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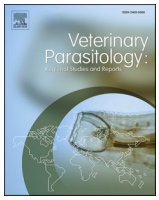

Original Article

Molecular survey for tick-borne pathogens and associated risk factors in sheep and goats in Kano Metropolis, Nigeria

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ABSTRACT

Tick-borne pathogens (TBPs) pose an increased health and productivity risk to livestock in sub-Saharan Africa. Information regarding TBPs infecting small ruminants in Kano metropolis is scarce. Therefore, we investigated the molecular epidemiology of tick-borne pathogens of economic importance from sheep and goats in Kano, Nigeria using Polymerase chain reaction (PCR). A total of 346 blood DNA samples were collected from small ruminants and analyzed for TBPs using PCR and sequencing. Risk of infection was determined for age, sex, breed and animal species. Our results indicate the absence of piroplasmids (*Babesia/Theileria*) and *Rickettsia* spp. infections. The overall prevalence for *Anaplasma* spp. was 9.25% (32/346) with a higher prevalence in goats 13.59% (25/184) compared with sheep 4.32% (7/162). With respect to age of animals, goats >4 years had the highest prevalence of 32.45% (11/37) which differs significantly ($P = 0.0059$) compared with other age categories. Cross breed goats had a prevalence of 15.63% (5/32) compared with Kano brown breed 14.08 (20/142). Sex significant difference ($P = 0.029$) was observed in the goats with females having the highest prevalence 20.89% (14/67) compared with males 9.40% (11/117). Furthermore, with regards to sheep, no significant difference ($P > 0.05$) was observed with respect to age and breed. Finally, no significant difference ($P > 0.05$) was observed with the prevalence of *Anaplasma* spp. due to Body condition score (BCS) in both sheep and goats. Conclusively, the occurrence of TBPs in small ruminants is low. Continuous efforts in tick control must be sustained to ensure high productive yield and reduced disease burden associated with TBPs of sheep and goats in Kano metropolis.

1. Introduction

Haemoparasites are a diverse assemblage of organisms (including bacteria, flagellates, protozoa, and mycoplasma) characterized by the occurrence of at least one developmental stage in the bloodstream of their vertebrate host (Benedikt et al., 2009). The presence of these haemoparasites in the bloodstream results in haemolysis of the red blood cells leading to anemia, anorexia, reduced reproductive capacity, emaciation and even death (Uilenberg, 1995). Generally, the effect of these haemoparasites in the host is determined by the interactions that exist between the type of pathogen within the geographical area, the life cycle of the pathogen, environmental conditions including weather pattern, farm management practices and host factors (Ukwueze and Kalu, 2015). Among the haemoparasites that infect small ruminants, anaplasmosis and babesiosis are considered to be the major impediments to ruminant production (Useh et al., 2006).

Over 40 species of ticks of veterinary importance have been registered in Africa (Walker et al., 2003), among which ticks belonging to the genera *Hyalomma*, *Amblyomma*, and *Rhipicephalus*, sub-genus *Boophilus* are the most important infesting domestic small ruminants in Nigeria (Ofukwu and Akwuobu, 2010; Adam, 2019; Orpin et al., 2020). In Northern Nigeria, tick infestation has been documented with varying

Abbreviations: TBP, Tick-borne pathogens; PCR, Polymerase chain reaction; DNA, Deoxyribonucleic acid; EDTA, Ethylene diamine tetraacetic acid; BCS, Body condition score.

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prevalence's in sheep and goats in some states such as Kaduna (Adam, 2019), Benue (Ofukwu and Akwuobu, 2010) and Katsina (Orpin et al., 2020). The difference in prevalence's found in these studies may be associated with the difference in the climatic conditions such as environmental temperature, relative humidity, and geographical location. This factor helps in the ecological structure for the reproduction and growth of tick populations (Pegram et al., 1981). Tick abundance in the dry season is usually low but dramatically increases following the first scattered rains, with peak abundance after one month after the heavy rains (Bayer and Maina, 1984; Maina, 1986; Lorusso et al., 2013).

