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Seroprevalence of *Babesia bigemina* antibodies in cattle in North Kordofan state, the Sudan

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Abstract: *This cross-sectional study was conducted to elucidate the prevalence of anti-babesia antibodies in cattle in North Kordofan state, the Sudan. A total of 803 thin blood smears and serum samples were collected from January to December 2008, and examined using blood smear technique (BST) or microscopy, indirect-Immunofluourescent antibody test (iIFAT) and indirect-enzyme linked immunosorbent assay (iELISA). The overall prevalences were 5.20% (CI = 95%, 3.88 - 6.52), 68.7% (CI = 95%, 60.40 - 77.00) and 28.4% (CI = 95%, 25.14 - 31.66), respectively. In the univariate analysis, season (chi-square = 11.908, p-value = 0.003), locality (chi-square = 30.412, p-value = 0.000) and breed (chi-square = 9.426, p value = 0.05) were significantly associated with BST-positive status, whereas, locality (chi-square = 18.050, p-value = 0.001) and breed (chi-square = 17.546, p-value = 0.002) were significantly associated with positive-iELISA for B. bigemina. The factors that were significantly associated with odds of BST-positivity for babesia-infection in the multivariate analysis were rainy (Exp(B) = 5.37, p≤0.015) and hot (Exp(B) = 5.53, p≤0.015) seasons, and Baggara (Exp(B) = 10.82, p≤0.035) and cross (Exp(B) = 9.626, p≤0.044) breeds. For iELISA-positivity, were Al-Rahad (Exp(B) = 2.761, p≤0.001), Al-Nihood (Exp(B) = 1.818, p≤0.022), and Al-Khoway (Exp(B) = 1.720, p≤0.035) localities. Also, Kenana (Exp(B) = 2.909, p≤0.002), Baggara (Exp(B) = 3.136, p≤0.001), cross (Exp(B) = 3.867, p≤0.000), and Foga (Exp(B) = 3.626, p≤0.001) breeds, in addition to the age group >2 – 3 years old (Exp(B) = 2.280, p≤0.000) and male animals (Exp(B) = 1.684, p≤0.006). It can be concluded that antibodies against bovine babesiosis were prevalent in North Kordofan state and the agreement between the BST and the iELISA was slight (0.13). Epidemiological studies on babesiosis, in the state and the whole country, are warranted, as well as the molecular characterization of the parasite.*

Keywords: *Seroprevalence, antibodies, babesia, cattle, iELISA, Sudan*

1. INTRODUCTION

The Sudan has a hug wealth of animal resources. It has an estimated population of cattle of about 41.43 million heads (Anon, 2008). The size of this cattle population is ranking as the second biggest in Africa and the sixth worldwide, and breeds are either indigenous ecotypes of zebu cattle which include Foga, Baggara, Kenana, Gaash and Butana or cross-breeds of these ecotypes with exogenous breeds mainly Friesian, and others like Ayrshire and Holstein (Fadlelmoula *et al.*, 2007). In the Sudan, nomads raise various species of domesticated animals as dictated by ecological conditions; in Kordofan they are either cattle herders, whom are locally known as Baggara or camel herders or the so-called Abbala. These two nomadic pastoralism types are distinct from each other in production systems and culture. Baggara raise basically cattle with few sheep and goats, however, Abbala usually have camels and sheep (IGAD, 2007). In the Sudan, the largest population of cattle is in Kordofan (IGAD, 2007; Anon, 2008).

Babesiosis is one of the most not uncommon infections of free-ranging animals. It is a cosmopolitan disease and recently acquiring more attention and interest as an emerging zoonosis (Homer *et al.*, 2000). The disease is caused by the intraerythrocytic parasites of the genus *Babesia.* In cattle the protozoan parasites *B. bovis* and *B. bigemina* are the most important species (Homer *et al.*, 2000; Bock *et al.*, 2004; Radostits *et al.*, 2007; OIE, 2008). But infections with *B. divergens*, *B. major*, *B. ovate*, and *B. jakimovi* were also detected (Radostits *et al.*, 2007; OIE, 2009). Bovine babesiosis covers vast areas of Africa and other parts of the world (Bock *et al.*, 2004; Radostits *et al.*, 2007; OIE, 2008). The disease is transmitted by ticks; *Rhipicephalus microplus* is the principal vector of *B. bovis* and *B. bigemina*. This tick is widespread in the tropics and subtropics. Other important vectors include *Ixodes ricinus*, *Haemaphysalis* species, *Rhipicephalus* species and members of the subgenus *Boophilus* (Homer *et al.*, 2000; Bock *et al.*, 2004; Radostits *et al.*, 2007; OIE, 2008; OIE, 2010). For the proper diagnosis of bovine babesiosis thorough case history should be taken and subsequent analysis must include examination of stained blood smears with light microscope as it can give a definitive clue for the infection when the parasite is detected within the stained blood films. However, parasitemias are often too low in subclinical and chronic infections for microscopic diagnosis. Due to this reason, several serological assays like indirect immunofluorescent antibody test (IFAT) and enzyme linked immunosorbent assay (ELISA) have to be used to detect antibodies in infected cattle (Levine, 1988; Homer *et al.*, 2000; Radostits *et al.*, 2007). Prevention and control of bovine babesiosis is by means of 1- chemotherapy by injecting drugs like diminazene aceturate, amicarbalide, and imidocarb intramuscularly (Radostits *et al.*, 2007), 2- tick control by regular dipping (Norimine *et al.*, 2002; Norimine *et al.*, 2003), and 3- immunization by administration of babesiosis vaccines (Echaide *et al*., 1993; de Waal, 1996; de Waal and Combrink, 2006).

