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Research Article

Seroprevalence of *Brucella* antibodies in Foreign-breed Police-Kennel Dogs and Citizen-owned Dogs in Khartoum State-Sudan, Using the Rose Bengal Test

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Abstract

Dogs may acquire and transmit various Brucella spp., but their role in the epidemiology of brucellosis in Sudan is poorly characterized. This study evaluated serological evidence of Brucella exposure among two management groups of foreign-breed dogs in Khartoum State. 77 mature dogs were sampled: 45 Police-Kenneled Dogs (PKD) and 32 Citizen-Owned Dogs (COD). Whole blood was collected by venipuncture, sera separated, and screened for anti-Brucella antibodies using the Rose Bengal Plate Test (RBPT) and a modified RBPT protocol. Data were summarized with frequencies and percentages; associations between management type or sex and serostatus were tested with chi-square ($\alpha = 0.05$). 61 out of the 77 dogs (79.2%)were tested positive by RBPT. Seropositivity was markedly higher in PKD (44/45; 97.8%) than in COD (17/32; 53.1%). The association between dog management and RBPT serostatus was highly significant ($\chi^2 = 22.652$, df = 1, $p < 0.001$). No significant difference in seropositivity was observed between males and females (78.1% versus 80.0%, $\chi^2 = 0.040$, $p = 0.842$). The findings reveal substantial serological evidence of *Brucella* exposure in the sampled population, particularly among police-housed dogs. Because RBPT detects antibodies to smooth LPS antigens and cross-reactivity or non- *Brucella canis* infections are possible.

Keywords

Brucellosis, *Brucella*, Dog, Sudan, Rose Bengal

1. Introduction

Brucellosis is a significant zoonosis affecting both humans and animals worldwide particularly in developing countries in Africa such as Sudan. The disease is endemic in many developing countries and remains a major public health problem due to inadequate control programs [1, 3]. Brucellosis is characterized by reproductive losses in animals and undulant fever in humans [2, 3]. It is caused by bacteria of the genus *Brucella*, which infect a wide range of domestic and wild

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animals, resulting in significant economic and public health impacts [4, 5].

In dogs, *Brucella canis* is the primary cause of infection, although dogs may also acquire *Brucella abortus* and *Brucella melitensis*, especially in regions where livestock brucellosis is endemic [6-9]. The species of *Brucella* known to infect humans include *Brucella melitensis* (from goats), *Brucella suis* (from pigs), *Brucella abortus* (from cattle), and *Brucella canis* (from dogs) Transmission to humans typically occurs through direct contact with infected animals, ingestion of unpasteurized dairy products, or occupational exposure [3]. The close contact between dogs and humans pose a potential hazard to dog owners, veterinary workers, and police personnel at risk of infection. In Sudan, the persistence of brucellosis is linked to limited public awareness, uncontrolled animal movements, and insufficient surveillance measures [10, 11].

The disease is endemic among small ruminants (goats and sheep) and other livestock as demonstrated by multiple seroprevalence and isolation studies in various states in Sudan. In Khartoum State brucellosis was detected in goats [12]; in Eastern Sudan, *Brucella abortus* was isolated from sheep [13]. Brucellosis affects camels across Sudan, maintaining the pathogen in the livestock population over decades [14]. The disease also affects cattle [10, 11]. These data confirm that brucellosis remains a major public health and livestock production problem in Sudan. However, published data on *Brucella* infection in dogs in Sudan are lacking, highlighting an important gap.

PKD generally receive structured care and regular veterinary supervision, whereas COD vary widely in management, such differences in management may influence the level of exposure to *Brucella* spp. Assessing the prevalence of *Brucella* antibodies in dogs under different management systems is therefore essential for understanding their epidemiological significance and their potential zoonotic impact. This study compares PKD and COD in Khartoum State and evaluates whether management system or sex is associated with *Brucella* seropositivity. The findings will contribute to improved surveillance and public health planning in Sudan.

2. Materials and Methods

2.1. Study Area

The investigation was carried out in Khartoum State, Sudan, covering several urban and peri-urban districts, including Alsafia, Kafouri, Alsamrab, Burri, Western Elgeraif, Hillat Khojaly, Bahri, and Almurabaat. Khartoum lies in central Sudan between latitudes 15-16 ℃N and longitudes 31.5-34 ℃E. Fieldwork and sampling were conducted within four summer months.