The genus *Anaplasma* comprises of microorganisms which are obligate intra-erythrocytic bacteria. Species of importance within this genus in livestock include *Anaplasma ovis*, *A. marginale*, *A. phogocytophilum* and *A. platys* (Cabezas-Cruz et al., 2019). Tick bites have been generally considered as the main means of transmission but other modes have been reported such as bites of hematophagous insects (Hornok et al., 2011) and through blood contaminated instruments (Friedhoff, 1997). Anaplasmosis is widely prevalent in tropical and subtropical areas of the world among small ruminants where it causes chronic disease with rare outbreaks of acute disease which could be triggered by stressful conditions (Friedhoff, 1997). *Anaplasma ovis* is the most common cause of anaplasmosis in small ruminants and is considered to be less pathogenic compared with other species of *Anaplasma*. In Nigeria, few epidemiological studies have reported the presence of this pathogen using microscopy in small ruminants, and this is a well-known technique for parasitological diagnosis of *Anaplasma* in small ruminants (Jatau et al., 2011; Igwenagu et al., 2018; Kasozi et al., 2019; Berthelsson et al., 2020). On the other hand, *A. marginale* and *A. centrale* are species of *Anaplasma* that primarily infect cattle causing bovine anaplasmosis (da Silva et al., 2018). *Anaplasma centrale* is manifested clinically with progressive anemia and has been used against the more virulent *A. marginale* in live vaccine (Rjeibi et al., 2017). The close interaction between cattle and sheep/goats as well as their vectors, the DNA of this bacterium (*A. marginale*) has been detected in sheep and goats in Nigeria (Anyanwu et al., 2016), Iran (Yousefi et al., 2017) and Brazil (da Silva et al., 2018).

Several species of pathogenic *Babesia* and *Theileria* infect domestic ruminants. Ovine theileriosis is one important protozoal infection of sheep and goats caused by several species of *Theileria* (Altay et al., 2007). On the other hand, ovine babesiosis caused by several species of *Babesia* are pathogenic and infect small ruminants with resultant health implications manifested as economic losses to the owners (Anyanwu et al., 2016). Also, due to limited epidemiological studies, it is currently unknown if small ruminants or ungulates are reservoirs for *Rickettsia* pathogens in Nigeria.

Generally, research and diagnostics which focus on the prevalence of infectious agent caused by TBPs is hampered by inadequate tools that lack sensitivity and specificity, which is particularly lacking in small ruminants. Furthermore, to monitor the efficiency of therapeutic and prophylactic measures against TBPs, there is a need for rapid and efficient diagnostic tests. Conventionally, the most commonly used approaches involve clinical signs, microscopic examination of blood film and serological detection of antibodies (Kamani et al., 2010). Lack of sensitivity and specificity of these diagnostic approaches enumerated limits their use (Salih et al., 2015). Molecular diagnosis offers the best practical method to mitigate the pitfalls associated with the traditional approaches offering rapid specific and sensitive way to ascertain the animal infection status. Currently, there is paucity of data on the current status of TBPs in Nigeria especially in the northern part of the country. Therefore, this study was undertaken using polymerase chain reaction (PCR) based technique to detect and identify the presence of some selected tick-borne pathogens of sheep and goats in Kano state metropolis, Nigeria.

2. Materials and methods

2.1. Study area

The study was carried out in Kano state in north western Nigeria. Kano state is the most populated state in Nigeria estimated at 9000, 000 (National Population Commission, 2006). It has a land area of about 20,760 km^2 and located between latitudes 12^o 4′ and 10^o 3′ N and longitude 7^0 4′ and 9^0 3′ E. This state has a savannah type of vegetation. The temperature ranges from 18 °C to 45 °C with a mean of 27 °C. There is a single rainy season (May to October) with mean annual rainfall of 508–1016 mm and dry season (October to April) (Kabiru, 2011). The metropolitan council of Kano state comprises of 6 core local governments namely Fagge, Nassarawa, Tarauni, Gwale, Dala and Municipal (see Fig. 1).

2.2. Study design and sampling

Cross sectional study was undertaken in February and March 2020. Due to Covid-19 pandemic, sampling was interrupted. Additional samples were collected in August 2020. Household (backyard) flocks were visited at several points within the metropolis. Sampling was carried out using convenient sampling after owner consent and approval granted. Samples were collected from 346 small ruminants comprising of 162 sheep and 184 goats.