Development and exploitation of cattle population in Kordofan state are challenged by many constrains including the prevalence of many infectious and parasitic diseases. Tick-borne diseases (TBDs) like babesiosis and trypansomiasis are among those diseases. According to the data viewed from the records of the veterinary clinic of the veterinary teaching hospital of El-Obeid, North Kordofan, and it's diagnostic laboratory, babesia-infection was prevailing in cattle in the state, regularly since 1999 up to the time of conducting this study, in a rate of at least three cases per month. This highlighted the need to investigate the prevalence of the disease and to study the individual risk factors that contribute to the spread of the disease.

2. MATERIALS AND METHODS

2.1. Study area

The study was carried out in North Kordofan state (Fig. 1), which lies between latitudes 11° 15′ – 16° 45′ N and longitudes 27° 5′ – 32° 15′ E. North Kordofan state has an area of 185,302 Km² and inhabited by nomads and pastoralists. The state is divided into administrative units called localities. Agriculture and livestock comprise about 70% of the economic activities. The state is covered by a wide range of vegetation and green grasses especially in the season of rainfall (Anon, 2008). Soil types are about 55.0% sand or gouze, 20.0% gerdud, 15.0% alluvial land and 10.0% clay land. The annual rainfall is concentrated in a single relatively short summer season from June to September and the amount of rainfall is up to 500 mm. The state is located in the grass- and wood-land savannahs. In rainy seasons, animals are trekked by pastoralists to the northern part of the state while during dry seasons animals are trekked to Bahar Al-Gazal River in South Sudan. A mixture of farming systems are practiced in the state including nomadic, sedentary and semi-sedentary animal production systems. Furthermore, Kordofan has an estimated livestock population of 24,665,761 animals (Anon, 2008; ILRI, 2009).

2.2. Study design and sampling strategy

A cross-sectional study was carried out during the period from January to December 2008 (Thursfield, 2007). As in Fig. 1, four localities, namely; Al-Rahad, Shiekan, Al-Khoway, and Al-Nihood, were conveniently selected as well as herds and individual cattle which were sometimes randomly sampled.

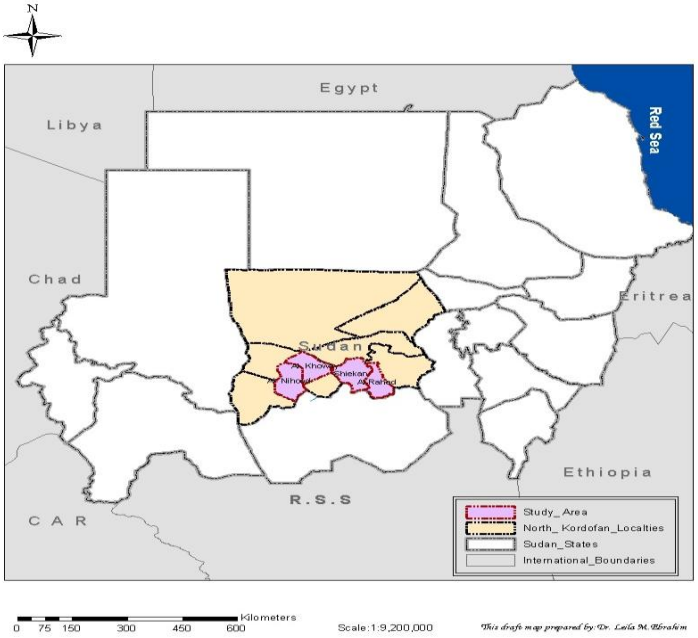

Fig. 1. *Map of the study area*

2.3. Calculation of sample size

The sample size (n) was determined according to Thursfield (2007) by using the following formula

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

Where:

n = required sample size

P_{exp} = expected prevalence = 15% (records of the veterinary clinic, El-Obeid)

d = desired absolute precision = ±5%

$(1.96)^2$ = confidence interval of 95%

According to the aforementioned formula, 195 cattle serum samples were collected from each locality to determine the seroprevalence of babesiosis.

