western blot also play crucial roles in diagnosis (12,13). Immunoassay evaluations, especially the PrioCHECK® Besnoitia Ab V2.0(a commercial ELISA kit), have nearly 100% sensitivity and excellent specificity, making them appropriate for epidemiological research (14). Serological diagnosis of carrier cattle remains essential for preventing and control of disease (15,16). BB was extensively documented in Africa (17), Asia (18), and Europe (19-21). In the Middle East area, the disease has been reported in Turkey (22), Jordan (23), and Egypt (24). Bb deoxyribonucleic acid was present in seventy-four (16.09) and forty-nine (10.65%) of the bovine blood and skin specimens, respectively, in Iraq (25). Although many surveys of enzootic diseases have been conducted in Mosul, Iraq (26-39).

However, no epidemiological survey of BB and related risk factors has been conducted in Iraq. The goal of the present research was to determine the seroprevalence of anti-*Besnoitia besnoiti* antibodies in cattle in Mosul city (Northern Iraq) and identify associated risk factors, such as age, sex, breed origin, herd size, and season.

Materials and methods

Ethical approval

The College of Veterinary Medicine Committee/ University of Mosul approved this study with the approval number UM.VET.2024.090.

Area of study

Mosul is one of the largest cities in Iraq, situated in the northern part of the country, just about 400 km (250 miles) north of Baghdad on the Tigris river. The area of the city is 32,308 km^2. This city has four discrete seasons with dissimilar rainfall periods in autumn, winter, and early spring that range between 400-860 millimeters. This zone is branded by diverse districts, such as rivers, fields, pastures, and agricultural lands with biological variety.

Sample size determination

A cross-sectional study was performed on 20 farms by obtaining 300 sera between April 2024 and February 2025 from animals (clinically normal or asymptomatic) aged 1 -7 years. The appropriate sample size was measured with a standard error of 5%. As there is no currently available information on the prevalence of disease in Iraq, the sample size was computed with a formula that included a 20% expected prevalence, a 7% desirable absolute precision, and a 95% confidence interval. By using the following formula: number of samples=$z^2p(1-p)/d^2$ (40). Z = (1.69)= Value of the normal distribution for a 95% confidence level. P = Expected prevalence. d = Absolute error. N=246. In order to improve the accuracy, the number of sera was increased to 300.

Sample collection

Following appropriate restraint, a simple vacutainer tube and needle were used to aseptically draw five milliliters of blood from each animal's jugular vein. Standardized data sheets were used to document each animal's age (≤ 1, 2-4, and ≥ 5 years), sex, breed origin (native and imported breeds), herd size (Small ≤ 15 and Large ≥25), and season. The samples were brought to the lab in the ice-packed cooler. The sera from the clot-filled blood samples were collected and kept at -20°C until needed after being centrifuged for five minutes at 3000 rpm.

Serological analysis

A PrioCHECK®Besnoitia Ab 2.0 ELISA kit (Product No.: 7610530, Version: 2.1_e) (Prionics AG, Schlieren, Switzerland) was utilized in accordance with the manufacturer's recommendations to identify specific antibodies (It has a sensitivity of 100% and specificity of 98.8%). At a wavelength of 450 nm, the plates were examined in an ELISA microplate reader (Bio-Tek Instruments, MicroQuant). The following formula was used to calculate the results: Test sample OD450 divided by positive control OD450 × 100 = 100% positivity. A seropositive result was defined as a percentage positivity (PP) ≥23%.

Statistical analysis

A STATA 13.0 (StataCorp., College Station, TX, USA) was utilized to examine the data that were produced. Chi-Square (x^2) was used to determine the statistical differences between seroprevalence rates based on risk factors. Logistic regression analysis was utilized by observing the relationship among various epidemiological parameters with seroprevalence of anti- *B. besnoiti* seropositivity. The preliminary sorting was completed utilizing univariable analysis (odds ratio, OR). With a multivariable analysis, variables with a value of $P < 0.20$ were further examined. The adjusted odds ratio (AOR) was computed to compare the degree of association among potential risk factors with seropositivity animals. For all analyses, a confidence interval (CI) of 95% and a p-value of less than 0.05 was taken as statistically significant.

