Prevalence and Associated Risk Factors of Bovine Babesiosis in West Kordofan State, Sudan

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SUMMARY

Bovine babesiosis is a tick-borne disease with great economic impacts on the cattle sector globally, caused by *Babesia* species and transmitted by *Rhipicephalus* and Ixodes ticks. This study aimed to determine its prevalence and risk factors in West Kordofan State, Sudan. A cross-sectional study was conducted from June to December 2022. Blood samples from 154 cattle were randomly collected from Mayram (n=50), Muglad (n=54), and Debab (n=50). *Babesia* spp. were detected using microscopic examination of Giemsa-stained blood smears, and risk factor data were collected thorough a structured questionnaire. The overall prevalence of Babesia spp. Infection was 37.0%. prevalence was highest in Mayram (48.0%), followed by Muglad (35.2%) and Debab (28.0%). Univariate analysis revealed significant associations with age, purpose of raising, cross-border movement, treatment history, and tick infestation. Multivariate analysis identified strong associations with cattle age (OR = 3.048, 95% CI: 1.219-7.621), cross-border movement (OR = 11.912 95% CI: 2.627-54.011), and lack of previous antiparasitic treatment (OR = 2.706). No significant associations were found for location, sex, breed, body condition, herd size, grazing system, or owner awareness. Bovine babesiosis is highly prevalent in West Kordofan. Key risk factors include cattle age, cross-border movement, and no prior treatment. The study recommends movement restrictions, regular antiparasitic treatments, improved veterinary surveillance, and molecular diagnostic methods for accurate species identification.

Keywords: Bovine babesiosis; *Babesia* species; cattle disease; risk factors; prevalence; tick-borne diseases; Sudan.

Article History

***Submitted: 20 April* 2026**
Revised: 15 May 2026
Accepted: 6 June 2026
Published: 3 July 2026

Tanzania Veterinary Journal Vol. 41(2)2026
https://doi.org/10.4314/tvj.v41i2.1
ISSN: 0856 - 1451 (Print)
ISSN: 2714-206X (Online)
https://tvj.sua.ac.tz

INTRODUCTION

Bovine babesioses are economically important tick-borne hemoprotozoan diseases with global distribution and some of them with zoonotic challenges (Ozubek *et al.*, 2020). They are caused by

parasites of genus *Babesia*, transmitted primarily by *ixodid* ticks during blood feeding (Abdela et al., 2016). Among the pathogenic species, *B. bigemina* is the most widespread, while *B. bovis* is the most virulent (Jacob *et al*., 2020). The major vectors include *Rhipicephalus microplus*, responsible for transmission in tropical and subtropical regions and other species such as *Haemaphysalis spp*. (WOAH, 2022).

Clinical disease is often characterized by fever, anorexia, and a tendency for affected animals to isolate themselves from the herd. Additional signs may include weakness, depression, reluctance to move, hemoglobinuria, and anaemia, while central nervous system involvement is more prevalent in cattle infected by B. bovis (WOAH. 2022). Diagnosis of bovine babesioses traditionally relies on microscopic examination of Giemsa-stained thin blood smears, which remains widely used field diagnostic method due its cost-effectiveness and rapid results (Bock *et al*., 2004; WOAH 2023). However, this method exhibits limited sensitivity in chronic infections and often fails to detect sub-clinical carrier animals (Criado-Fornelio *et al*., 2009). Complementary diagnostic approaches include serological and molecular techniques, which offer enhanced sensitivity and specificity for parasite detection and species identification (Sharma *et al.*, 2013).

The disease imposes major economic losses through reduced milk, meat production, control costs, while also restricting international cattle trade, and via cattle mortality (Abdelmoen *et al.*, 2020; Bock *et al.*, 2004). Sudan is home to one of Africa's largest livestock populations, estimated at 149 million (Wilson, 2018), though more recent figures suggest 140 million (SUNA, 2025). West Kordofan State, one of Sudan's 18 states, covers an area of 111,373 km² and held a population of approximately 1.3 million residents (WKU, 2025; Gadalkareem *et al*., 2017). The state is recognised as one of the country's most important livestock-producing regions, housing an estimated 25 million animals, of which 5.9 million are cattle (MARF 2020). The climate patterns vary from one area to another and from season to season. The wet season lasts at least five months, from June to October, characterized by high rainfall, while the remaining months are dry seasons with generally high temperatures. The regional economy is predominantly based on pastoralism and rain-fed agriculture, with cattle, sheep and goat production serving as primary livelihood sources (Fadlelmoula *et al*., 2007; IGAD, 2007). Seasonal livestock movement, coupled with cross-border trade, increases the risk of disease spread (El Sammani, 1993).