2.4. Collection of samples

Whole blood samples were collected from the jugular vein according to OIE (2010). A total of 803 blood smears were first made. Then the same number of serum samples was prepared by allowing the blood to clot in plain test tubes at room temperature overnight. After that, the plain test tubes containing the clotted blood were centrifuged for 3 minutes at 1500 rpm. Sera were finally separated and put into cryovials after labeling and stored at -20ºC until tested.

2.5. Testing of the collected samples

2.5.1. Blood smear technique (BST) or microscopy

The preparation of Giemsa stained blood smears and viewing them under light microscope (microscopy) were carried out according to Salih *et al.* (2007), Preast (2007), OIE (2008) and OIE (2010).

2.5.2. Detection of antibodies against B. bigemina

The commercial indirect-ELISA (iELISA) kits for detection of anti-*B. bigemina* antibodies were purchased from SVANOVIR® Laboratory (Uppsala, Sweden). The test procedure was carried out according to the manufacturer's instructions.

The cut-off point for the iELISA of *B. bigemina* was calculated using the formula: mean of the optical density of the negative control plus three standard deviations (Wright *et al.*, 1993; Salih *et al.*, 2009). Rogan and Gladen (1978), estimator (P_{RG}) was used to correct the apparent prevalence:

$$P_{RG} = \frac{AP + Sp - 1}{Se + Sp - 1}$$

Where:

AP = apparent prevalence

Sp = specificity

Se = sensitivity

The sensitivity and specificity of the *B. bigemina* iELISA provided by the manufacturer were 96.0% and 97.5%.

2.5.3. Detection of antibodies against B. bovis

The commercial indirect-IFAT (iIFAT) kits for detection of anti-*B. bovis* antibodies were purchased from Fuller Laboratory (Fullerton, California, USA). The test was carried out according to the manufacturer's instructions.

2.6. Data management and statistical analyses

The generated data were initially manipulated on Microsoft® Excel 2007 before being exported to the Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Inc, USA). Appropriate statistical analyses were carried out with a confidence interval of 95% and *p-value* of $p \leq 0.05$ for significance. Descriptive statistics were used to estimate the prevalences. Moreover, hypotheses of differences of location, age group, breed, sex, and season between test-positive and test-negative animals were first tested by univariate analysis using 2-tailed chi-square test. Then in a second step, a logistic regression model was used to assess the association between the potential risk factors, which were significant in the univariate analysis, and the outcome babesia-infection. Potential risk factors with *p-value* of $p \leq 0.25$ in the univariate analysis were entered into the regression model.

3. RESULTS

3.1. The overall prevalences of babesiosis

Generally, the disease under investigation was detected in all of the selected localities with variations observed between different age groups, seasons, breeds and males and females. The overall prevalences were found to be 5.20% (CI = 95%, 3.88 - 6.52) using BST, 68.70% (CI = 95%, 60.40 - 77.00) using iIFAT, and 28.40% (CI = 95%, 25.14 - 31.66) using iELISA to be reduced to 27.7% after adjustment of the misclassification of the iELISA test and correcting the apparent prevalence.

3.2. Prevalences of babesiosis by locality, age, breed, seasons and sex

There were no significant variations at $p \leq 0.05$ between the prevalences estimated across the surveyed localities and among the different age groups, breeds, seasons and between sexes (Table 1). Al-Rahad locality showed the highest prevalences which were 13.1% (CI = 95%, 8.21 – 17.99) and 39.6% (CI = 95%, 32.51 – 46.69), while Al-Khoway and Shiekan localities showed the lowest prevalences which were 2.1% (CI = 95%, 0.05 – 4.15) and 20.0% (CI = 95%, 14.89 – 25.11) using microscopy and iELISA. Animals of the age groups >1 - 2 and >2 - 3 years old showed the highest prevalences (8.2%; CI = 95%, 2.74 - 13.66 and 37.2%; CI = 95%, 26.47 – 47.39) whereas animals of the age group ≤1 years old showed the lowest prevalences (3.9%; CI = 95%, 1.65 - 6.15 and 25.0%; CI = 95%, 19.74 – 30.36). Furthermore, 8.7% (CI = 95%, 4.49 – 12.91) and 33.6% (CI = 95%, 24.65 – 42.55) were the highest prevalences that were detected in cross and Foga breeds but 0.80% (CI = 95%, -0.70 - 3.36) and 12.4% (CI = 95%, 6.32 - 18.48) were the lowest prevalences estimated in Butana breed by using microscopy and iELISA. In addition, 7.6% (CI = 95%, 4.39 – 10.81) and 29.5% (CI = 95%, 23.69 – 32.35) prevalences were reported as the highest prevalences in the rainy and hot seasons whilst 1.2% (CI = 95%, -0.16 – 2.56) and 26.7% (CI = 95%, 23.69 – 35.31) were reported as the lowest prevalences in the hot and cold seasons by using microscopy and iELISA. Females showed the highest prevalences of 7.3% (CI = 95%, 3.47 - 11.13) and 34.2% (CI = 95%, 26.87 - 41.53) using microscopy and iELISA, respectively.

