J. Protozool. Res. 33. 1-17 (2023)
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Molecular detection of selected tick-borne hemo-parasites in small ruminants from Seno and Oudalan provinces, Burkina Faso

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ABSTRACT

Tick-borne diseases (TBDs) restrict livestock farming and have significant economic impact in sub-Saharan Africa. In endemic areas livestock are exposed to different tick species carrying various pathogens and they transmit them to livestock. Small ruminants in Sahel region plays important role in human livelihood, however, there is scarcity of information on the TBDs epidemiology in this region. Therefore, our study was aimed at providing an overview of the occurrence of TBDs in Seno and Oudalan provinces of Burkina Faso. We used PCR and sequencing to analyze 79 blood samples collected from sheep and goats to detect the tick-borne pathogens (TBPs). The PCR assays used to analyze samples were targeting *Theileria ovis* SSU rRNA, *Ehrlichia ruminantium* pCS20, *Anaplasma ovis* AoMSP4, *Anaplasma phagocytophilum* Epank1, *Babesia ovis* SSU rRNA and *Theileria* spp. 18S rRNA genes. PCR screening revealed that *T. ovis* and *A. ovis* were the only pathogens detected in this study. The prevalence rates for *T. ovis* and *A. ovis* were 29/79 (36.7%) and 33/79 (41.8%), respectively. Mixed infections were detected in 22/79 (27.9%) samples of this study. Sequence analysis of *T. ovis* SSU rRNA gene indicated that the gene is conserved among *T. ovis* isolates in the study area with sequence identity values of 100%. The *A. ovis* AoMSP4 gene sequences showed that the gene is conserved among the *A. ovis* isolates in the study area with the sequence identity values of 100%. All *A. ovis* appeared in the same clade on a phylogenetic tree based on AoMSP4 gene sequences. Likewise, the *T. ovis* sequences of this study appeared in the same clade of the phylogenetic tree based on SSU rRNA gene. This study provides an overview of the presence of important tick-borne pathogens in small ruminants of Seno and Oudalan provinces of Burkina Faso.

Keywords: Tick-borne diseases, small ruminants, *Theileria ovis*, *Anaplasma ovis,* Burkina Faso

INTRODUCTION

Tick- borne diseases remain an economic burden for the livestock industry in the sub-Saharan Africa. The parasites such as *Theileria* spp., *Babesia* spp. and *Anaplasma* spp. are the most important tick-borne protozoan and rickettsial pathogens of small ruminants and have considerable economic impact in the livestock industry (Jongejan and Uilenberg, 2004; Torina and Santo, 2012; Bilgic et al., 2017).

Caprine and ovine theileriosis is mainly caused by *Theileria lestoquardi, T. ovis, T. recondita* and *T. separata*. Among these species, *T. lestoquardi* is the most pathogenic *Theileria* spp. in small ruminants causing malignant ovine theileriosis. The disease is characterized by fever, lethargy, cough, lymphadenopathy and weight loss (Durrani et al., 2012; Ringo et al., 2018; Al-Hosary et al., 2021). The pathogen is transmitted by ticks of the genus *Hyalomma*. The other *Theileria* species causes benign theileriosis in sheep and goats (Chae et al., 1999; Altay et al., 2007). Several species of *Babesia* have been reported to cause ovine and caprine babesiosis including *Babesia ovis*, *B. motasi* and *B. crassa* (Schnittger et al., 2003). Among the three species, *B. ovis* is the most virulent species especially in sheep and causes severe anemia, fever, hemoglobinuria, icterus and occasionally death with the case fatality ranging between 30 – 50% (Aktas et al., 2007). Ticks of the genus *Rhipicephalus* have been implicated to be a vector of *B. ovis.*

Ovine anaplasmosis is caused by Gram-negative bacterial organisms, namely, *Anaplasma ovis* and *A. phagocytophilum* (Yousefi et al., 2017). However, *A. ovis* is reported as the main cause of small ruminant anaplasmosis (Stuen, 2016) resulting in severe disease in goats and bighorn sheep (Tibbitts et al., 1992; Renneker et al., 2013). The *A. ovis* infection is transmitted biologically and mechanically by ticks, blood-sucking insects and contaminated fomites (Ringo et al., 2018). Ovine anaplasmosis is often sub-clinical in health animals, but under stressful condition such as poor health condition, climatic change, pregnancy, mixed infections, heavy tick burden, worm infestation, vaccination and transportation in long distances may trigger the development of clinical signs (Stuen, 2016).

