Vol.3, No.5, Page 1171- 1177, 2025 https://doi.org/10.59628/jast.v3i5.1984 ISSN 2958-9568 JAST

SUN PUBLISHER

مجلة جامعة صنعاء للعلوم التطبيقية والتكنولوجيا

Sana'a University Journal of Applied Sciences and Technology

https://journals.su.edu.ye/index.php/jast/

Brucellosis among exported livestock from the Horn of Africa to Yemen: Seroprevalence study associated with risk factors

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Abstract

The international trade of livestock plays a significant role in the global dissemination of transboundary animal diseases and associated crisis risks. Brucellosis, a silent zoonotic transboundary disease, affects most livestock species. This study investigated the prevalence of brucellosis among camels, cattle, sheep, and goats exported from Djibouti and Somalia to Yemen. From April 2024 to April 2025, 1500 blood samples were tested using WOAH-officially recognized sero-agglutination techniques. Overall, 34 of 1500 samples (2.26%) tested positive for Brucella. Our analysis revealed significant differences in brucellosis positivity across animal types, age groups, and origins. Cattle showed the highest positivity rate, with 24 of 375 samples (6.4%). In contrast, sheep had 6 positive samples (1.6%), goats had 4 (1.1%), and camels had no positive samples (0%). When examining age, the older group (2-3 years) exhibited the highest prevalence, with 17 of 408 samples (4.2%). The positivity rates varied slightly across seasons, peaking in spring (3%) and autumn (2.6%), with lower rates in summer (2.2%) and winter (1.3%). Regarding origin, the Hirshabella district recorded the highest positivity in 13 of 125 samples (10.4%). This was followed by Southwest Somalia (5.5%) and Jubaland (4.4%). This study represents the first reported index of brucellosis among animals exported to Yemen, underscoring a significant risk, particularly for cattle and older animals. These findings highlight the urgent need for comprehensive epidemiological investigations to provide policymakers with the data necessary to formulate effective regulations, thereby reducing the spread of this transboundary animal disease.

Article Info

Keywords:
Brucellosis, Horn of Africa, Transboundary disease , Livestock, Export and Import animal

Article History:
Received: 29-June-2025,
Revised: 04-August-2025,
Accepted: 07-August-2025,
Available online: 28 October 2025.

1. INTRODUCTION

International animal trade facilitates the movementof livestock populations, posing a significant risk to the transboundary spread of animal diseases (TADs) between countries. TADs are highly contagious epidemics that pose significant threats to trade, economics, and food security, particularly in developing countries [1]. TAD poses a significant global health challenge, causing widespread illness and death in animals. Furthermore, certain TADs have emerged as infectious diseases, foodborne illnesses, and zoonoses. These diseases are responsible for over one billion cases of illness and millions of deaths annually, accounting for more than 50% of all human infectious diseases and 70% of emerging infectious diseases worldwide. This highlights the urgent need for effective surveillance and control strategies to mitigate their devastating impact on both animal and public health [1, 2]. Among these TADs, brucellosis stands out because of its widespread impact and the challenges it presents in its control and prevention. It is a zoonotic disease affecting both animals and humans worldwide. The disease is endemic to numerous developing regions, particularly Mediterranean Europe, Central America, Italy, Near Eastern countries, Mexico, South America, Central Asia, Africa, and India. Brucellosis causes significant economic loss, particularly in the livestock sector, and has a substantial global impact. Approximately 500,000 human cases are reported annually [3]. The World Health Organization (WHO) has designated brucellosis as one of the most neglected zoonotic diseases [4]. It affects a range of animals including cattle, pigs, goats, sheep, dogs, camels, horses, and other ruminants. *B. abortus* is mainly associated with cattle, whereas *B. suis* and *B. melitensis* are prevalent in sheep and goats. In addition, *B. suis* and *B. canis* are known to affect pigs and dogs [5]. Humans become infected with *Brucella* through consumption of raw milk, contact with an aborted fetus, or vaginal discharge from infected animals. This can lead to an acute illness known as undulant fever or Malta fever, which can last weeks or months. Symptoms included chills, headaches, weakness, weight loss, and general aches [6, 7]. In Latin America, brucellosis causes economic losses exceeding $600 million annually owing to its impact on cattle reproduction. Similarly, in India, the disease leads to nearly $3.4 billion in economic losses [8]. Although brucellosis has a global presence, its effects are particularly significant in East African countries and Yemen. The disease is widespread among livestock in East African countries (EAC), human patients in hospitals, and individuals exposed to pastoral communities in these regions [7, 9]. Additionally, brucellosis in Djibouti poses a significant threat to human health, especially to those who consume cattle products. Moreover, the disease is endemic to Ethiopian livestock and remains a persistent challenge in Somalia [10–14]. East African countries are particularly vulnerable to the spread of animal diseases because of the large livestock population in rural areas with inadequate infrastructure and disease control efforts [15, 16]. The prevalence of brucellosis among animals and humans in Yemen has not been well documented because of the lack of published research on this disease [17]. To the best of our knowledge, only cattle in Dhamar and camels in Al Hodeida governorates have been studied, showing very low and high prevalence of brucellosis, respectively [17, 18]. While Brucella infection is present in the country's animal population, it has little epidemiological importance in Yemenis [19]. Therefore, this study aimed to investigate the prevalence of brucellosis associated with various risk factors in camels, cattle, sheep, and goats exported from the Bossaso, Berbera, and Djibouti regional quarantines to Yemen.