Although bovine babesioses has been reported in several parts of Sudan, including Darfur and North Kordofan (Abdelmoen *et al.*, 2020; Idris *et al.*, 2018; Shuaib *et al.*, 2015; Alkareem *et al.*, 2012), no detailed epidemiological studies have been conducted in West Kordofan despite its strategic importance for cattle production and trade. This lack of data hampers the design of effective control and prevention measures. Therefore, the present study was conducted to (1) estimate the prevalence of bovine babesioses in three major localities of West Kordofan State (Mayram, Muglad, and Debab), and (2) identify risk factors associated with its occurrence. Findings from this study will provide critical epidemiological evidence to guide disease control strategies and livestock health policies adapted to the region.

MATERIALS AND METHODS

Study area

The study was carried out in three localities: Mayram, Muglad, and Debab in West Kordofan State, Sudan (Figure 1). This area (formerly Greater Abyei Locality) is situated in the southwestern region of West Kordofan State, bordered by Al Salam Locality to the north, East Darfur to the west, South Kordofan to the east, and South Sudan to the south (Braima, 2006; Alkareem *et al*., 2012). The region is characterized by diverse ecosystems including forests, valleys, and natural water sources such as Buta, Rahad, and Bahr al Arab rivers in the southern area. Sad Al Wadi dam serves as the primary water reservoir in Muglad. The area features numerous livestock markets and traditional migration routes (Masarat) facilitating north-south animal movement patterns.