The small ruminant population in Burkina Faso is estimated to be more than 23 million, most of these animals are owned by the small-holder farmers (Dahourou et al., 2021). Predominantly, different ethnic groups of Burkina Faso keep small ruminants as source of protein, income security and poverty alleviation. Furthermore, small ruminants play important economic role in the traditional farming system of Burkina Faso and are used in a number of customary transactions (Bennison et al., 1997).

Sheep and goats in the Sahelian region are mainly reared outdoors in a transhumant system due to the unreliable pastures and water scarcity caused by seasonal variation characterized by short rainfall seasons and long dry seasons (Lesnoff,1999). Consequently, animals are exposed to a number of vector-borne infections transmitted by arthropods such as ticks and other blood-sucking insects. In Burkina Faso, several studies related to tick-transmitted diseases have been conducted and reported the presence of tick-borne diseases (Adjou Moumouni et al., 2021; Ouedraogo et al., 2021; Zannou et al., 2021; Compaoré et al., 2022). However, these studies have mainly focused on large ruminants. Therefore, the present study aimed to investigate the occurrence of *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp. and *Theileria* spp. in apparently healthy sheep and goats from Oudalan and Seno provinces of Burkina Faso.

MATERIALS AND METHODS

Study area

Samples were randomly collected in different farms of Oudalan and Seno provinces. The two provinces are in the Sahelian region located in the north of Burkina Faso (Fig. 1). The Seno province lies on 13°55’0” N, 0°10’0” W and Oudalan is positioned at 14°40’0.12” N, 0°19’59.98” W. The provinces are located in a semi-arid zone of Sahel region with low population density mainly the transhumant livestock keepers. The region receives about 200 – 800 mm of rains a year. rainy season starts in May and ends in October followed by a long intense dry season from November to May (Ibrahim et al., 2013). The annual average temperatures ranges between 15 - 45℃. The vegetation is characterized by low growing grasses, with tall herbaceous perennials including thorny shrubs, acacia and baobab trees (Rasmussen et al., 2001).

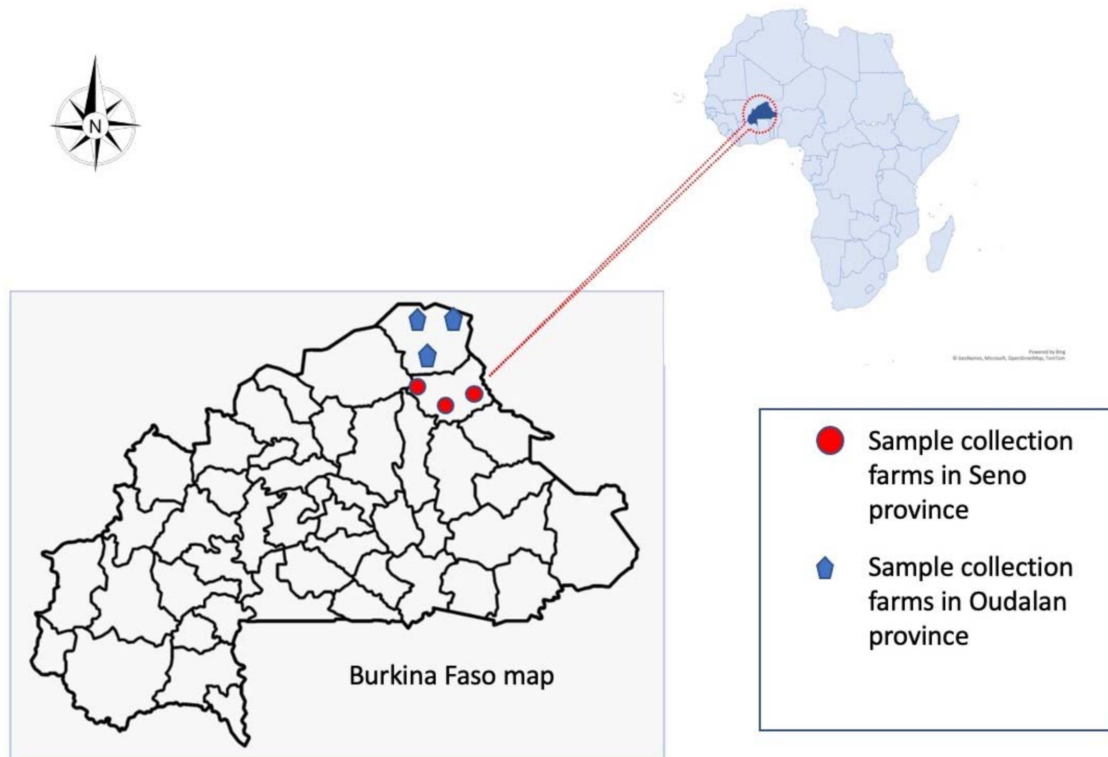

Fig. 1. Map of Burkina Faso showing sample collection farms in Seno and Oudalan provinces.