Table 1. *Microscopy and iELISA prevalences of babesia-infection in cattle by individual animal risk factors in North Kordofan state during the period January to December 2008*

Risk factor	No. of samples (%)	No. of positive (%)			
		microscopy with 95% CI		iELISA wit 95% CI	
Locality					
Al-Rahad	183 (22.8)	24 (13.1)	8.21–17.99	72 (39.6)	32.5-46.7
Shiekan	235 (29.3)	9 (3.8)	1.36–6.24	38 (20.0)	14.9-25.1
Al-Khoway	188 (23.4)	4 (2.1)	0.05–4.15	48 (27.7)	21.3-34.1
Al-Nihood	197 (24.5)	5 (2.5)	0.32–4.68	51 (26.7)	20.5-32.9
Age Group					
≤ 1	285 (35.5)	11 (3.9)	1.65-6.15	65 (25.0)	19.74-30.3
> 1 - 2	97 (12.1)	8 (8.2)	2.74-13.7	25 (28.4)	18.98-37.8
> 2 - 3	87 (10.8)	6 (6.9)	1.57-12.2	29 (37.2)	26.47-48.0
≥ 3	334 (41.6)	17 (5.1)	2.74-7.76	90 (29.0)	23.95-34.1
Breed					
Kenana	231 (28.8)	12 (5.2)	2.34-8.06	61 (29.6)	23.37-35.8
Baggara	155 (19.3)	7 (4.5)	1.24-7.76	46 (32.6)	24.86-40.3
Cross	172 (21.4)	15 (8.7)	4.49-12.9	52 (30.8)	23.84-37.8
Foga	120 (14.9)	7 (5.8)	1.62-9.98	36 (33.6)	24.65-42.6
Butana	125 (15.6)	1 (0.8)	-0.76-3.36	14 (12.4)	6.32-18.48
Season					
Rainy	262 (32.7)	20 (7.6)	4.39 –10.81	68 (29.2)	23.36-35.04
Cool	295 (36.7)	19 (6.4)	3.61 – 9.19	71 (26.7)	21.38-32.02
Hot	246 (30.6)	3 (1.2)	-0.16 – 2.56	70 (29.5)	23.69-35.31
Sex					
Male	177 (22.0)	13 (7.3)	3.47-11.13	55 (34.2)	26.9-41.5
Female	626 (78.0)	29 (4.6)	2.06-7.14	154 (26.8)	23.2-30.4

3.3. Univariate association with microscopy and iELISA

The proportions of being BST and iELISA positive for babesia-infection varied between age groups, localities, seasons, breeds and sex categories.

In the univariate analysis using chi-square, to assess the association of babesia-infection and the potential risk factors one by one, season (chi-square = 11.908, *p value* = 0.003), locality (chi-square = 30.412, *p value* = 0.000) and breed (chi-square = 9.426, *p value* = 0.05) were significantly associated with BST-positive status (Table 2).

Table 2. *Univariate association of microscopy positive status with individual animal risk factors in cattle in North Kordofan state during the period January to December 2008*

Risk factor	No. of samples (%)	No. of positive	% positive	Chi square	df	*p-value*
Season				11.908	2	0.003
Rainy	262 (32.7)	20	7.6			
Cool	295 (36.7)	19	6.4			
Hot	246 (30.6)	3	1.2			
Locality				30.412	3	0.000
Al-Rahad	183 (22.8)	24	13.1			
Shiekan	235 (29.3)	9	3.8			
Al-Nihood	188 (23.4)	4	2.5			
Al-Khoway	197 (24.5)	5	2.1			
Breed				9.426	4	0.051
Kenana	231 (28.8)	12	5.2			
Baggara	155 (19.3)	7	4.5			
Cross	172 (21.4)	15	8.7			
Foga	120 (14.9)	7	5.8			
Butana	125 (15.6)	1	0.8			
Age groups (yrs)				3.362	3	0.339
≤1	285 (35.5)	11	3.9			
>1 - 2	97 (12.1)	8	8.2			
>2 - 3	87 (10.8)	6	6.9			
>3	334 (41.6)	17	5.1			
Sex				2.047	1	0.152
Male	177 (22.0)	13	7.3			
Female	626 (78.0)	29	4.6			