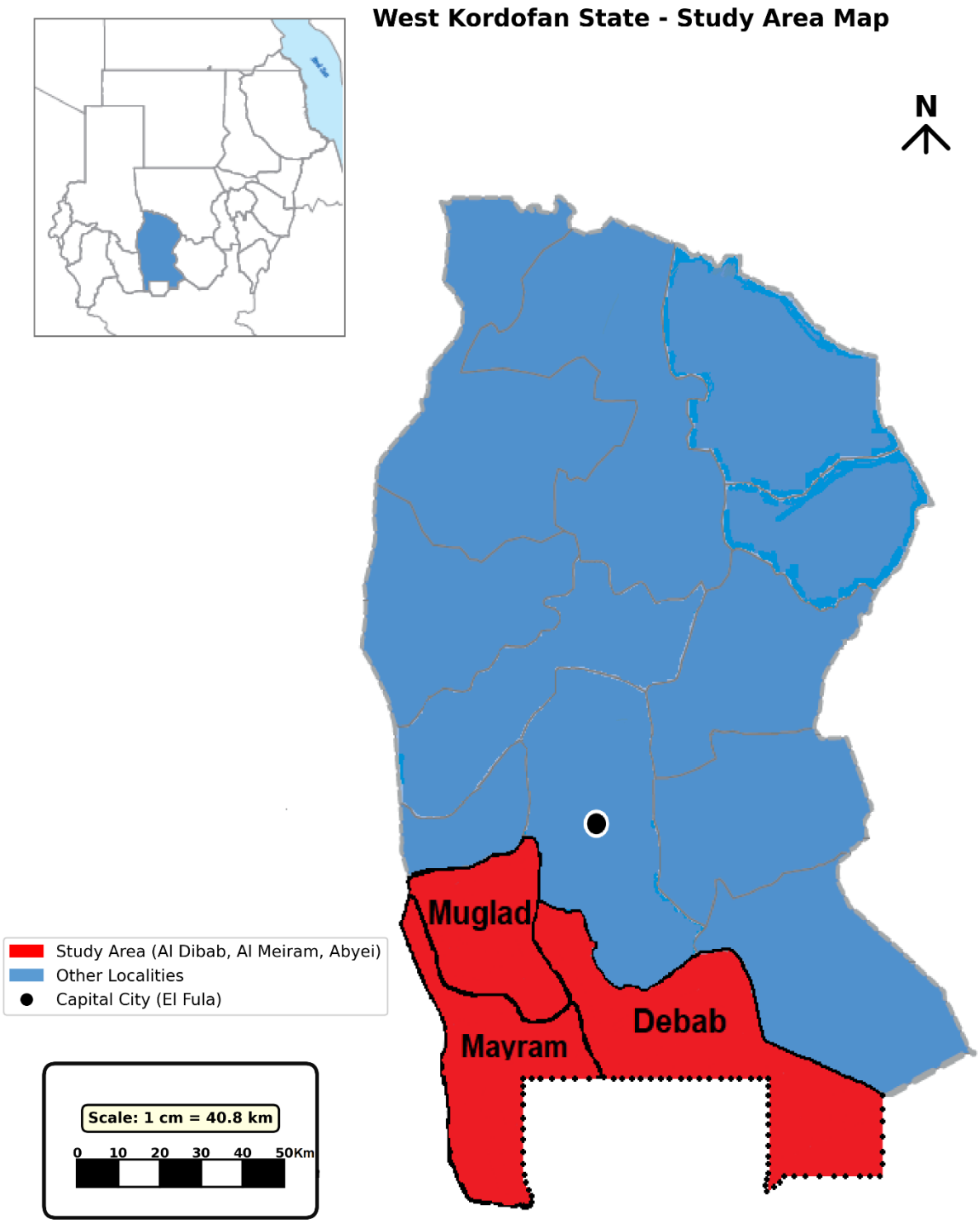

Figure 1: Map of Sudan, West Kordofan State showing the study area including Mayram, Muglad (Abyei), and Debab. The map modified form (OCHA, 2022)

Study design

A cross-sectional study was conducted from June to December 2022 to estimate the prevalence of bovine babesioses and identify potential risk factors associated with the infection in the study area.

Sampling method

A three-stage random sampling approach was employed to select study animals across the three target localities (Mayram, Muglad, and Debab). Stage one involved purposive selection of localities based on cattle population density; stage two

comprised random selection of five villages/areas, two herds of each were selected per locality; and stage three, of identified herds random selection of five cattle of all age, sex, and breed was done. Samples were collected from settled and nomadic herds to ensure representative sampling process.

Sample size determination

The sample size was calculated using the formula outlined by Thrusfield, (2005):

$$n=\frac{(1.96)2\times Pexp\times(1\text{-}Pexp)}{d^2}$$

The expected prevalence of bovine babesioses (5.2%) was obtained from Shuaib *et al.,* (2015) article. To enhance the precision, the sample size was doubled (Thrusfield, 2005), resulting in a target of 152 samples. A total of 154 animals were ultimately sampled.

Sample collection and blood smear preparation

Blood samples were collected from 154 cattle distributed across Mayram (n=50), Muglad (n=54), and Debab (n=50). The prepared smears were air-dried, fixed in 70% methanol for 20 minutes, and stored in slide boxes. All samples were transferred to the Parasitology Laboratory at the College of Veterinary Medicine, Sudan University of Science and Technology. Slide were prepared according to technique presented at CDC (2020).

Microscopic blood examination

Fixed blood smears were stained using 10% Giemsa solution for 15 minutes, rinsed with distilled water to remove excess stain, and allowed to dry. Examination was performed under a 100× oil immersion lens to detect *Babesia* parasites based on their morphological features. At least 300 microscopic fields were examined before considering negative case. Two independent examiners reviewed each slide to confirm results, in case of disagreement a third examiner was consultant.