Sample collection and DNA extraction

Blood samples were collected randomly from clinically health sheep and goats. The sampling farms were all in Seno and Oudalan provinces. Samples were collected from 15th to 17th October, 2016. The age and sex of the sampled animals were recorded. A total of 79 small ruminants aged 5 months and above were sampled from different farms. Forty-two animals were from Oudalan and 37 were from Seno province. The sampled animals included 48 goats and 31 sheep. Age category of the sampled animals was set at under 1 year (14 animals) and 1 year and above were 65 animals. In general, 65 animals were female and 14 were males. Approximately 3 ml of blood was drawn from the jugular vein into vacutainer tubes coated with ethylenediaminetetraacetic acid (EDTA) and were kept in cool boxes until transportation to the laboratory for DNA extraction. Genomic DNA was extracted from 200 µl of whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, German) based on the manufacturer' s protocol, and stored at -30℃ until used.

Ethical statement

Farmers were informed about the study and they gave their consent under the condition that blood should be drawn by experienced veterinarian and proper restraint procedures should be applied. The sampling procedures were carried out based on ethical guidelines for the use of animal samples permitted by Obihiro University of Agriculture and Veterinary Medicine (Permit for animal experiment: 280080; DNA experiment 1219-2; Pathogens: 2017700).

Molecular detection of tick-borne pathogens

Polymerase chain reaction (PCR) was used to screen all the samples using species specific assays for *Babesia ovis*, *Anaplasma ovis*, *A. phagocytophilum*, *Ehrlichia ruminantium* and SFG Rickettsia (Table 1). *Theileria* group was screened using the *Theileria* spp. 18S rRNA primers and the detected positive samples were then screened by species specific primers. The reaction mixture had a final volume of 10 µl, containing 0.5 mM of each primer, 1 µl of 10 x standard *Taq* buffer, 1 µl of deoxynucleotide triphosphate mix (dNTPs mix) and 0.1 µl of Ex Taq polymerase (Takara, Japan), a 1 µl of DNA template and a 5.9 µl of double distilled water. The positive controls were positive samples from the previous study (Ringo et al., 2019), while double distilled water was used as negative control. The thermal cycling condition used in this study were obtained from the previous studies (Table 1). PCR were run in a thermal cycler (Bio rad, USA). The PCR products were electrophoresed on a 1.5% agarose gel and stained with ethidium bromide for visualization on a UV trans-illuminator.

Table. 1. List of primers used for PCR assays

Target gene	Assay	Primer sequences (5' ⟶ 3') Forward	Reverse	Fragment size (bp)	Annealing temp. (°C)	References
Babesia ovis (SSU rRNA)	PCR	TGGGCAGGACCTT GGTTCTTCT	CCGCGTAGCGCC GGCTAAATA	549	62	Rjeibi et al., 2016
Anaplasma ovis (AoMSP4)	PCR	TGAAGGGAGCGG GGTCATGGG	GAGTAATTGCA GCCAGGCACTCT	347	62	Torina et al., 2012
A. phagocytophilum (epank1)	PCR	GAGAGATGCTTAT GGTAAGAC	CGTTCAGCCATC ATTGTGAC	444	54	Walls et al., 2000
Rickettsia spp. (OmpA)	PCR	ATGGCGAATATTT CTCCAAAA	GTTCCGTTAATG GCAGCATCT	590	60	Eremeeva et al., 1994
Ehrlichia ruminantium (pCS20)	PCR	ACTAGTAGAAATT GCACAATCYAT	RCTDGCWGCTTT YTGTTCAGCTAK	400	61	Farougou et al., 2012
	nPCR	ACTAGTAGAAATT GCACAATCYAT	TGATAACTTGG WGCRRGDARTC CTT	278	61	
Theileria spp. (18S rRNA)	PCR	GAAACGGCTACC ACATCT	AGTTTCCCCGTG TTGAGT	778	55	Cao et al., 2013
	nPCR	TTAAACCTCTTCC AGAGT	TCAGCCTTGCGA CCATAC	581	55	
Theileria ovis (SSU rRNA)	PCR	TCGAGACCTTCGG GT	TCCGGACATTGT AAAACAAA	520	60	Aktas et al., 2006