The univariate analysis further showed significant association between positive-iELISA status for *B. bigemina* and locality (chi-square = 18.050, *p value* = 0.001) and breed (chi-square = 17.546, *value* = 0.002) (Table 3).

Table 3. *Univariate association of iELISA positive status with individual animal risk factors in cattle in North Kordofan state during the period January to December 2008*

Risk factor	No. of samples (%)	No. of positive	% positive	Chi square	df	*p-value*
Season				0.603	2	0.740
Rainy	262 (32.7)	68	29.2			
Cool	295 (36.7)	70	26.7			
Hot	246 (30.6)	71	29.5			
Locality				18.050	3	0.001
Al-Rahad	183 (22.8)	72	39.6			
Shiekan	235 (29.3)	38	20.0			
Al-Nihood	188 (23.4)	48	27.7			
Al-Khoway	197 (24.5)	51	26.7			
Breed				17.546	4	0.002
Kenana	231 (28.8)	61	29.6			
Baggara	155 (19.3)	46	32.6			
Cross	172 (21.4)	52	30.8			
Foga	120 (14.9)	36	33.6			
Butana	125 (15.6)	14	12.4			
Age groups (yrs)				4.496	3	0.213
≤1	285 (35.5)	65	25.0			
>1 - 2	97 (12.1)	25	28.4			
>2 - 3	87 (10.8)	29	37.2			
>3	334 (41.6)	90	29.9			
Sex				3.368	1	0.066
Male	177 (22.0)	55	34.2			
Female	626 (78.0)	154	26.8			

3.4. Multivariate association with microscopy and iELISA

Results of the logistic regression assessing the collective relationship between season, locality, breed, and sex and being BST positive for babesia-infection are presented in Table 4. The regression coefficients (Exp (B)) express 'odds ratios' (OR) (= the increased or decreased probability (OR ≠1)) of seropositivity occurrence in comparison to the reference (OR = 1). The factors that were significantly associated with odds of BST-positivity for babesia-infection were rainy (Exp(B) = 5.37, 95% CI = 1.38 - 20.86, $p \leq 0.015$) and cool (Exp(B) = 5.53, 95% CI = 1.39 - 21.81, $p \leq 0.015$) seasons and Baggara (Exp(B) = 10.82, 95% CI = 1.17 - 89.74, $p \leq 0.035$) and cross (Exp(B) = 9.626, 95% CI = 1.06 - 87.77, $p \leq 0.044$) breeds.

Table 4. *Multivariate association of microscopy positive status with individual animal risk factors in cattle in North Kordofan state during the period January to December 2008*

Risk factor	No. of samples (%)	No. of positive	Exp(B)	*p-value*	95% CI for Exp(B)
					Lower - Upper
Season					
Hot	246 (30.6)	3 (1.2)	Ref		
Rainy	262 (32.7)	20 (7.6)	5.369	0.015	1.38 - 20.86
Cool	295 (36.7)	19 (6.4)	5.525	0.015	1.39 - 21.81
Locality					
Al-Khoway	197 (24.5)	5 (2.1)	Ref		
Al-Rahad	183 (22.8)	24 (13.1)	2.249	0.191	0.66 - 7.956
Shiekan	235 (29.3)	9 (3.8)	0.621	0.486	0.16 - 2.374
Al-Nihood	188 (23.4)	4 (2.5)	0.692	0.610	0.16 - 2.843
Breed					
Butana	125 (15.6)	1 (0.8)	Ref		
Kenana	231 (28.8)	12 (5.2)	7.439	0.069	0.85 - 64.50
Baggara	155 (19.3)	7 (4.5)	10.82	0.035	1.17 - 89.74
Cross	172 (21.4)	15 (8.7)	9.626	0.044	1.06 - 87.77
Foga	120 (14.9)	7 (5.8)	7.581	0.072	0.83 - 69.08
Sex					
Female	626 (78.0)	29 (4.6)	Ref		
Male	177 (22.0)	13 (7.3)	1.642	0.171	0.80 - 3.343