2.3. Blood sample collection

Exactly 5 mL of whole blood was collected from the jugular veins of sampled animals using sterile hypodermic needles and syringes after proper restraining of the animals. Collected blood samples were dispensed into 5 mL EDTA coated blood tubes and placed in cooler maintained at 4 °C with ice pack and transported to the laboratory. About 125 μL of blood was carefully dispensed into Whatman Chromatography FTA™ (Flinders Technology Associate) card (Sigma-Aldrich, USA). The cards were air-dried in dust-free environment for 4 h and then stored in sealed pouches at room temperature until used.

2.4. Epidemiological questionnaire

To assess the possible risk factors associated with exposure to tick-borne pathogens in sheep and goats, a closed-ended, semi-structured questionnaire was used to collect information of each animal on possible factors that might influence positivity to any of the screened pathogens. Information obtained includes species of animal (sheep or goat); sex (male or female); breed; age and body condition score (BCS 1 = extremely thin with no fat reserves, BCS 2 = moderate looking, BCS 3 = healthy looking, BCS = 4 good looking and BCS 5 = excessively fat/obese).

2.5. Extraction of genomic DNA from filter paper

Genomic DNA was extracted using high pure PCR template preparation kit (Roche ® Diagnostic GmbH, Mannheim, Germany) following the manufacturer's instructions with some modifications. Briefly, two punches of about 6 mm diameter were carefully excised from the dried blood spot into a 2 mL labelled Eppendorf tube. Thereafter, 200 μL of Tissue Lysis Buffer and 20 μL of Proteinase K were added. Mixing was carried out by vortexing and the tubes were transferred to a TissueLyser (Qiagen, Hilden, Germany) for 10 min at an oscillation frequency of 30 Hz. An additional 20 μL of Proteinase K were added before incubation was carried at 56 °C for 1 h (h). Exactly 200 μL of Binding Buffer was added after incubation. The mixture was immediately vortexed and incubated for 10 min at 70 °C. After incubation, 100 μL of isopropanol was added and the mixture was mixed well by vortexing. The whole mixture was thereafter transferred onto High Pure Filter Tube (column)

Fig. 1. Map of Kano metropolis showing the six local governments where samples were collected.

and centrifuge at 8000 *g* for 1 min. Flow-through was discarded with a collection tube and the column was transferred to a new collection tube. Exactly 500 μL Inhibitor Removal Buffer was added to the column and thereafter centrifuge at 8000 *g* for 1 min. Flow-through was discarded with collection tube and the column transferred to a new collection tube. Exactly 500 μL Wash Buffer was added to the column and centrifuged at 8000*g* for 1 min and the flow-through was discarded. The last step was repeated once more. The column was transferred to a new collection tube and was centrifuged at 13000*g* for 10 mins to remove any remnant buffer. Elution of genomic DNA was carried out by placing the High Pure Filter Tube (column) into a new 1.5 ml Eppendorf tube and the addition of 100 μL Elution Buffer into the center of the column and allowed to incubate for at least 5 mins and centrifuged at 8000 13,000*g* for 1 min to elute pure genomic DNA. Purified template DNA was stored at −20 °C until further usage. The quality and quantity of extracted DNA from blood (FTA cards) were measured using Thermo Scientific™ NanoDrop Lite Spectrophotometer.

2.6. Molecular detection of pathogens using PCR

PCR assays were conducted used for amplification of DNA of *Rickettsia* spp., *Anaplasma/Ehrlichia* spp., and *Babesia/Theileria* spp. from blood DNA using primer sequences shown in Table 1. All reactions were performed using DreamTaq PCR master mix (Thermo Scientific) comprising of (2× DreamTaq buffer, 0.4 mM of each dNTP and 4 mM $MgCl_2$) in total volume of 25 μL. The reaction consisted of 12.5 μL DreamTaq Master mix, 1.0 μL of each primer (10 μM each of both forward and reverse primer), 5.5 μL Nuclease-Free Water and 5 μL template DNA. A Bio-Rad C1000 Touch™ Thermal Cycler PCR machine (Bio-Rad, Hemel Hempstead, UK) was used for the PCR cycling with the following conditions: initial denaturation at 95 °C for 3 mins, followed by 35 cycles of 95 °C for 30 s, annealing at temperature depending on pathogen, see Table 1) for 30 s, 72 °C for 2 min and a final extension at 72 °C for 7 mins. Cooling was carried out at 4 °C until the PCR products were used. Every reaction set had a positive and negative control (molecular grade

Table 1
PCR condition and primer set used for DNA amplification and sequencing in this study.

