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PREVALENCE AND RISK FACTORS ASSOCIATED WITH CAPRINE BRUCELLOSIS IN AL NUHOD LOCALITY, WEST KORDUFAN STATE, SUDAN

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ABSTRACT

Background: This study aimed to assess the prevalence of Caprine Brucellosis in Al Nuhod Locality, West Kordofan State- Sudan, from March to May 2019. Stratified random sampling covered Al Nuhod city and surrounding regions, collecting 507 serum and 325 milk samples from apparently healthy, non-vaccinated local breed goats of varying ages and sex. Diagnostic tests included RBPT and BAPA for serum, and MRT for milk sample. **Results:** Prevalence rates were (60.4%) using RBPT test, (25.6%) BAPA test, and (23.7%) when MRT test wasused. RBPT revealed significant differences between Al Nuhod city (46.6%) and areas around Al Nuhod (70.7%). Age-wise, RBPT showed significant differences (p-value 0.001), with rates of 53.9% (≤12 months) and 68.6% (>12 months). Gender-wise, RBPT indicated rates of 62.5% (females) and 55.5% (males). Comparison between RBPT and BAPA showed that there was a significant difference (p- value 0.002) between results of the two tests. Comparison between RBPT and MRT showed that there wasn't a significant difference (p- value 0.335) between results of the two tests. **Conclusions:** The study indicated a high prevalence of Caprine brucellosis using RBPT particularly in areas around EnNahud compared to EnNahud city. The disease's occurrence was notably higher in females and those aged over 12 months, demonstrating a significant association with caprine brucellosis seropositivity. The results highlighted the importance of considering regional variations and age-related factors in caprine brucellosis prevalence assessments.

KEYWORDS: Caprine, Brucellosis, West Kordofan State, Sudan.

INTRODUCTION

Sudan is a home to approximately 41 million goats (Anon, 2003), with an estimated 4.291.13 million residing in West Kordofan State (UNDP, 2006). These animals are of great economic importance and are kept for meat, milk, hair and skin. Brucellosis is an important zoonotic disease of wild and domestic animals and it has a worldwide distribution, Caprine brucellosis is the most important zoonotic disease caused by gram- negative bacteria named *Brucella melitensis* (*Br. melitensis*) which belong to the genus Brucella and family Brucellaceae (Bergey *et al.*, 1984). The clinical signs in goats are abortions most often during the last trimester, stillbirths and the birth of weak offspring, most animals abort only once, and subsequent pregnancies are usually normal, reductions in milk yield are common when mastitis has been reported in small ruminants infected with large doses of *B. melitensis*, but clinically apparent mastitis is uncommon in the field, retention of the placenta and secondary metritis are possible complications, acute orchitis and epididymitis are sometimes seen in males, also infertility, arthritis and hygromas have been reported, deaths are rare except in the fetus or newborn (OIE, 2018). Presumptive evidence of Brucella is provided by the demonstration, by modified acid-fast staining of organisms typical of Brucella in abortion material or vaginal discharge, especially if supported by serological tests, the polymerase chain reaction (PCR) methods provide additional means of detection, whenever possible, Brucella spp should be isolated using selective or non-selective media by culture from uterine discharges, aborted fetuses, udder secretions or selected tissues, such as lymph nodes, spleen, uterus, testes and epididymes. Species and biovars should be identified by phage lysis, and by cultural, biochemical and serological criteria, molecular methods have been developed that could also be used for complementary identification based on specific genomic sequences (OIE, 2008). The serological tests were used included Rose Bengal Plate Test

(RBPT), Serum or Tube Agglutination Test (SAT or TAT), Complement Fixation Test (CFT), Card Test, Plate Agglutination Test, Enzyme linked Immune Sorbent Assay (ELISA), Milk Ring Test (MRT), Whey Agglutination Test (WAT) and Allergic Skin Test (AST)(WHO, 1992).