Results of the logistic regression assessing the collective relationship between localities, breed, age, and sex and being and iELISA positive for babesia-infection are presented in Table 5. Al-Rahad (Exp(B) = 2.761, 95% CI = 1.66 - 4.297, $p \leq 0.001$), Al-Nihood (Exp(B) = 1.818, 95% CI = 1.09 - 3.029, $p \leq 0.022$), and Al-Khoway (Exp(B) = 1.720, 95% CI = 1.03 - 2.849, $p \leq 0.035$) localities. Also, Kenana (Exp(B) = 2.909, 95% CI = 1.05 - 5.632, $p \leq 0.002$), Baggara (Exp(B) = 3.136, 95% CI = 1.59 - 6.174, $p \leq 0.001$), cross (Exp(B) = 3.867, 95% CI = 1.89 - 7.909, $p \leq 0.000$), and Foga (Exp(B) = 3.626, 95% CI = 1.84 - 7.140, $p \leq 0.000$) breeds, in addition to the age group >2 – 3 (Exp(B) = 2.280, 95% CI = 1.26 - 4.106, $p \leq 0.000$) and male animals (Exp(B) = 1.684, 95% CI = 1.19 - 2.901, $p \leq 0.006$) were significantly associated with odds of being iELISA-positive.

Table 5. *Multivariate association of iELISA positive status with individual animal risk factors in cattle in North Kordofan state during the period January to December 2008*

Risk factor	No. of samples (%)	No. of positive	Exp(B)	*p-value*	95% CI for Exp(B)
					Lower - Upper
Locality					
Shiekan	235 (29.3)	38 (20.0)	Ref		
Al-Rahad	183 (22.8)	72 (39.6)	2.761	0.000	1.66 - 4.297
Al-Nihood	188 (23.4)	48 (27.7)	1.818	0.022	1.09 - 3.029
Al-Khoway	197 (24.5)	51 (26.7)	1.720	0.035	1.03 - 2.849
Breed					
Butana	125 (15.6)	14 (12.4)	Ref		
Kenana	231 (28.8)	61 (29.6)	2.909	0.002	1.05 - 5.632
Baggara	155 (19.3)	46 (32.6)	3.136	0.001	1.59 - 6.174
Cross	172 (21.4)	52 (30.8)	3.867	0.000	1.89 - 7.909
Foga	120 (14.9)	36 (33.6)	3.626	0.000	1.84 - 7.140
Age groups					
≤1	285 (35.5)	65 (25.0)	Ref		
>1 - 2	97 (12.1)	25 (28.4)	1.444	0.001	0.80 - 2.585
>2 - 3	87 (10.8)	29 (37.2)	2.280	0.000	1.26 - 4.106
>3	334 (41.6)	90 (29.9)	1.526	0.000	0.98 - 2.366
Sex					
Female	626 (78.0)	154 (26.8)	Ref		
Male	177 (22.0)	55 (34.2)	1.684	0.006	1.19 - 2.901

4. DISCUSSION

The current study has shown that babesiosis, due to *B. bigemina* and *B. bovis* infection, is prevalent in North Kordofan state with prevalences of 5.2%, 27.7% and 68.7% by using BST, iELISA and iIFAT, respectively. Different prevalences of babesia-infection, ranging from 1.75% to 42%, were reported using BST in Ethiopia, Malaysia, Pakistan, and Brazil (Ahmad and Hashmi, 2007; Rahman *et al.*, 2010; Atif *et al.*,2012; Amorim *et al.*, 2014; Hamsho *et al.*, 2015). But contrary, Mtshali *et al*. (2004) found no blood parasites (0/386), including babesia species, in the thick and thin blood smears prepared from cattle in South Africa. Presence of infected vectors together with susceptible animals in the same place as well as the amount of interaction between them and their numbers would result in variations in the detection rate of babesia parasites in blood smears. The sero-prevalence reported herein using iELISA was higher than the overall and by-region seroprevalences reported by Salih *et al.* (2009) in the Sudan. The overall seroprevalence was 10.7% in the whole country while the by-region seroprevalences were 17.5% in Northern Sudan, 11.9% in the Blue Nile, 11.7% in Eastern Sudan, 7.5% in the White Nile, and 6.7% in Western Sudan, in addition to 7.5% in Shiekan locality, North Kordofan (Salih *et al.*, 2009). The prevalence estimated in this study by using iELISA was also higher than those observed by Salih *et al.* (2007) using polymerase chain reaction (PCR) and reverse line blot (RLB). These dissimilarities could be attributed to the different number of animals (sample size or n) involved in each study and to the different ecological characteristics of each study area, too. Moreover, the prevalence reported by using iIFAT was higher than that reported by Anon (1983) who found a prevalence of 58.5% using the same diagnostic technique. This higher seroprevalence of *B. bovis*-infection could possibly be due to the cross-reactivity between babesia and theileria species in this test. The findings of this study concur with the results of previous studies that were conducted in tropical countries. In Eastern and Southern Africa, studies have shown that bovine babesiosis occurred

with a sero-positivity ranging from 19.5% to 94.0% by using different diagnostic techniques (Dreyer *et al.*, 1998; Mbati *et al.*, 2002; Mtshali *et al.*, 2004; Martins *et al.*, 2008; Jonsson *et al.*, 2008).