2. MATERIALS AND METHODS

2.1. Study area

Djibouti and Somalia are located in the Horn of Africa on the eastern coast of Africa, along the southern approach to the Babel Mandeb Strait and along the route through the Red Sea. Djibouti has a land area of 8,400 square miles (21,883 square kilometers), while Somalia covers 637,657 square kilometers, with an area of 23,200 square kilometers for Djibouti. Somalia is located within the latitude and longitude of 10° 00' N and 49° 00' E, and Djibouti is within the latitude and longitude of 11° 30 N and 43 °00 E. Somalia has two dry seasons, summer and winter, and two rainy seasons, spring and autumn, with an average annual temperature of 28.5°C. The climatic conditions of Djibouti exhibit notable seasonal temperature variations. The lower season (October to April) recorded an average minimum temperature of 18.98°C in January and a maximum of 33.25°C in October. Conversely, temperatures are much higher during the warmer period (June to September), ranging from 25.95°C to a maximum of 38.85°C in July [20, 21].

Figure 1. Geographical border of the study area

2.2. Sample collection procedures

A total of hundred samples were collected equally (375 samples from each) from camels, cattle, sheep, and goats exported to Yemen from Bossaso and Berbera Animal National Quarantines, and Djibouti Regional Livestock Quarantine. Blood samples were collected by venipuncture from the jugular vein using a double -ended needle to fill the vacuum plane with a clot activator in a blood collection tube containing 3–5 mL of blood. The tube was labelled with a special number. In separate forms, we recorded details such as date, age, type, origin, and current season of the samples. The collected samples were stored in a cool box at 4 °C and transferred to the laboratory. Upon arrival at the laboratory reception unit, we matched the data from the tube with the data from the sheet in accordance with the laboratory's sample reception policy. The serum was separated by centrifugation at 3000 rpm for 5 min using a centrifuge model 80-2 (Xiangshui Fada Medical Apparatus Factory) [22]. The separated serum was transferred into labeled Eppendorf tubes and stored in a refrigerator at 20 °C until needed for serological analysis.

2.3. Risk factors:

These factors included different animal types, with 375 samples collected for each animal type. Age was also divided into three groups according to the age of the animals sampled: $\leq$ one year, 1-2 years, and 2-3 years. We ensured a balanced representation of the samples collected during the four seasons to account for seasonal factors. In addition, the animal origin factor was addressed by directly inquiring owners and herders during the sampling process to clarify any ambiguity