Risk factor Investigation

A structured questionnaire was administered to livestock owners and/or herders to collect information on potential risk factors associated with bovine babesiosis occurrence, it was translated to Arabic language, the questionnaire was pre-tested, the investigators who implement the questionnaire were received two day workshop training, and validated with 20 cattle owners prior to the study. and the information included: (1) Demographic factors including animal age category (≤5 years vs ≥6 years), sex (male vs female), and breed (Baggara, Butana, Kenana, and crossbred); (2) Management factors with primary purpose of cattle rearing (milk production, meat production, or social/cultural purposes), management system (rural sedentary, nomadic, or semi-intensive farm), and herd size categorized as small (≤25 animals), medium (26-75 animals), or large (≥76 animals); (3) Health and treatment factors comprising history of treatment for blood parasites (treated vs untreated), visible tick infestation status (present vs absent), and body condition score assessed using a 5-point scale (1=poor, 2=moderate, 3=good, 4=very good, 5=excellent); (4) Environmental and geographic factor such as study locality (Mayram, Muglad, or Debab), presence of forested areas near grazing sites (yes vs no), presence of other animal species in the herd (yes vs no), and owner awareness of blood parasite diseases (aware vs unaware); and (6) Movement factors including history of cross-border movement to South Sudan within the previous 12 months (crossed vs not crossed).

Statistical analysis

The data of this study were statistically analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Univariate analysis of categorical variables was conducted using Chi-square test. In addition, to identify independent risk factors a multivariate logistic regression analysis was employed on variables that demonstrated statistical significance in the univariate analysis ($p \leq 0.05$). The strength of association was quantified using odds ratios (OR) with 95% confidence intervals (CI). Statistical significance was set at $p \leq 0.05$.

RESULTS

Overall prevalence of bovine babesioses

Out of 154 examined cattle blood samples, 57 sample tested positive for *Babesia* spp., giving an overall prevalence of 37.0% (95% CI: 29.4-45.2%). While there were no statistically significant differences of the disease prevalence among cattle on the three localities; the highest prevalence recorded on Mayram followed by Muglad, and Debab with an estimated prevalence rates of 48.0% (95% CI: 33.7-62.6%), 35.2% (95% CI: 22.7-49.4%), and 28.0% (95% CI: 16.2-42.5%); respectively(Table 1).

Table 1: Prevalence of Babesia infection in cattle by locality in West Kordofan State, Sudan

Locality	Total examined	Positive cases	Prevalence%	95% CI
Mayram	50	24	48	33.7-62.6
Muglad	54	19	35.2	22.7-49.4
Debab	50	14	28	16.2-42.5
Total	154	57	37	29.4-45.2

CI, confidence interval

Univariate analysis of risk factors

Univariate analysis identified several risk factors significantly associated with bovine babesioses infection (Table 2). Five variables showed significant associations ($P \leq 0.05$): animal age, primary purpose of cattle rearing, cross-border movement history, treatment history, and tick infestation status.

Multivariate analysis

Variables with *P*-values ≤0.05 in Univariate analysis were included in the multivariate logistic regression model. The final multivariate model identified three independent risk factors significantly associated with Babesia infection (Table 3): animal age ($P=0.017$), cross-border movement ($P<0.001$), and previous anti-parasitic treatment history ($P=0.036$).

Table 2: Univariate analysis of risk factors associated with bovine Babesia infection in West Kordofan State, Sudan

Risk factors	Category	Total	Positive cases, n (%)	χ^2	*P-value*
Age group (years)	≤ 5	65	16 (24.6)	7.415	0.006*
	≥ 6	89	41 (46.1)		
Rearing purpose	Dairy	20	5 (25.0)	31.538	0.000*
	Beef	80	16 (20.0)		
	Social/cultural	54	36 (66.7)		
Cross-border movement	Yes	52	39 (75.0)	48.595	0.000*
	No	102	18 (17.6)		
Previous anti-parasitic treatment history	Treated	87	22 (25.3)	11.793	0.001*
	Untreated	67	35 (52.2)		
Tick infestation	Yes	74	35 (47.3)	6.463	0.011*
	No	80	22 (27.1)		
Location	Mayram	50	24 (48.0)	4.409	0.110
	Muglad	54	19 (35.1)		
	Debab	50	14 (28.0)		
Sex	Male	61	23 (37.7)	0.021	0.885
	Female	93	34 (36.6)		
Breed	Baggara	103	36 (35.0)	2.704	0.433
	Kenana	20	8 (40.0)		
	Butana	27	10 (37.0)		
	Crossed breed	4	3 (75.0)		
Body condition score	Poor	65	26 (40.0)	2.401	0.493
	Moderate	35	13 (37.1)		
	Good	42	16 (38.1)		
	Very good	12	2 (16.7)		
Herd size	Small (≤ 25)	39	15 (38.5)	0.927	0.629
	Medium (26-75)	42	13 (31.0)		
	Large (≥ 76)	73	29 (39.7)		
Management system	Sedentary	45	14 (31.1)	1.695	0. 429
	Nomadic	88	37(42.0)		
	Semi-intensive	21	6 (28.5)		
Forests presence	Yes	102	37 (36.3)	.790	0.462
	No	52	20 (38.5)		
Presence of other animal species	Present	118	47 (39.8)	1.719	0.190
	Absent	36	10 (27.8)		
Awareness	Aware	74	22 (29.7)	3.241	0.072
	Unaware	80	35 (43.8)		