Risk factors associated with BST-positive status for babesia-infection in the univariate analysis were season, locality, and breed. As did in this study, Atif *et al*. (2012) investigated the relationship of age, sex, breed, and herd size to BST-positive status and found no association between age (chi-square = 7.56; *p-value* = 0.109) and sex (chi-square = 0.63; *p-value* = 0.720) and BST-positive status, not like breed (chi-square = 6.49; *p-value* = 0.039) which was statistically related to babesia infection. Once more, agreeing with the findings of this study, Atif *et al*. (2012) observed that the prevalence of tick-transmitted diseases was significantly dependent on season, where the infection in summer (chi-square = 6.22, *p-value* = 0.044) was greater than in autumn, winter and spring. Hamsho *et al.* (2015) investigated the relationship between Kebele or village, sex, age, body condition, and treatment with BST-positive status. Village (chi-square = 11.41; *p-value* = 0.01) and body condition (chi-square = 35.09; *p-value* = 0.001) showed a significant statistical association with positivity for babesiosis, while sex (chi-square = 0.951; *p-value* = 0.758), age (chi-square = 3.82; *p-value* = 0.148), and treatment (chi-square = 3.16; *p-value* = 0.076) did not. Amorim *et al.* (2014) found no relation between sex (chi-square = 11.06; *p-value* = 0.10) and babesia-infection.

iELISA-seropositivity for *B. bigemina*-infection was statistically associated, by means of univariate analysis, with locality and breed. Likewise, Tembue *et al.* (2011) observed significant association between origin of animals with seropositivity for either *B. Bigemina* or *B. bovis* whereas association between both parasites and sex and age did not occur. Furthermore, 91.5% of the animals investigated for *B. bigemina* by IFAT were sero-positive (chi-square = 92.64, df = 1, prob = 0.001) in relation to the location of animal (Mtshali *et al.*, 2004). Costa *et al.* (2013) found a number of risk factors including method of application of acaricide, presence of horse flies (Tabanidae), use of injectable anti-helminthics and number of animals introduced into the herd as statistically ($p \leq 0.20$) coupled variables with the presence of at least one serologically positive cow for *B. bigemina*. However, divergent from the findings of this study, Amorim *et al.* (2014) could not establish statistical relation between breed (chi-square = 0.21; *p-value* = 0.64) and babesiosis in cattle but did with age (chi-square = 14.98; *p-value* = 0.00).

Multivariate analysis using logistic regression was used for assessing the combined effect of all risk factors that were significant in the univariate analysis with positive BST and cELISA statuses for babesia-infection. Some potential risk factors with *p-value* of ≤ 0.25 in the univariate analysis were also entered into the multivariate analysis. These factors were thought to be biologically important and may influence the occurrence of babesia-infection. The multivariate analysis showed association between a BST positive status for babesia-infection and season with animals during the seasons of rainfall (Exp(B) = 5.37) and winter (Exp(B) = 5.53) having been at increased risk of becoming positive. This typified the findings of Qin *et al.* (2015) who considered season as a risk factor associated with *B. bigemina*-infection in China and observed that white yaks encountered higher risk of being exposed to *B. bigemina* in spring (OR = 3.52, 95% CI = 1.899 - 6.538, $p \leq 0.001$) and in summer (OR = 3.44, 95% CI = 1.909 - 6.193, $p \leq 0.001$) than that in winter. Kenana, Baggara, and Foga and cross breeds were at increased risk of becoming BST- and IELISA-positive with odds ratios ranging from 2.90 to 10.82 compared to Butana breed, confirming the results of Muhanguzi *et al.* (2010) and Tembue *et al.* (2011), who both, found a significant distinction in susceptibility to babesia parasites between breeds. Muhanguzi *et al.* (2010) noted that Zebu cattle were more resistant to *B. bigemina* infection compared to exotic breeds and their crosses while Tembue *et al.* (2011) indicated that there were variations in the susceptibility to *B. bigemina* infection among cattle breeds and showed that *Bos indicus* species are considered to be more resistant to tick infestation and thus babesia-infection and other TBSs. The analysis further showed that there was a significant association between being iELISA-positive for babesia-infection and locality, breed, age and sex. This did confirm the findings of Swai *et al.* (2007) who noted that three factors were significantly associated with variation in antibody prevalence to *B. bigemina* in the multivariable model. These factors were history of grazing, age and geographical location of the animal. Muhanguzi *et al*. (2010) found also a generally increased rate of infection with age in Uganda. Amorim *et al.* (2014) indicated age of animals was significant in the multivariate analysis with calves being more susceptible than adult animals (OR = 2.18, 95% CI = 1.24 - 3.81, $p \leq 0.006$). However, age, gender and the numbers of pregnancies of white yaks were not significant risk factors in the multivariate logistic regression