Target	Gene target	Primer sequence (5′-3′)	Annealing temperature	Product size(bp)	Reference
Babesia/Theileria	18S rRNA	BJ1: GTCTTGTAATTGGAATGATGG BN2: TAGTTTATGGTTAGGACTACG	55 °C 30 s	411–452	Casati et al., 2006
Anaplasma spp.	16S rRNA	16SAna-F- CAGAGTTTGATCCTGGCTCAGAACG 16S Ana-R-GAGTTTGCCGGGACTTCTTCTGTA	60 °C 30s	467	Stuen et al., 2003
Rickettsia spp.	*glt*A	Rsfg877: GGGGGCCTGCTCACGGCGG Rfsg1258: ATTGCAAAAAGTACAGTGAACA	56 °C 30 s	381	Regnery et al., 1991

water). Table 1 summarizes the primer sequences, gene targets and annealing temperatures.

2.7. Gel electrophoresis and sequencing

Agarose gel (1%) (*w/v*) stained with ethidium bromide was used to separate the PCR products. Visualization was carried out under UV trans-illuminator (Gene Genius Imaging System (Syngene, UK). Selected positive amplicons were sequenced at Inqaba Biotechnical Ltd., in both directions using the forward and reverse primers.

2.8. Statistical analysis

The prevalence (P) was calculated using the formula: P (%) = number of positive samples/total number of animals × 100. A chi-squared test was used to investigate the association within different variables using GraphPad Software Inc. version 5 (San Diago, CA, USA). *P*-values <0.05 were considered to indicate statistically significant differences.

3. Results

3.1. Molecular detection of tick-borne pathogens from blood DNA

All of the screened samples from goats and sheep were negative for PCR targeting the *18S* rRNA gene of piroplasmids (*Babesia/Theileria* spp.) (Appendix 1), and the *glt*A gene of *Rickettsia* spp. (Appendix 2), respectively.

In terms of *Anaplasma*, the *16S* rRNA gene PCR yielded bands of approximately 467 bp from the small ruminant DNA (Appendix 3). Unfortunately, the sequenced products were extremely short and unsatisfactory, and therefore could not be compared with those from published *Anaplasma* sequences.

3.2. Prevalence of Anaplasma spp. and associated risk factors

In total, three hundred and forty-six (346) small ruminants comprising of 162 sheep and 184 goats were sampled from Kano metropolitan council in Kano state, Nigeria. Of the 346 samples screened from sheep and goats, a total of 32 were PCR positive for *Anaplasma* spp., with an overall prevalence of 9.25% (95% CI: 6.6–12.8).

Of the 162 sheep blood DNA samples screened by PCR for *Anaplasma* spp. infection, 7 were positive with a prevalence of 4.32% (95% CI: 2.1–8.7) (Table 2). Concerning age, sheep >4 years of age had the highest prevalence of 5.45% (6/110, 95% CI: 2.5–11.4) (Table 2). Only the Ouda breed of sheep was positive for *Anaplasma* spp. with a prevalence 4.76% (7/147, 95% CI: 2.3–9.5). *Anaplasma* spp. prevalence was non-significantly ($P > 0.05$) higher in females 4.71% (4/85, 95% CI: 1.9–11.5) compared with males 3.89% (3/77, 95% CI: 1.3–10.8) (Table 2). Finally, sheep with a body condition score of 3 had a slightly higher prevalence of 5.41% (95% CI: 1.5–17.7) compared with those with body condition score of 4 (5.13%; 95% CI: 2.0–12.5) or 5 (2.13%; 95% CI: 0.4–11.1) (Table 2). No significant difference ($P > 0.05$) was observed for any of the risk factors analyzed in the sheep.