*$P \leq 0.05$ indicates statistical significance; *df*, degrees of freedom; n, number of animals. χ^2 test was used for all comparisons.

Table 3: Multivariate logistic regression analysis of independent risk factors for *Babesia* spp. infection in West Kordofan State, Sudan

Risk factor	Category	Positive n (%)	Adjusted OR	95% CI	*P-value*
Age group (years)	≤5	16 (24.6)	-	-	-
	≥6	41 (46.1)	3.048	1.219-7.621	0.017*
Rearing purpose	Milk production	5 (25.0)	-	-	-
	Meat production	16 (20.0)	2.095	0.495-8.876	0.315
	Social/cultural	36 (66.7)	2.527	0.914-6.987	0.074
Cross-border movement	No	18 (17.6)	-	-	-
	Yes	39 (75.0)	11.912	2.627-54.011	<0.001*
Previous antiparasitic treatment history	treated	22 (25.3)	-	-	-
	Not treated	35 (52.2)	2.706	1.066-6.827	0.036*
Tick infestation	Absent	22 (27.5)	-	-	-
	Present	35 (47.3)	0.461	0.190-1.122	0.088

**$P \leq 0.05$ indicates statistical significance; *OR*, odds ratio; *CI*, confidence interval

DISCUSSION

The overall prevalence of 37.0% observed in this study indicates that bovine babesiosis is highly endemic in West Kordofan State. This finding aligns with recent studies from similar ecological zones, including a 37.0% prevalence reported in Iran (Hasheminasab *et al.*, 2018), suggesting comparable epidemiological patterns in semi-arid pastoral systems. However, our results substantially exceed previous estimates from the broader Kordofan region, where prevalences of 5.1% in Abyei area (Alkareem *et al*., 2012) and 5.2% in North Kordofan (Shuaib *et al*., 2015) were reported. This seven-fold increase may reflect temporal changes in vector populations, climate patterns, or livestock management practices over the past decade.

Conversely, our findings were lower than those reported in Sudan's Darfur region, where prevalences of 59.3% and 58.5% were documented in South and North Darfur, respectively (Abdelmoen *et al.*, 2020; Idris *et al*., 2018). These regional variations likely reflect differences in ecological conditions, vector species composition, seasonal rainfall patterns, and livestock management intensity. The observed prevalence range across our study sites (28.0-48.0%) suggests significant spatial heterogeneity, potentially related to local environmental factors and livestock density patterns.

The variance in overall prevalence of bovine babesiosis could be related to environmental factors, genetic variation, and seasonal changes. The presence of infected vectors near susceptible animals, as well as the degree of interaction between vectors and animals, influences fluctuations in *Babesia* parasite prevalence (Nasir *et al*., 2000; Homer *et al*., 2000).

Univariate and multivariate analyses revealed a significant association between disease prevalence and cattle age, with a higher prevalence in older animals (>5 years) (P=0.006 in univariate analysis; OR=3.048, P=0.017 in multivariate analysis). These results are consistent with findings by Hamsho *et al*. (2015) and Abdela *et al.* (2018), who found that

cattle of advanced age were more susceptible to bovine babesiosis. However, this conclusion contradicts the findings of Zulfiqar *et al*. (2012), who showed that calves were more infected with *B. bovis* (28%) than adult animals (15%). This variance can be attributed to two factors. First, young animals have been demonstrated to be less susceptible to *Babesia* infection in endemic areas due to passive immunity (James, 1988). Second, older animals are more likely to have been exposed to the disease and have a higher rate of tick infestation than younger animals (Homer *et al*., 2000).