Animal brucellosis was conducted in many parts of Sudan in which *Br. Melitensis* was isolated from the milk of cattle, sheep and goats (Daffalla and Khan, 1958). Furthermore many studies were conducted to study the prevalence rate of Caprine Brucellosis in Khartoum State, Hayfa found that the prevalence rate in Khartoum State by Rose Bengal Plate Test (RBPT) and the Tube Agglutination Test (TAT) were 1.5% and 2% respectively whereas by the Milk Ring Test was 0.5% (Hayfa, 2001). Azza in Omdurman Area found that the rate of positive reactors for caprine brucellosis was (16.57%) by RBPT and a much lower percent of positive reactors 9.28% was obtained with Milk Ring Test (Azza, 2006). Nisreen in Khartoum north found that the positive reactors were 10.5% by RBPT and a higher percentage of positive reactors 16% obtained with MRT (Nisreen, 2006). Musa in Darfar States found that the rate of positive reactor of caprine brucellosis was 10.5% by RBPT, cELISA, MRT, SAT and CFT (Musa, 2006). Ahmed *et al.* (2015) determined that ovine brucellosis is not a significant public health problem in North Kordufan. However, there has been no investigation of the disease in the Al Nuhod locality in West Kordufan. Hence, this study was specifically designed to address this gap and shed light on the prevalence of Caprine Brucellosis infection in goats in Al Nuhod, West Kordufan State. Notably, there had been no vaccination program in practice in this area. The study aims to detect the prevalence of Caprine Brucellosis infection in Al Nuhod and identify associated risk factors.

MATERIAL AND METHODS

Study area: Al Nuhod Locality, situated in West Kordufan State, spans latitudes 12.0 to 14.0 North and longitudes 27.0 to 30.0 East and boasting the largest goat population in the State.

Samples: Blood and milk samples were meticulously collected through stratified random sampling, considering the area, age, and sex of apparently healthy non-vaccinated goats during the period of March to May 2019 (Table 1).

Collection of samples

Serum Samples: A volume of five milliliters of blood was aseptically drawn into sterile tubes from the jugular vein, utilizing a disposable syringe as per established protocols (Alton *et al*., 1988).

Milk samples: Collection of milk samples followed established procedures (Alton *et al*., 1988).

Antigens: Antigens utilized for RBPT, BAPA, and MRT were sourced from the Central Veterinary Research Laboratories (CVRL) in Soba.

Procedures of the tests

Milk Ring Test (MRT): This test relies on the agglutination of antibodies secreted into the milk (OIE, 2009).

Serological Tests: Sera collected were screened for the presence of Brucella antibodies through the Rose Bengal Plate Test (RBPT) (Alton *et al*., 1988) and Buffered Acidified Plate Antigen Test (BAPA) (OIE, 2012).

Data analysis: Data underwent comprehensive analysis using the Statistical Package for Social Sciences (SPSS) for Windows® version 20 (SPSS Inc., Chicago, Illinois). The analytical process encompassed descriptive statistics, frequencies, and cross-tabulations for each potential risk factor. Univariate and multivariate analyses, employing the 2-tailed chi-square test, were conducted. Associations deemed significant in the chi-square test met the criterion of $p \leq 0.05$.

RESULTS

Out of 507 serum samples tested, 306 (60.4%) were positive using RBPT and 130 (25.6%) using BAPA (Table 2). Among the 325 examined milk samples, 77 (23.7%) tested positive using MRT (Table 2). The prevalence of caprine brucellosis in Al Nuhod locality using RBPT was 46.6% in Al Nuhod city and 70.7% in areas around Al Nuhod, showing a statistically significant difference (p-value: 0.004). For BAPA, the prevalence was 27.11% and 25.2% in Al Nuhod city and areas around Al Nuhod, respectively, with an insignificant difference (p-value: 0.09) (Table 3 and 4).

By using RBPT, the prevalence in age less than 12 months was 153 (53.9%) out of 284 serum samples, and in age more than 12 months, it was 153 (68.6%) out of 223 serum samples, with a statistically significant difference (p-value: 0.001) (refer to Table 5). By using BAPA, the prevalence in age less than 12 months was 26.4% out of 284 serum samples, and in age more than 12 months, it was 24.7% out of 223 serum samples, showing an insignificant difference (p-value: 0.366) (refer to Table 6). By using MRT the prevalence in age less than or equal 12 months was 6.3% out of 284 tested, and in age more than 12 months, it was 23.3% out of 223, with a statistically significant difference (p-value: 0.000) (refer to Table 7). RBPT results indicated a prevalence of 62.5% (203 out of 325) in females and 55.5% (101 out of 182) in males, with no statistically significant difference (p-value: 0.882) (Table 8). For BAPA, the prevalence in female goats was 25.2% (82 out of 325), and in males, it was 28% (51 out of 182), with no statistically significant difference (p-value: 0.280) (Table 9).