A total of 184 blood DNA samples from goats was screened by PCR, with 25 samples being positive, giving a prevalence of 13.59% (95% CI: 9.4–19.3) (Table 3). Concerning age of animals, goats >4 years had the highest prevalence of 32.45% (11/37, 95%CI: 17.5–45.8) which differs significantly ($P = 0.006$) compared with other age categories (Table 3). Crossbreed goats had a slightly higher prevalence of 15.63% (5/32, 95% CI: 6.9–31.8) compared with Kano brown breed 14.08 (20/142, 95% CI: 9.3–20.8). Sex significant difference ($P = 0.029$) was observed in the goats with females having the highest prevalence of 20.89% (14/67, 95% CI: 12.9–32.1) compared with males at 9.40% (11/117, 95% CI: 5.3–16.1) (Table 3). Finally, no significant difference was observed with

Table 2
Prevalence and risk factors associated with infection of sheep with *Anaplasma* spp. in Kano metropolis, Nigeria.

Variable	Number examined	Number positive (%)	χ2	*P*-value
Age (years)				
0–1	7	0 (0)	1.137	0.566
2–3	45	1 (2.22)		
> 4	110	6 (5.45)		
Total	162	7 (4.32)		
Breed				
Balami	14	0 (0)	0.747	0.689
Ouda	147	7 (4.76)		
Cross breed	1	0 (0)		
Total	162	7 (4.32)		
Sex				
Male	77	3 (3.89)	0.064	0.800
Female	85	4 (4.71)		
Total	162	7 (4.32)		
BCS				
3	37	2 (5.41)	0.775	0.679
4	78	4 (5.13)		
5	47	1 (2.13)		
Total	162	7 (4.32)		

BCS - Body Condition Score.

Table 3
Prevalence and risk factors associated with infection of goats with *Anaplasma* spp. in Kano metropolis, Nigeria.

Variable	Number examined	Number positive (%)	χ2	*P*- value
Age (years)				
0–1	95	9 (9.47)	10.28	0.0059
2–3	52	5 (9.62)		
> 4	37	11 (32.45)		
Total	184	25 (13.59)		
Breed				
Kano brown	142	20 (14.08)	1.72	0.424
Sahel	10	0 (0)		
Cross breed	32	5 (15.63)		
Total	184	25 (13.59)		
Sex				
Male	117	11 (9.40)	4.79	0.029
Female	67	14 (20.89)		
Total	184	25 (13.59)		
BCS				
3	106	12 (11.32)	5.23	0.07
4	52	5 (9.62)		
5	26	8 (30.77)		
Total	184	25 (13.59)		

BCS - Body Condition Score.

the prevalence of *Anaplasma* spp. due to body condition score.

4. Discussion

This study attempted to provide insight into molecular epidemiology of selected tick-borne protozoal (*Babesia/Theileria* spp.) and bacterial (*Anaplasma* spp. and *Rickettsia* spp.) pathogens in blood DNA of sheep and goats from Kano metropolis, Nigeria. The data presented here was arrived following robust and strict laboratory procedures including controls (both positive and negative) to validate the test outcome.

None of the blood DNA samples was positive for piroplasms (*Babesia/Theileria* spp.). Specifically, *Babesia* parasites are seldom detected in small ruminants across Africa (Gebrekidan et al., 2014; El Imam et al., 2016; Aouadi et al., 2017; Lee et al., 2018; Ringo et al., 2019; Hassan et al., 2019), with the exception for a single study in Tunisia (Rjeibi et al., 2014). Nonetheless, *B. ovis* has been widely reported from several epidemiological surveys in small ruminants in Turkey and Iran (Esmaeilnejad et al., 2012; Shahzad et al., 2013; Ozubek and Aktas, 2017). The only molecular evidence in Africa of the detection of DNA of *B. ovis* in small ruminants was in Tunisia (Rjeibi et al., 2014). Similarly, there is no report so far in west Africa or Nigeria on the presence of DNA of *Theileria ovis* and other species of *Theileria* from sheep and goats. This study observed no positive sample for *Theileria* species from blood DNA from sheep and goats. Nonetheless, several species of *Theileria* infecting small ruminants including *T. ovis*, *T. lestoquardi* and *T. separata* have been sparsely reported in Africa with major concentration in North Africa (Taha et al., 2013; Gebrekidan et al., 2014; El Imam et al., 2016; Rjeibi et al., 2016; Aouadi et al., 2017). Furthermore, *Rhipicephalus turanicus* and *R. bursa*, are known competent vectors responsible for the transmission of these piroplasmids in northern Africa and Mediterranean region (Erster et al., 2016), but are absent in Nigeria. Further studies with large sample size from other districts within the metropolis are needed to further provide more clarity to our current observation.