2.4. Screening Sero agglutination Test for Brucellosis

We used Rose Bengal Brucellosis Antigen solution purchased from (IDEXX Laboratories. Inc. One IDEXX Drive Westbrook, Maine 04092 USA). The reagent was allowed to reach room temperature (18-21 °C) before use. A clean whiteboard was manually divided into a grid of five rows and ten columns, creating 50 equal squares of 2 cm each. Each intersection point serves as an individual testing site. A sterile glass plate was placed over the grid to serve as a test surface. We determined the first angle for distributing the sample on the glass plate and added 35μl of the serum and the same volume of Rose Bengal brucellosis antigen solution, and then gently mixed them well to produce a circular or oval zone approximately 2 cm in diameter. The plate was gently shaken and incubated on its surface for exactly four minutes. Subsequently, the glass plate was placed on a custom-designed illuminated reading box to enhance the visibility of agglutination. The illuminated surface also facilitated identification of weak agglutination reactions. A positive result was considered if the samples showed any agglutination, even slight agglutination (indicating the presence of anti-brucellosis antibodies), while samples showing no agglutination were considered negative (indicating the absence of anti-brucellosis antibodies). The prevalence of disease, expressed as a percentage, was calculated using the following formula:

$$\textbf{Prevalence}\ (\%)\left(\frac{\text{Number of positive animals}}{\text{Total number of animals tested}}\right)\times 100$$

[22]

2.5. Statistical analysis

After conducting serological screening using the agglutination test, the results were organized and coded in Microsoft Excel Sheet 2021, and then transferred to IBM SPSS Statistics version 24 package for analysis. The data were displayed and expressed in numerical form and percentages. The relationship between variables was assessed using the chi-square test, Fisher's exact test, and post-hoc tests, which were conducted following the significant chi-square results. Statistical significance was determined when the *p*-value was less than 0.05.

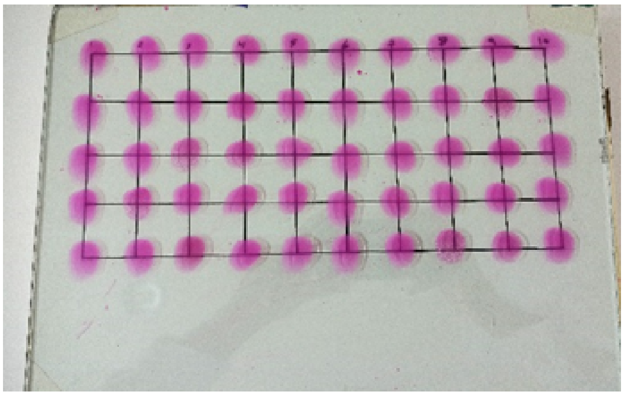

Figure 2. Sterile glass plate on a divided whiteboard for the brucellosis test

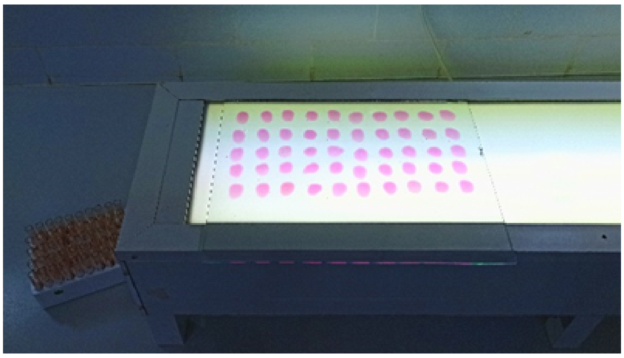

Figure 3. Custom-designed illuminated reading box

3. RESULTS

3.1. Prevalence of Brucellosis related to animal species

Animal type is a crucial determinant that can affect the prevalence of infection as different species have varying levels of susceptibility. Consequently, infection rates have been evaluated across various animal types to investigate potential differences. The overall prevalence showed that 34 of 1500 samples (2.26%) tested positive. Among the samples examined, cattle had the highest positivity rate, with 24 out of 375 samples (6.4%) testing positive, followed by other animals with positivity rates of 6 out of 375 (1.6%), 4 out of 375 (1.1%), and 0 out of 375 (0%) for sheep, goats, and camels, respectively (Table 1). Statistical analysis revealed a significant association

Table 1. The prevalence of Brucellosis among different animal types

Type	Positive No/Total	Percentage %	P-Value
Cattle	24 / 375	6.4	
Sheep	6 / 375	1.6	
Goat	4 / 375	1.1	0.0001
Camel	0 / 375	0	

between RBP test results and animal type ($p < 0.05$).