Results

A total of 300 clinically normal (asymptomatic) animals were examined to estimate the seroprevalence of BB and its associated risk factors in Mosul city, Iraq. Among the examined animals, 69 were found seropositive with an overall prevalence of 23% (95%, CI= 17.9 to 29.1). Higher prevalence of anti-Bb antibodies showed in animals with ≥ 5 years old (42.3%, 95%, CI=31.97 – 59), during spring 40.0% (95%, CI= 20.2-42.8) and in imported animals 37.7% (95%, CI= 33.7-61.4) compared to those with ≤ 1year, other seasons of year and native breeds respectively(P=0.0001),

while a lower prevalence rate in animals with ≤ 1year old (Table 1 and Figure 1). Cattle with ≥5 years old (OR = 6.6; 95% CI: 3.0–14.1), imported breeds (OR = 3.9; 95% CI: 2.21–6.9), and those sampled in the spring (OR = 4.7; 95% CI: 2.0–10.4) showed a significantly greater probability of being seropositive, according to univariable analysis. There was no significant association between infection and either sex or herd size (Table 2). Multivariable logistic regression model of risk factors analysis indicated that animals with ≥ 5 had a significant association with seroprevalence of BB. However, compared to seasons and native breeds, the probabilities (AOR) of seropositivity were 4.0 times higher during spring and 3.1 times higher for imported breeds (Table 3).

Table 1: Distribution of seroprevalence of bovine besnoitiosis according to some risk factors

Factors	Categories	Examined (n)	Positive n(%) Total=69	95% CI	x^2	P-value
Age (Years)	≤ 1	100	10(10.0)	4.8 to18.4	Reference	
	2-4	96	15(15.6)	8.8 to 25.8	1.4	0.2
	≥ 5	104	44(42.3)	31.97 to 59	27.1	0.0001
Sex	Male	154	37(24.0)	26.0 to 51.0	2.0	0.7
	Female	146	32(21.9)	21.9 to 45.2	Reference	
Breed origin	Native breeds	176	23(13.0)	14.6 to 34.5	Reference	
	Imported breeds	124	46(37.1)	33.7 to 61.4	23.8	0.0001
Herd size	Small ≤ 15	143	31(21.7)	21.0 to 44.0	Reference	
	Large ≥25	157	38(24.2)	27.0 to 52.2	0.3	0.6
Seasons	Winter	80	10(12.5)	4.8 to18.4	Reference	
	Spring	75	30(40.0)	20.2 to 42.8	15.2	0.0001
	Autumn	90	14(15.6)	7.7 to 23.5	0.3	0.56
	Summer	55	15(27.3)	8.4 to 24.7	4.7	0.03

Table 2: Association of bovine besnoitiosis with various risk factors using univariable logistic regression in 300 animals

Variables	Categories	Positive (n) N=69	Negative (n) N=231	Odds ratio	p-value
Age	≤ 1	10	90	Reference	
	2-4	15	81	1.7(0.7 to 3.9)	0.12
	≥ 5	44	60	6.6(3.0 to 14.1)	0.000001
Sex	Male	37	117	0.9 (0.5 to 1.5)	0.43
	Female	32	114	Reference	
Breed	Native breeds	23	153	Reference	
	Imported breeds	46	78	3.9 (2.2 to 6.9)	0.000001
Herd size	Small ≤ 15	31	112	Reference	
	Large ≥25	38	119	0.87 (0.5 to 1.5)	0.30
Seasons	Winter	10	70	Reference	
	Spring	30	45	4.7(2.0 to,10.4)	0.00001
	Autumn	14	76	1.3 (0.5 to 3.0)	0.20
	Summer	15	40	2.6 (1.0 to 6.4)	0.02

Table 3: Multivariable logistic regression analysis of various risk factors associated with bovine besnoitiosis

Variables	Categories	Adjusted Odds Ratio (95% CI)	P value
Age (years)	≤ 1	Reference	
	2-4	0.48(0.22 to 1.03)	0.42
	≥ 5	6.01(2.80 to 9.27)	0.002
Breed	Native breeds	Reference	
	Imported breeds	3.1 (2.15 to 9.85)	0.001
Seasons	Winter	Reference	
	Spring	4.0 (1.89 to 23.47)	0.0001
	Autumn	0.16 (0.08 to 0.32)	0.21
	Summer	0.82 (0.47 to 1.46)	0.34