In the current study, cattle reared for social reasons exhibited a higher prevalence of bovine babesiosis (66.7%) than cattle reared for milk (25.0%) or meat (20.0%) (P<0.001). Although limited studies have examined the association between rearing purpose and blood parasite prevalence, our findings are consistent with research linking blood parasites to animals raised for milk production (P=0.011) (Michael *et al*., 1989; Safieldin *et al.,* 2011). We hypothesize that cattle raised for milk production or social purposes spend more time in herds than cattle raised for meat production. Furthermore, the highest bovine babesiosis prevalence among cattle maintained for social purposes could be attributed to lack of veterinary services (Foil *et al*., 2004), traditional management practices common in the study area, and migration into endemic areas (WOAH, 2022). However, rearing purpose was not independently significant in multivariate analysis (P=0.074), suggesting confounding with other factors.

A significant association was found between disease prevalence and antiparasitic treatment history in both univariate (P=0.001) and multivariate analyses (OR=2.706, P=0.036). Bovine babesiosis was more prevalent in untreated cattle (52.2%) than in cattle treated with antiparasitic medications (25.3%) (P=0.001). This finding contradicts results from Shuaib *et al.* (2015) and Hamsho *et al*. (2015), who found no significant link between treatment and infection. This disparity could be attributed to variations in medication types used (Foil *et al*., 2004), as well as differences in animal owners' understanding of proper drug usage and financial constraints (Yusuf, 2017).

Furthermore, our findings revealed that cattle infested with ticks had a higher prevalence rate than non-infested cattle (P=0.011). This finding is consistent with other research demonstrating a strong relationship between tick infestation and *Babesia* infection (Idris *et al.,* 2018; Maiju, 2005; Abdela *et al.,* 2018). However, tick infestation was not independently significant in the multivariate model (P=0.088), likely because it is confounded with cross-border movement and lack of acaricide treatment.

In the current study, 75.0% of cattle that crossed into South Sudan tested positive for bovine babesiosis, compared to 17.6% of animals that did not cross the border. Both univariate (P<0.001) and multivariate analyses (OR=11.912, P<0.001) demonstrated a significant association between border crossing and bovine babesiosis infection. Although we currently have no information about the area beyond the border, we presume that herds did not receive veterinary care there. Furthermore, these animals may have come into contact with infected animals, whether domestic or wild.

In this study, males had a slightly higher infection rate (37.7%) than females (36.6%), although the difference was not statistically significant (P=0.885). This finding was consistent with Idris et al. (2018), who found higher prevalence of babesiosis in male cattle (65.3%) than female cattle (58.2%). Conversely, this contrasts with Kocan *et al*. (2010), who found higher prevalence in female cattle (11.2%) than males (6.96%). The difference in our results could be due to hormonal differences between males and females *(Travi* et al., 2002); however, more investigation is needed to determine the relationship between animal sex and bovine babesiosis.

The highest prevalence was recorded in Mayram (48.0%), followed by Muglad (35.2%) and Debab (28.0%), although these differences were not statistically significant (P=0.110). This finding was consistent with Alkareem *et al.*, (2012), who observed that Mayram had a greater prevalence of bovine babesiosis than other locations in the southern section of West Kordofan (8.82%). This observation could be attributed to the concentration of animals of various species in the Mayram area during the wet season. Furthermore, most cattle herds relocated to Bahr al Gazal and Bahr al Arab, where no veterinary services were accessible.

This study discovered that crossbreeds had a higher prevalence of bovine babesiosis (75.0%) than local breeds (Baggara, Kenana, and Butana); however, this difference was not statistically significant (P=0.433). This outcome is in alignment with the fact that Bos taurus cattle are more susceptible than Bos indicus (zebu) cattle to babesiosis (Patterson *et al.,* 2017). Furthermore, Kenana cattle were found to be the most susceptible to bovine babesiosis among local breeds, with a prevalence of 40.0%, whereas

Baggara cattle were found to be more resistant (35.0%). This finding could be attributed to the fact that the indigenous breed develops inherent resistance to disease (Bock *et al.*, 1999).