3.2. Prevalence of Brucellosis related to the age groups

After assessing the prevalence among different animal species, it is important to consider how age influences the susceptibility to infection. Factors such as immune system maturity, physiological stress, and the duration of exposure to pathogens can vary significantly with age. Consequently, infection rates were analyzed across different age groups. Animals aged 2-3 years exhibited a higher rate of positive *brucellosis*, with 17 of 408 samples (4.2%), compared to those aged $\leq$ one year, which had 6 of 363 samples (1.7%). The age group between 1-2 years showed the lowest rate, with 11 of 729 samples (1.5%) (Table 2). Statistical analysis revealed a significant relationship ($p < 0.05$) between the brucellosis test results and the age group.

Table 2. Prevalence of brucellosis according to age

Age	Positive No/Total	Percentage %	*P*-Value
2 to 3 years	17/408	4.2	
$\leq$ One year	6/363	1.7	0.010
1 to 2 years	11/729	1.5	

3.3. Prevalence of Brucellosis related to the season

Seasonal variations, along with age, significantly influence brucellosis transmission dynamics in both humans and animals. The results indicated that spring had the highest positivity rate (11/317 samples, 3%), followed by autumn (11/317 samples, 3%), summer (10/451 samples, 2.2%), and winter (5/317 samples, 1.3%) (Table 3). No statistically significant association was identified between brucellosis test results and seasonal factors (p = 0.05).

Table 3. Prevalence of Brucellosis related to season

Season	Positive No/Total	Percentage %	P-Value
Spring	11 / 371	3.0	
Autumn	8 / 307	2.6	
Summer	10 / 451	2.2	0.495
Winter	5 / 371	1.3	

3.4. Prevalence of Brucellosis related to the origin

The origin of animals is crucial for brucellosis, as different species in various environments show varying susceptibility. The current evaluation of animal origins revealed that the Hirshabella district had the highest prevalence, with 13 of 125 samples (10.4%) testing positive. Following this, Southwest Somalia, Jubaland, and Somaliland showed 3 out of 55 samples (5.5%), Jubaland had 7 out of 158 samples (4.4%), and Somaliland had 6 out of 247 samples (2.4%). Conversely, Khatomu and Puntland recorded the lowest rates, with one out of 121 samples (0.8%) and four out of 589 samples (0.7%), respectively. Notably, Ogaden District reported no positive results, with 0 of 205 samples (0%) (Table 4).

Table 4. Prevalence of Brucellosis related to the origin

Livestock Origin	Positive No/Total	Percentage %	*P*-Value
Hirshabelle	13 /125	10.4	
Southwest Somalia	3 /55	5.5	
Jubaland	7 /158	4.4	
Somaliland	6 /247	2.4	0.000
Khatomu	1 /121	0.8	
Puntland	4 /589	0.7	
Ogaden	0 /205	0	

We found a statistically significant association between the brucellosis test results and the origin of the livestock ($p < 0.05$).