The absence of *Rickettsia* infection among sheep and goats blood DNA in Kano metropolis was interesting given the recent evidence on the existence of Spotted Fever Group (SFG) *Rickettsia* from blood fed-ticks of camels in Kano state (Kamani et al., 2015; Onyiche et al., 2020a), blood-fed ticks from horses in Kano state ("Onyiche Unpublished results") and blood-fed ticks from sheep and goats in Ethiopia, Kenya and South Africa (Teshale et al., 2015; Omondi et al., 2017; Jongejan et al., 2020). Furthermore, results from this study may probably suggest that domestic small ruminants (sheep and goats) are unlikely amplifying host for tick-borne pathogens of the genus *Rickettsia*. Therefore, detection of SFG *Rickettsia* species should be preferably carried out using their tick vectors which serves as reservoir host for the maintenance of the pathogen rather than blood.

Generic primers were used for the amplification of *Anaplasma* spp. from blood DNA of sheep and goats. Although, it was not possible to generate sequences, nonetheless, all positives' bands at the correct amplicons size were considered as most likely to be *Anaplasma* spp. *Anaplasma ovis* is the principal species of *Anaplasma* known to infect small ruminants. The prevalence of *Anaplasma* spp. in this study was low in both sheep (4.32%) and goats (13.59%). Low prevalence of *A. ovis* (11.5%) was also reported in Senegal (Djiba et al., 2013) and Egypt (9.1%) (Tumwebaze et al., 2020a). On the other hand, higher prevalence of 69.7% has been reported in Niger republic (Dahmani et al., 2017), 83.8% in Sudan (Lee et al., 2018) and 89.1% in Uganda (Kasozi et al., 2021). It has been hypothesized that low or high prevalence is associated with low or high genetic diversity respectively using the *Msp*4 gene (Han et al., 2017; Tumwebaze et al., 2020b). Generally, discrepancies in prevalence could be attributed to differences in climatic conditions, abundance of tick vectors, farm management, and differences in susceptibilities among different animals in different geographical areas (Belkahia et al., 2014; Tumwebaze et al., 2020a).

The present study revealed that the prevalence rates of anaplasmosis are associated with certain risk factors which differs significantly with regards to age and sex in the goats. In goats, the infection rates were higher in females compared with males. This observation is in agreement with the findings of other workers in Tunisia (Belkahia et al., 2014) and Egypt (Tumwebaze et al., 2020b). Older animals were more infected compared to young ones. This corroborates previous observation of higher prevalence in older animals (Belkahia et al., 2014). Furthermore, these findings could be attributed to the likelihood of more exposure to ticks during previous tick seasons (Ben Said et al., 2015). The complete absence of infection among young animals (lambs) could be attributed to the protective effect of antibodies from colostrum which has been observed to protect animals during the first three months of life (Friedhoff, 1997). Only the Ouda breed (one of the local breed of sheep) was found to be infected with *Anaplasma* spp. With respect to goats, none of the Sahel breeds was positive to *Anaplasma* spp., as this could be connected to genetic resistance among the local breeds. Contrary to previous reports of higher prevalence of *A. ovis* in sheep compared to goats (El Imam et al., 2016; Ringo et al., 2019; Kasozi et al., 2021), this study reported higher prevalence in goats compared with sheep. This observation corroborates another study in Sudan on anaplasmosis in small ruminants where a higher prevalence was registered in goats comapred with sheep (Eisawi et al., 2020).

Molecular based surveys are still in their infancy in Nigeria and very few studies have employed this diagnostic technique to study the molecular epidemiology of TBPs in the country (Ogo et al., 2012; Kamani et al., 2015; Onyiche et al., 2020a; Onyiche et al., 2020b). As from available literature review (Tables 4 and 5) on the molecular epidemiology of *Anaplasma ovis* in sheep and goats in Africa, its presence has been confirmed by PCR and sequencing with sequences deposited in the NCBI database. Prevalence ranges from 9.1% to 83.9% in Sheep (Table 4) while in goats, its occurrence ranges from 25.4% to 80.5% (Table 5). Most studies registered high prevalence in both sheep and goats suggesting the endemicity of the pathogen across all regions of Africa (Ben Said et al., 2015; Lee et al., 2018; Dahmani et al., 2019; Kasozi et al., 2019). On the other hand, previous studies have reported higher prevalence in Sheep compared with goats (Aouadi et al., 2017; Lee et al., 2018; Kasozi et al., 2021).