2.2. Study Population

A total of 77 dogs of foreign breed, which was believed to be pure German Shepherd, were enrolled for serological examination for *Brucella* antibodies. The study population comprised two management categories: 45 PKD aged 1–3 years and weighing 25–40 kg. and 32 COD from private households which was the maximum available, given the difficulty of finding pure-bred German Shepherds owned by citizens.

2.3. Management Conditions

PKD were kept individually in divisions measuring approximately 6 m^2 and maintained under a unified feeding schedule, receiving one meal daily at 8:30 AM consisting mainly of cooked pasta and meat. In contrast, COD were subject to varied husbandry practices dictated by individual owners, resulting in differences in feeding, housing, and overall care.

2.4. Sample Collection and Processing

From each animal, approximately 3 mL of blood was collected aseptically from the cephalic vein using sterile disposable syringes. Samples were allowed to clot at room temperature, after which serum was separated into sterile Eppendorf tubes. When necessary, samples were centrifuged at 30 cycles/second to ensure complete serum clarification. All sera were processed and tested within 24 hours of collection.

2.5. Serological Assays

Detection of antibodies against smooth *Brucella* species was carried out as described by the manufacturer and [15] using the Rose Bengal Plate Test (RBPT) and the Modified Rose Bengal Plate Test (mRBPT). Antigens stained with Rose Bengal dye were sourced from Lillidale (London). Equal volumes of antigen and serum were mixed on a white porcelain plate, gently rotated for 4 minutes, and results were interpreted immediately. Any visible agglutination was considered evidence of a positive reaction.

2.6. Data Analysis

Data were entered and analyzed using IBM SPSS Statistics version 26. Descriptive statistics were presented as frequencies and percentages. Associations between categorical variables were assessed using the Chi-square test, with statistical significance defined as $p < 0.05$.

3. Results

A total of 77 dogs were sampled. PKD represented 58.4% (n=45) of the study population, while COD accounted for 41.6% (n=32). Seropositivity for *Brucella* antibodies of PKD was found to be 97.8% and that of COD was found to be 53.1% (Table 1).

A chi-square test showed a highly significant association between dog management type and *Brucella* antibody status ($\chi^2 = 22.652$, df = 1, $p < 0.001$). Fisher's exact test also confirmed significance (p = 0.000) (Table 2).

The distribution of male and female dogs did not differ significantly between PKD and COD (Table 1); The association between dog management and sex was not significant ($\chi^2 = 0.020$, p = 0.889). Seropositivity was similar between males and females with a positivity 78.1% and 80.0% in males and females respectively. No significant association ($\chi^2 = 0.040$, p = 0.842) between dog sex and *Brucella* antibody status.

Table 1. *Distribution of Brucella antibody test results by dog management system and sex.*

Dog management			Positive (%)	Negative (%)	Total (%)
PCD	SEX	Male	18 (94.7)	1 (5.3)	19 (42.2)
		Female	26 (100.0)	0 (0.0)	26 (57.8)
	Total		44 (97.8)	1 (2.2)	45 (100.0)
COD	SEX	Male	7 (53.8)	6 (46.2)	13 (40.6)
		Female	10 (52.6)	9 (47.4)	19 (59.4)
	Total		17 (53.1)	15 (46.9)	32 (100.0)
Total	SEX	Male	25 (78.1)	7(21.9)	32 (41.6)
		Female	36 (80.0)	9 (20.0)	45(58.4)
	Total		61 (79.2)	16 (20.8)	77 (100.0)

Table 2. *Association of dog management and Brucella antibodies.*

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	22.652[a]	1	.000		
Continuity Correction[b]	20.020	1	.000		
Likelihood Ratio	24.869	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	22.357	1	.000		

0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.65
Computed only for a 2 x 2 table

4. Discussion

Brucellosis is a zoonotic disease that is classified among the top seven world neglected zoonotic diseases [5] and public health problems in developing countries with adverse negative effects on both human and animals as well as economic implications [4]. Dog brucellosis including that caused by *Brucella canis*, is part of brucellosis public health hazard [16]. Development of a rapid, accurate, and widely available identification method is required for diagnosing of this disease [17].