Out of 507 serum samples examined, the percentage of negative agreement between RBPT and BABA were 164(32.3%) while samples negative for RBPT and positive for BABA were 37(7.3%), the percentage of positive agreement between the two tests was 93(14%) and the percentage of the positive for RBPT and negative for BABA were 213(42%). The overall percentage agreement between the two tests was (46.3%). The different was statistically significant with P-value 0.002 (Table 10). The negative percentage agreement between RBPT and MRT were 95(29.2%) while samples negative for RBPT and positive for MRT were 27(8.3%), the positive agreement between the two tests was 50(15.4%) and positive for RBPT and negative for MRT were 153(47%). The overall agreement between the two tests was (44.6%). The difference was statistically insignificant with P-value 0.355 (Table, 11).

Table 1: Areas, number of samples and sex of examined Goats.

Area	No. Samples	Sex	
		Female	Male
EnNahud City	193	97	96
Areas Around EnNahud	314	228	86
Total	507	325	182

Table (2): Seroprevalence of Caprine Brucellosis in Al Nuhod Locality.

Tests	Type of Samples	No. Examined	No. Positive	No. Negative
RBPT	Serum	507	306 (60.4%)	201 (39.6)
BABA	Serum	507	130 (25.6%)	377 (74.4%)
MRT	Milk	325	77 (23.7%)	248 (76.3%)

Table (3): Seroprevalence of Caprine Brucellosis in different Areas.

Area	No. Examined	Tests					
		RBPT			BABA		
		+ve	-ve	p.value	+ve	-ve	p.value
Al Nuhod city	193	90 (46.6)	103 (53.4)	0.004	54 (27.11)	139 (72.0)	0.009
Areas Around Al Nuhod	314	222 (70.7	92 (29.3)		79 (25.2)	235 (74.8)	

Table (4): Prevalence rate of Caprine Brucellosis in different areas using MRT.

Area	No. Examined	Result		P.Value
		Positive	Negative	
Al Nuhod City	97	27(27.8)	70(72.2%)	0.389
Areas Around Al Nuhod	228	51(22.4%)	177(77.6%)	
Total	325	78(24%)	247(76%)	

Table (5): Prevalence of caprine Brucellosis using RBPT to different ages.

Age	No. Examined	Result		Df	P.Value	Risk Estimate
		Positive	Negative			
Less than 12 Months	284	153 (53.9%)	131 (46.1%)	1	0.001	1.303
More than 12 Months	223	153 (68.6%)	70 (31.4%)			0.697
Total	507					

Table (6): Prevalence of caprine Brucellosis using BABA to different ages.

Age	No. Examined	Result		Df	P. Value	Risk Estimate
		Positive	Negative			
Less than 12 Month	284	75 (26.4%)	209 (73.6%)	1	0.366	0.961
More than 12 Month	223	55 (24.7%)	168 (75.3%)			1.053
Total	507					

Table (7): Prevalence of caprine Brucellosis using MRT to different ages.

Age	No. Examined	Result		Df	P. Value	Risk Estimate
		Positive	Negative			
Less than 12 month	284	18 (6.3%)	266 (93.7%)	1	0.000	2.367
More than 12 Month	223	52 (23.3%)	171 (76.7%)			0.527
Total	507					

Table (8): Prevalence of Caprine Brucellosis using RBPT to different sex.

Age	No. Examined	Result		Df	P. Value	Risk Estimate
		Positive	Negative			
Female	325	203 (62.5%)	122 (37.5%)	2	0.882	0.918
Male	182	101 (55.5%)	81 (44.5%)			1.164
Total	507	304	203			

Table (9): Prevalence of Caprine Brucellosis using BAPA to different sex.

Sex	No. Examined	Result		Df	P. Value	Risk Estimate
		Positive	Negative			
Female	325	82 (25.2%)	243 (74.8%)	1	0.280	1.054
Male	182	51 (28%)	131(72)			0.913
Total	507	131	374			

Table (10): Comparison between RBPT and BAPA tests to determine the prevalence rate of caprine Brucellosis.

RBPT	BABA		P. Value	Total
	Negative	Positive		
Negative	164 (32.3%)	37(7.3%)	0.002	201 (39.6%)
Positive	213 (42%)	93 (18.4%)		306 (60.4%)
Total	130 (25.6%)	377 (74.4%)		**507 (100%)**

Table (11): Comparison between RBPT and MRT tests to determine the prevalence rate of caprine Brucellosis.