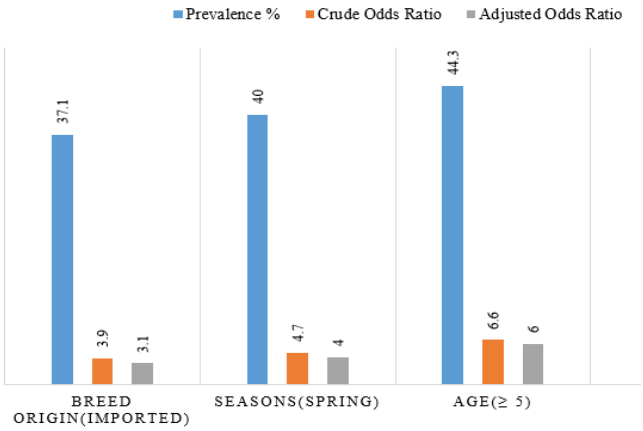

Figure 1: Important risk factors associated with *B. besnoiti*.

Discussion

The purpose of this study was to explore the seroprevalence of besnoitiosis in cattle as well as the risk factors associated with its emergence in Mosul city, Iraq, using the PrioCHECK® Besnoitia Ab 2.0 ELISA Kit. Additionally, Lunar *et al.* (40,41) recommend this kit as a reliable diagnostic tool capable of identifying BB-specific antigens that do not cross-react with *Toxoplasma gondii* or *Neospora caninum*. According to García Lunar *et al.* (14), PrioCHECK® Besnoitia Ab V2.0 revealed 100% Sensitivity and 98.8% specificity. Despite the absence of any clinical symptoms, 23% of the animals in this study had anti- *B. besnoiti* antibodies. BB has been reported in various countries with variable prevalence rates (42).

In endemic regions, only a few animals in a BB-infected herd exhibited clinical symptoms, whereas the majority were seropositive or sub-clinically infected (14). During the outbreak of the disease in Spain, the majority of animals, 90.5%, tested seropositive, but only 43% displayed clinical signs (43). According to Frey *et al.* (16), carrier animals in endemic areas may contribute significantly to disease transmission.

Also, the prevalence of infection in this study was lower than the 25, 26.6, and 28.7% reported by Kyari *et al.* (44), Ocal *et al.* (45), and Talafha *et al.* (23) in cattle at the Maiduguri Central Abattoir in Nigeria, Turkey, and Jordan, respectively, and is generally identical to a 22.1% was recorded previously by Kuraa *et al.* (4) in Egypt. According to Coelho *et al.* (11), the prevalence in Portugal was 16.89%. Higher prevalence rates of besnoitiosis were observed in France at 89% (46), Spain at 90% (43), and Italy at 36.51% (47). In an extremely recent study carried out in Iraq, the BB was molecularly detected in 74 (16.09%) bovine blood samples and 49 (10.65%) skin biopsies (25). The variation in prevalence rates between prior research and the findings of this survey can be attributed to the number and type of samples tested, the diagnostic technique applied, the availability of transmitting vectors, and the importation of animals from disease-endemic areas.

Based on our conclusion, the age of the cattle had a substantial impact on the seroprevalence of besnoitiosis. Larger animals (≥ 5 years) were more likely to become seropositive than those with < 1 and 2-4 years old (AOR 6.0). Similar findings were reported by Kuraa *et al.* (4) and Coelho *et al.* (11). This can be explained by prolonged and continuous vector exposure, as well as the possibility of transmission through direct contact between animals (during natural mating or artificial insemination), and the fact that most infected animals are carriers and remain seropositive for life (47). Blood-sucking insects, which are likely disease vectors, may be attracted to larger animals due to the high levels of CO_2 released as the animal grows (48). Furthermore, younger animals had higher rates of defense behaviors against these arthropods; thus, the chance of being bitten should be decreased (49). Additionally, age-related immune system alterations may also play a role. Older cattle exhibit lowered innate and adaptive immunological responses, especially decreased levels of essential cytokines that promote inflammation, like interferon-gamma (IFN-γ), which play a vital role in controlling infection (50).