4. DISCUSSION

Brucellosis is a zoonotic infectious disease that is widespread in Africa and causes significant economic losses. It is present in various regions of the world, including Asia, Africa, Europe, and the Americas [23]. In East Africa, where many countries export livestock to Arab Gulf states and Yemen, brucellosis is a major concern, as it can lead to the rejection of livestock shipments because of its contagious nature [24]. In our study, the results revealed that 34/1500 samples (2.26%) were positive, which is lower in prevalence rate when compared with previous studies conducted in East African exporting countries such as Djibouti [10], Somalia [13], Tanzania, [25] and Ethiopia [26]. Moreover, the prevalence rates found in the expected importing countries, such as Oman [27] and Yemen [18], [28] were less than our findings, while the same results were observed in Saudi Arabia [29]. Understanding these prevalence rates is crucial, particularly when considering their impacts on different livestock species. In our study, cattle showed the highest positive results, consistent with findings from previous studies in Djibouti, Somalia, and Ethiopia [10, 13, 26]. However, these values were lower than those reported in the Thamar governorate in Yemen and Saudi Arabia [28, 30]. The relatively low overall incidence of brucellosis observed in this study can be attributed to a combination of factors related to the animal population, diagnostic techniques, and environmental conditions [18, 24]. Specifically, the lower prevalence observed in our study may be attributed to the focus on clinically healthy male animals selected from managed herds destined for export at veterinary quarantine facilities. This finding contrasts with other studies that have included both sexes. This finding underscores the need for targeted interventions and control measures that focus on cattle populations, particularly in exporting countries. Goats showed the highest positive rates in studies conducted in Tanzania [25], Southern Oman [31], and Ethiopia [26]. However, a study conducted in Oman revealed that camels had the highest number of positive results [27]. These variations may be attributed to several factors including environmental conditions, variations in host immunity, age, nutritional status, and management practices [32]. Moving on to camels, this study did not produce positive results, which is consistent with studies conducted in the Al-Hodeida governorate of Yemen [18] and Ethiopia [33]. In contrast, the Igad Member Countries, the Al-Qassim Region in KSA, and Sudan [7, 24, 34] reported a higher prevalence than our findings. The results obtained may be attributed to awareness among animal owners who routinely check their herds for brucellosis. Differences from other studies could be due to various factors such as environmental conditions, health protocols, and genetic variation among camels in different regions [35]. These findings have significant implications for imported Yemen animals, highlighting the need for enhanced health certification and quarantine measures to ensure the safety and quality of imported camels. Understanding the prevalence among animals offers valuable insights into host susceptibility, while understanding how age influences brucellosis prevalence further deepens our understanding of disease dynamics. Animals aged 2-3 years had the highest positive brucellosis rate, with 17 out of 408 samples (4.2%) testing positive, indicating that age is a significant factor. These findings are consistent with those of studies conducted in Somalia and Saudi Arabia, and previous research that has shown *brucellosis* to be more common in older animals [13, 29, 30, 35]. In contrast, studies in Tanzania and Dhamar in Yemen did not find a significant difference in brucellosis prevalence among different age groups [21, 28]. The varying conclusions drawn by the two studies likely stem from differences in the sample sizes and distinct geographical research areas [36]. These findings highlight the critical need to integrate age-specific screening and management strategies into quarantine and disease control protocols to prevent the entry of infected animals into Yemen. There were no significant variations in seasonal trends, with a prevalence ranging from 2% to 3% across all seasons, which is consistent with the results of a study conducted in Iran [37]. This finding contrasts with those of previous studies in the Republic of Benin [38], [39] possibly due to differences in climatic conditions and challenges in sustainable agricultural practices [37]. This underscores the importance of continuous surveillance throughout the export process, independent of season. While seasonal trends did not show significant variation, it is crucial to consider the factors of animal origin. Regarding livestock origin, Hirshabelle district exhibited the highest prevalence of brucellosis among the different districts. Conversely, the Ogaden district showed no positive results among the different animals. This result is consistent with that of a study conducted in Ogaden [40] and another study conducted in the Somali and Ethiopian regions, which showed a 0.3% seroprevalence [33]. Our results revealed a lower prevalence in the Puntland District than in studies conducted in the same region [41, 42]. To our knowledge, the lack of previous studies using similar methodologies in these regions underscores the importance of current research. This study provides critical baseline data on the seroprevalence of brucellosis among various livestock species in these areas. By addressing this significant knowledge gap, this study establishes a foundation for future research and can directly inform policymaking related to regional animal health monitoring and control strategies.

5. LIMITATIONS

Our study has several limitations. Financial constraints were a primary limit, impacting both the sample size we could achieve and our ability to acquire essential diagnostic tools such as ELISA kits. In addition, social challenges significantly impede access to animals for sample collection. These challenges stem from the lack of awareness among livestock owners and traders.

6. CONCLUSIONS AND RECOMMENDATIONS

The study concluded that cattle were more susceptible to brucellosis, with a higher prevalence rate despite a low overall prevalence. Prevalence was lower in sheep and goats, whereas camels showed no positive results. Older animals are more susceptible to disease than younger animals. Factors, such as age, animal type, and origin, were significantly associated with the prevalence of brucellosis. This study recommends comprehensive epidemiological investigations to evaluate the burden of the disease and to inform evidence-based policymaking. Moreover, before exporting animals, they must undergo laboratory testing and quarantine procedures within official quarantine facilities, which play a crucial role in minimizing the transmission of transboundary animal diseases to Yemen. However, strict post-arrival testing protocols should be enforced at the Yemeni ports of entry to reduce the risk of cross-border transmission and prevent local transmission.

7. ACKNOWLEDGMENT

We extend our sincere appreciation to Prima International Company for generously providing access to quarantine and laboratory facilities.

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