5. Conclusions

In this study, the epidemiology of tick-borne pathogens of sheep and goats in Kano metropolis, Nigeria was studied using conventional PCR based molecular method. Our results revealed the presence of *Anaplasmataceae* in small ruminants in the study area with higher prevalence in goats compared to sheep. Additionally, age and sex were observed as significant risk factors in the goats to infection with *Anaplasma* species. No piroplasmids (*Babesia/Theileria* spp.) and *Rickettsia* spp. DNA was detected in any of the blood DNA from sampled animals. We recommend that further studies need to be conducted to ascertain the tick species infesting sheep and goats in the study areas and their possible role in the epidemiology of anaplasmosis in the study area. Also, the role of season in the prevalence of TBPs warrants further studies.

Ethical approval

Local permission to carry out sampling was sort and approval was granted by the Kano state Ministry of Agriculture and Natural Resources with reference number RPPR/3/17-7. The Animal Welfare and Ethical Review Board (AWERB), University of Edinburgh provided additional approval with reference number OS3-20.

Authors contribution

TEO and ETM designed the study. TEO carried out the field survey and DNA extraction. TEO and LM carried out PCRs. TEO and ETM carried out data analyses, the statistical analyses, and interpreted the data. OT provided some reagents. ETM supervised the study. TEO drafted the

Table 4
Overview on molecular detection of *Anaplasma ovis* in blood from sheep in Africa.

Country	No. tested	No. positive	Prevalence (%)	Sequencing of PCR products	Accession number	Reference
Algeria	120	74	61.67	+	NS	Aouadi et al., 2017
Tunisia	204	143	70.09	+	KC432641, KC432642, KC432643, KC432644	Belkahia et al., 2014
Tunisia	260	198	76.15	+	KM285218, KM285219, KM285220, KM285221, KM285222	Ben Said et al., 2015
Senegal	136	76	55.88	+	MN317237, MN284924, MN317249	Dahmani et al., 2019
Senegal	120	38	31.67	+	NS	Djiba et al., 2013
Sudan	62	52	83.87	+	MG383900, MG383901, MG383902	Lee et al., 2018
Egypt	66	6	9.09	+	MN882167, MN882168, MN882169, MN882170	Tumwebaze et al., 2020a
Kenya	76	26	34.21	+	MG637125, MG637126, MG637127	Ringo et al., 2019
Sudan	200	65	32.50	+	NCS	Eisawi et al., 2020
Uganda	46	12	26.10	–	–	Kasozi et al., 2021

NS - Not stated.
NCS - Not Clearly Separated.

Table 5
Overview on molecular detection of *Anaplasma ovis* in blood from goats in Africa.

Country	No. tested	No. positive	Prevalence (%)	Sequencing of PCR products	Accession number	Reference
Algeria	120	65	54.17	+	NS	Aouadi et al., 2017
Tunisia	303	244	80.53	+	KM285217	Ben Said et al., 2015
Botswana	100	76	76.00	+	NS	Berthelsson et al., 2020
Sudan	116	55	47.41	+	MG778617, MG778618, MG778619, MG778620, MG383897, MG383898	Lee et al., 2018
South Africa	61	28	45.90	+	NS	Ringo et al., 2018
Sudan	198	71	35.85	+	NCS	Eisawi et al., 2020
Uganda	666	169	25.40	–	–	Kasozi et al., 2021
Uganda	201	11	5.5	+	MT247053, MT267793, MT247052, MT247055, MT247051, MT247054	Tumwebaze et al., 2020b

NS - Not stated.
NCS - Not Clearly Separated.

paper. LM, OT and ETM critically revised the manuscript. All authors read and approved the final version of the submitted manuscript.

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Declaration of Competing Interest

No conflict of interest exists among the authors involved in this submission. Thank you.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2022.100753.

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