analysis for determining the risk factors associated with *B. bigemina* seroprevalence (Qin *et al.*, 2015).

Some management risk factors were found to significantly influence the occurrence of babesia-infection in cattle in Brazil and Puerto Rico (Rodríguez, 2007; Costa *et al.*, 2013). When independent variables were subjected to multivariate analysis ($p \leq 0.05$), use of spraying for acaricide application and presence of horse flies on the farm were significantly associated with babesia infection (Costa *et al.*, 2013). Rodríguez (2007) found 15 factors that were significantly associated with high *B. bovis* seropositivity at $p \leq 0.20$. The final multivariable logistic regression model demonstrated that farms located in the north coastal region (OR = 0.21, 95% CI = 0.05 - 0.86), dairy farms with calf raising facilities (OR = 16, 95% CI = 3.0 - 86), having more than 4 neighbors (OR = 17, 95% CI = 1.6 - 176), same producer owing more than 1 farm (OR = 7.3, 95% CI = 1.7 - 32), and use of government services to apply amitraz on cattle (OR = 5.5, 95% CI = 1.5 - 20) had a significant association with high *B. bovis* seropositivity.

Dependencies between individual animal risk factors including location, age, sex, breed, and season and babesia-infection positive status might occur or not due to many reasons. Climate variations by season or geographical location influence the spread of babesia-infection. The pattern of the infection is not expected to be similar in arid or semi-arid regions and in high rainfall savannah, because the biology of tick vectors of babesia parasites and their different developmental stages is greatly promoted or demoted by ambient temperature and relative humidity. Moreover, babesia-infection was associated with sex where males were at higher risk than females. Sex unlikeness in response to parasitic diseases (susceptibility or resistance) is known. It has been demonstrated that male animals generally show lower immune responses than females. This is due to that phagocytic activity of macrophages and production of inflammatory cytokines are higher in females. Antibody production by B cells is also greater in females (Sasaki *et al.*, 2013). Another plausible explanation is that testosterone has been noticed to weaken and delay innate immunity, including splenic macrophage activation, resulting in failure of subsequent acquired immunity and lower production of IFN-γ, leading to a dramatic increase in parasitemia and severe symptoms (Sasaki *et al.*, 2013). Age-related immunity is an important factor that influences the occurrence of babesiosis; calves exposed to babesiosis during the first year of age rarely show clinical symptoms due to passively acquired resistance from colostrum and innate immunity (Bock *et al.*, 2004; Radostits *et al.*, 2007). Regarding breed susceptibility to babesia-infection, *Bos indicus* breeds or zebu cattle were more resistant to babesia-infection and almost invariably experience milder clinical symptoms to primary infections compared to *Bos taurus* breeds or exotic breeds and their crosses. This phenomenon is thought to be a result of the evolutionary relationship between *Bos indicus* cattle, *Boophilus* species and babesia (Bock *et al.*, 2004).

In the present study the sensitivity of the BST for detection of babesia parasites was low when compared to the iELISA. The agreement between the two techniques was slight and this might be attributed to the fact that detection of babesia parasites using microscopy requires a high parasitemia level while the iELISA can detect antibodies even after long time post infection. Carriers or sub-clinically infected cattle are important contributors to the transmission of the infection by tick vectors thus detecting them is important to cut the transmission cycle (Salih *et al.*, 2007). The sensitivity of detection was much increased when the same samples were subjected to iELISA (Bock *et al.*, 2004).

Conclusions and recommendations

It can be concluded that bovine babesiosis was prevalent in the study area. The iELISA showed that age and breed significantly influence the occurrence of the disease. Further epidemiological studies on babesiosis in the state and the whole country are warranted. In addition, the tick vectors and the factors affecting their population dynamics, and molecular characterization of the parasite causing bovine babesiosis should be studied.

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