In this study, imported animals were more likely to become seropositive (AOR =3.1) than regional breed animals. Transport, environmental changes, and adaptation stress can all minimize immune responses, making imported animals more susceptible to illness. Furthermore, imported breeds may have genetic features, causing them to be more predisposed to BB than native or cross-bred cattle that have adapted to the local environment (11).

Finally, in a multivariable regression analysis, the adjusted odds ratio (AOR) of seropositivity was 4.0 times higher for spring compared to other times of year. This is in agreement with Kuraa *et al.* (4), who found the seroprevalence of BB was highest in spring 42.9% (6/14). Spring is an essential time for disease dissemination because it allows for the spread of biting and blood-sucking insects, which play a significant role in transmitting disease from infected to healthy animals (8,9,10,51,52). In cattle, BB is transmitted via the ingestion of the sporulated oocysts shed in the feces of definitive hosts, through blood-sucking or biting insects like *Stomoxys* and *Tabanus* species (10,53), as well as reusing the same needles and, more frequently, by natural mating (8,9).

Conclusion

The current research provides the first sero-epidemiology insight into bovine besnoitiosis in Iraq, revealing a notable seroprevalence of 23%. The findings demonstrate that older cattle (≥5 years), imported breeds, and the spring season are significant risk factors for BB seropositivity. More studies are required to establish successful strategies for controlling this important parasite at the national level.

Conflict of interest

No conflicting interest to declare.

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الانتشار المصلي داء بيسنويتيا البقرية في مدينة الموصل، العراق

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الخلاصة

أصبح داء بيسنويتيا البقرية من الأسباب المهمة للخسائر التجارية الفادحة في المناطق الموبوءة على مستوى العالم. لا توجد معلومات كافية حول الانتشار المصلي للمرض في العراق. وبالتالي، كان الغرض من هذه الدراسة هو التحقيق في معدل انتشارها وعوامل الخطر المرتبطة بها في مدينة الموصل بالعراق. أجريت دراسة مقطعية على ٢٠ مزرعة من أكتوبر ٢٠٢٤ إلى فبراير ٢٠٢٥، وجمعت ٣٠٠ عينة من الحيوانات التي تتراوح أعمارها بين ١ إلى ٧ سنوات. كانت عوامل الخطر المحتملة التي تم التحقيق فيها هي العمر والجنس ومصدر الحيوانات وحجم القطيع والموسم. تم اختبار جميع المصل باستخدام اختبار الماص المناعي غير المباشر للإنزيم المتوفر تجاريًا. بلغ معدل انتشار المرض في الأبقار ٢٣%. أظهرت قطعان الأبقار ذات السلالات المستوردة ذات الأبقار الأكبر سناً (≤ ٥ سنة)، خلال الربيع، معدلات انتشار أعلى للأجسام المضادة ضد داء بوزناتيوزيس البقري(، في حين كان معدل الانتشار أقل في الحيوانات التي يبلغ عمرها ≥ ١ سنة. كشف التحليل أحادي المتغير ومتعدد المتغيرات أن إيجابية المصل المضاد الى ب ب كانت مرتبطة بالعمر وفصول السنة وأصل الحيوانات. في التحليل متعدد المتغيرات، كانت احتمـالات إيجـابية المصـل أعلى بمقدار ٦,٠ مرات

للحيوانات التي يبلغ عمرها ≤ ٥ سنوات وأعلى بمقدار ٤,٠ مرات في الربيع مقارنة بالحيوانات الأصغر سنًا وأوقات أخرى من العام. وجد أن الحيوانات المستوردة أكثر عرضة للإصابة بالإيجابية بمقدار ٣,١ مرة من تلك الموجودة في السلالات المحلية. تعد هذه الدراسة هي أولى الدراسات الوبائية التي كشفت مصليا عن نسب انتشار المرض في مدينة الموصل بالعراق. مثلت الحيوانات الكبيرة بالعمر والسلالات المستوردة وفصل الربيع اهم العوامل الخطورة للمرض مما يوفر معلومات مهمة سوف تساعد في توجيه إجراءات الترصد وإعداد خطط المكافحة المستقبلية للمرض في المنطقة.