Regarding body condition score, disease prevalence was 16.7%, 38.1%, 37.1%, and 40.0% for very good, good, moderate, and poor scores, respectively, with no significant relationship between disease and body condition (P=0.493). This finding is similar to previous research by Hamsho *et al*. (2015), Idris *et al*. (2018), and Abdela *et al.* (2018). This may be because animals in poor physical condition have weaker immune resistance, making them more vulnerable to infections by various parasites, including Babesia (Idris *et al*., 2018).

The current study found that large herd sizes had higher disease prevalence (39.7%) than small (38.5%) and medium herd sizes (31.0%); however, these differences were not statistically significant (P=0.629). This result agrees with previous research by Simking *et al*. (2014). This finding may be attributed to overpopulation within large herds and ineffective management practices. Furthermore, in nomadic environments, all animals feed together. Consequently, sharing grazing areas can increase tick spread, thereby elevating Babesia infection risk (Simuunza *et al.*, 2011).

The current study found that cattle raised without other animals in the same herd were more likely to be infected with babesiosis, with a prevalence of 38.5%compared to 36.3% in herds with other animal species, although this difference was not statistically significant (P=0.462). This finding contrasts with Theodoropoulos *et al.* (2006), who found that presence of other animals is a risk factor for babesiosis transmission. Although forest presence was not statistically associated with bovine babesiosis (P=0.190), the findings revealed higher infection prevalence in cattle grazing near forested areas (39.8%) than those without forest proximity (27.8%). This finding is consistent with the Maine Forest Tick Survey (MFTS, 2022), which stated that "tick habitats are associated with forest and forest edges."

Finally, prevalence of bovine babesiosis was lower in cattle owned by people with knowledge about blood parasite diseases (29.7%) than those without (43.8%); however, this difference was not statistically significant (P=0.072). Increasing knowledge about animal diseases among owners improves management practices and hygiene. Conversely, lack of disease knowledge can delay diagnosis and treatment (Zintl *et al.*, 2014).

CONCLUSIONS

This study establishes that bovine babesiosis is highly prevalent (37.0%) in West Kordofan State, Sudan, with three independent risk factors significantly associated with disease occurrence: advanced cattle age (>5 years, OR=3.048), cross-border movement to South Sudan (OR=11.912), and lack of previous antiparaitic treatment (OR=2.706). These findings provide critical baseline epidemiological data for this strategically important livestock-producing region and emphasize the urgent need for targeted interventions including: implementation of effective tick control strategies and integrated pest management; molecular diagnostic studies to identify specific Babesia species and detect subclinical infections; efficient border surveillance with mandatory screening and treatment protocols for cattle movement between Sudan and South Sudan; establishment of regular antiparasitic treatment programs targeting high-risk animals; capacity building through extension programs to increase owner awareness of disease recognition and proper management practices; and strengthening of veterinary service delivery in remote pastoral areas, particularly along migration routes and international borders.

Study limitations:

Firstly, the study only used Giemsa Stained smear which is less sensitivity than Molecular and serology techniques, so, the true prevalence may be higher than reported due to the limited sensitivity of microscopic examination in detecting chronic or sub-clinical infections and carrier infections. Secondly, the findings did not differentiate between B. bigemina spp. and B. bovis spp. and anther Babesia spp. that affects cattle in Sudan. Third, Cross-sectional design Prevents establishing temporal or causal relationships; can only describe associations at one time point, not direction or cause of infection. Ultimately, lack of molecular confirmation, which is make the findings less validate accurate.

AUTHORS`S CONTRIBUTIONS

Suleiman A. I. Hano and Sara B. Mohammed designed study, analyzed samples,analyzed data statistically, and wrote the manuscript draft. Mohammed I. A. Ibrahim, and Edris B. G. Alkareem revised the manuscript draft, designed the maps, and edited the text. All authors reviewed and approved the final version of manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors acknowledge the Lab of Parasitology, College of Veterinary Medicine, Sudan University of Science and Technology staff for their support and collaboration during samples analysis. Also, the authors acknowledge Prof. Adama Sow, the former ICRC livestock delegation for Sudan, for his valuable contribution on designing the questionnaire and during samples collection process.

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