While the overall seroprevalence of *Brucella* antibodies was high in both groups, PKD exhibited a higher seroprevalence (97.8%) than COD (53.1%). The high seropositivity among PKD and COD in our study suggests either a very high exposure to *Brucella*, highlighting the role of dogs as carriers and reservoirs for *Brucella* in addition to the high risk of infecting both human and livestock or potentially cross-reaction in the Rose Bengal test.

A chi-square test indicated a strong association between dog management type and *Brucella* antibody status ($\chi^2 = 22.652$, $p < 0.001$), suggesting that PKD are at substantially higher risk of exposure. Our results align with that of [18] who reported the higher seropositivity to antibodies to *Brucella* spp. was among confined than stray dogs in Nigeria and identified confinement as risk factor; in our study PKD are

more confined than the COD.

PKD generally receive structured care and regular veterinary supervision. However, their feeding regimen, often involving large quantities of raw ruminant meat and offal from slaughterhouses, may constitute a potential source of *Brucella* infection. Managing such large volumes of raw animal products is challenging, and non-wholesome or condemned parts may inadvertently be included, increasing the risk of exposure. In contrast, citizen-owned dogs, particularly foreign breeds that are highly valued by their owners, are typically fed small portions of household leftovers or purchased meat, which are less likely to harbor pathogens. COD do not roam freely, and mating is strictly managed by their owners, often with carefully selected dogs from friends or neighbors, targeting pure breeds. Although the owners are not specifically considering the risk of brucellosis, this controlled mating reduces the likelihood of infection. Additionally, the higher density of male dogs in Kennel settings may further contribute to the increased risk of transmission among PKD compared to COD. Similar seropositivity between males and females was found in this study indicating no significant association between sex and *Brucella* antibody presence. These results suggest that sex was not a determinant of *Brucella* seropositivity.

Using RBPT in our study revealed a high prevalence of *Brucella* antibodies compared to previous studies. No positive sample was detected among the dogs tested in State of Minas Gerais, Brazil [19]. In Nigeria, a large sero-epidemiological survey of 738 dogs reported 12.7% RBPT seroprevalence, with risk factors including age, confinement, and reproductive history such as infertility [18]. in Egypt, [20] reported about 2% prevalence of *Brucella* antibodies in dog sera.

RBPT was performed to detect antibodies against smooth *Brucella*. Although canine brucellosis is caused mainly by *Brucella canis*, dogs may be infected by smooth *Brucella* spp [21-23]. They have the potential to harbor *Brucella melitensis*, a species of particular concern due to its virulence in humans [23, 24] or B. abortus [21] highlighting the role of dogs as potential reservoirs for smooth *Brucella* species and underlines the importance of including dogs in brucellosis surveillance and control strategies to mitigate the zoonotic risk [25].

Conclusion: To the best of our knowledge, this study represents the first report of detection of *Brucella* antibodies in dog sera in Sudan. The markedly high seroprevalence detected- particularly among PKD highlights a potentially underestimated epidemiological and public health concern. Difference in management practices appears to influence exposure risk, with intensively managed dogs showing significantly higher seropositivity. Although RBPT detects antibodies primarily against smooth *Brucella* species, and *Brucella canis* infection cannot be ruled out without further confirmatory tests, the findings strongly suggest ongoing circulation of *Brucella* spp. among dog populations in Khartoum State.

Given the close interaction between dogs, humans, and livestock within the region, these results underscore the need to include dogs in national brucellosis surveillance and control programs. Strengthening diagnostic capacity, improving awareness among dog owners and veterinary personnel.

5. Limitation

A key limitation of this study is the reliance on a single serological test (Rose Bengal Test) for detecting *Brucella* antibodies, lack of species identification, and potential cross-reactivity and the possibility of false-positive results were not excluded, which may affect the accuracy of the reported seroprevalence.

Abbreviations

RBPT	Bengal Plate Test
mRBPT	Modified Bengal Plate Test

Conflicts of Interest

The authors declare that there is no conflicts of interest regarding the publication of this article.

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