RBPT	MRT		P. Value	Total
	Negative	Positive		
Negative	95 (29.2%)	27 (8.3%)	0.355	122 (37.5%)
Positive	153 (47%)	50 (15.4%)		203 (62.5%)
Total	248 (76.3%)	77 (23.7%)		325 (100%)

DISCUSSION

This study aimed to determine the prevalence rate of Caprine Brucellosis in Al Nuhod Locality, to investigate the risk factors associated with disease and to make a comparison between different tests used for detection of Brucellosis. The tests used were RBPT and BAPA tests for serum samples and MRT for milk samples. The results in this investigation indicated that the prevalence rate of the disease was 60.4%, 25.6%, and 23.7% using RBPT, BAPA and MRT respectively. The result of RBPT was higher than that detected by Hayfa (2001) in Khartoum State (1.5%), Eman *et al*. (2018) (11.4%), Nisreen (2006) in Khartoum North Locality (10.5%), Azza (2006) in Omdurman (16.57%), Solafa *et al.* (2014) in Jabel Aolia Locality, Sudan (36.0%), Musa (2006) in Darfur State (10.5%), Zein and Edris (2015) in Northern State of Sudan (16.3%), Agab (1997) in Eastern Sudan (0.0%), Khuzaima *et al.* (2018) in Gedarif State of Sudan (10.8%), Mokhtar *et al.* (2007) in Kassala State of Sudan (5.6%), Teferi and Yeshibelay (2019) in Babile Woreda, Eastern Hararghe, Ethiopia (1.56%), Gamal *et al.* (2014) in Egypt (0.26%) and Sajid *et al.* (2019) in Anhui province, China (3.9%). Soliman *et al.* (2018) in Dokki-Giza, Egypt detected higher percentage (81.74%). Aggad (2003) found that RBPT is more efficient and sensitive in screening and detection of Brucella infection. This

indicates that RBPT remains the most reliable serological test for large-scale surveillance and eradication purpose. The prevalence by using BAPA was 25.6% which was higher than that detected by Montasser *et al.* (2011) at South provinces of Egypt (5.0%) and lower than Soliman *et al.* (2018) in Dokki-Giza, Egypt (82.99%). By using MRT the prevalence was 23.7% which was higher than the recorded by Sajid *et al.* (2019) in Anhui province, China (11.67%), Islam *et al.* (2010) in Bangladesh (13.64%), Azza (2006) in Omdurman (9.28%), Musa (2006) in Darfur State (14.3%), Khuzaima *et al.* (2018) in Gedarif State (0.0%) and Nisreen (2006) in Khartoum North (16.0%).

In this study many risk factors were studied and the results showed that the prevalence rates of Caprine Brucellosis according to age were 53.9%, 6.3% and 26.4% by using RBPT, BAPA and MRT respectively in age less than 12 months and 23.3%, 0.9% and 68.6% by using RBPT, BAPA and MRT respectively in age more than 12 months. While the rates were 62.5%, 25.2% and 23.7% by using RBPT, BAPA and MRT respectively in females and 55.5% and 28% by using RBPT and BAPA respectively in males. The findings disagreed with Tigist *et al.* (2011) in Southern Ethiopia who found that sex has no effect on the prevalence of Brucellosis. However, Tigist *et al.* (2011) found that the prevalence of Brucellosis in aged females was 6.36% and 11.67% by RBPT and MRT respectively and 0.0% in young males and females (up to 9 months) and this disagreed with or findings. Islam *et al.* (2010) in Bangladesh found that aged goats (up to 4 years) had lower prevalence of Brucellosis (3.70%) than the age over 4 years (12.5%) which disagreed with our findings. Saikia *et al.* (2018%) in Assam State of Indian found that the prevalence of Brucellosis was higher in females (1.94%) than in males (1.06%) and this agree with our findings when used RBPT and disagree with BAPA. In relation to age, seroprevalence was highest in animals above 18 months (2.30%). Also disagree with waffa *et al.* (2016) in Baghdad, Iraq who found high percentage of disease in goat male which were 60% and agree with Maymona *et al.* (2013) in Abu Dhabi-UAE who found higher prevalence in females than males. According to area the prevalence was found to be 46.6% in EnNahud and 70.7% in areas around Al Nuhod. In this study the comparisons between tests used indicated that the prevalence was the highest when using RBPT and lower while using BABA the difference was significant (p-value 0.002) and not significant between RBPT and MRT (p-value 0.355).

CONCLUSION

Caprine Brucellosis prevalence varies based on the test used. The prevalence was high by using RBPT. Additionally, higher prevalence was observed in the areas around Al Nuhod compared to Al Nuhod Town. The disease was more prevalent in females and those aged over 12 months, indicating a significant association with Caprine Brucellosis seropositivity. The study emphasizes the importance of considering local conditions and test efficiency in prevalence studies.

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