Cloning and sequencing

For sequencing 5 – 10 PCR positive samples were randomly selected and amplicons were extracted for the agarose gel using QIAquick Gel Extraction Kit (Qiagen, German). The concentration of the selected samples was assessed using NanoDrop 2000 Spectrophotometer (Thermofisher, USA). The template (6 µl) was ligated into a pGEM-T easy vector (2 µl) (Promega, USA) with T4 DNA ligase and restriction buffer (each 1 µl) added and incubated at 16℃ for 3 hours and then at 4℃ overnight. Transformation of the plasmid in *Escherichia coli* DH5 α competent cells were performed. Lysogeny broth (LB) was added and incubated at 37℃ in a shaker incubator for 1 hour. Then inoculated on LB agar with antibiotic (Ampicilin) and incubated at 37℃ overnight. Plasmid was extracted using NucleoSpin® Plasmid quickPure (Macherey-Nagel-German) kit and sequencing PCR of the plasmid was performed with Dye Terminator Cycle Sequencing Kit (Applied biosystems, USA). For each agar plate a number of four clones were sequenced. Nucleotide sequences were produced using an ABI PRISM 3130xl genetic analyzer (Applied Biosystems, USA).

Phylogenetic analysis

Nucleotide sequences produced in the current study were initially aligned on nucleotide basic alignment search tool (BLASTn) of the National Center for Biotechnology Information (NCBI) to confirm identity of detected parasites. Genetic relatedness of positively detected tick-borne pathogen gene sequences of this study was analyzed against

those deposited in the GenBank database using MEGA version 11 (Tamura et al., 2021). The maximum likelihood method was used with bootstrap analysis set at 1000 replicates.

Nucleotide sequence accession numbers

Gene sequences produced in this study were deposited in the GenBank database of the National Center for Biotechnology Information using BankIt and the rRNA submission portal (submit.ncbi.nlm.nih.gov/subs/genbank/). The GenBank accession numbers were assigned to submitted sequences as follows: OP811308, OP811309, OP811310 and OP811311 for *T. ovis* (SSU rRNA) gene and MF945969, MF945970, MF945971, MF945972 and MF945973 for *A. ovis* (AoMSP4) gene.

Statistical analysis

Pearson's chi-square (X2) and Fisher's Exact test were used to statistically analyze the prevalence of the pathogens detected. The significance of the co-infections was determined by odds-ratio using MedCalc. Software. A p – value < 0.05 was considered statistically significant.

RESULTS

Overall results

Of the 79 blood samples of sheep and goats screened by PCR 40/79 (50.6%) were positive by at least one pathogen, while 39/79 (49.4%) animals were not infected by any of the screened pathogens. Only two pathogens were detected in this study, namely, *A. ovis* which was the most prevalent pathogen 33/79 (41.8%) and *T. ovis* 29/79 (36.7%) (Table 2).

Table. 2. Infection rates based on hosts

Animal species	Examined animals (n)	*Theileria ovis*	*Anaplasma ovis*	Co-infection
Sheep	31	13 (41.9%)	14 (45.2%)	11 (35.5%)
Goats	48	16 (33.3%)	19 (39.6%)	11 (22.9%)
Total	79	29 (36.7%)	33 (41.8%)	22 (27.9%)

n: Denote the number of examined animals

Infection rates based on hosts

The results further revealed that sheep 16/31 (51.6%) were more infected than goats 24/48 (50.0%) (Table 3). The prevalence of goats infected with *T. ovis* and *A. ovis* was 16/48 (33.3%) and 19/48 (39.6%), respectively. Meanwhile 13/31 (41.9%) of sheep were infected with *T. ovis* and 14/31 (45.2%) with *A. ovis*. However, the infection rates based on host were not statistically significant.

Table 3. Univariate analysis of tick-borne hemo-parasitic pathogens detected in sheep and goats

Variables	Categories	Examined animals (n)		*Theileria ovis*				*Anaplasma ovis*			
		Sheep	Goats	Sheep	*p*-value	Goats	*p*-values	Sheep	*p*-value	Goats	*p*-values
Age	Young	9 (29.1%)	8 (16.7%)	4 (44.4%)	0.83	1 (12.5%)	0.24	4 (44.4%)	0.73	2 (25.0%)	0.45
	Adult	22 (70.9%)	40 (83.3%)	9 (40.9%)		15 (37.5%)		10 (45.5%)		17 (42.5%)	
Sex	Males	4 (12.9%)	10 (20.8%)	3 (75.0%)	0.28	4 (40.0%)	0.71	3 (75.0%)	0.3	2 (20.0%)	0.28
	Females	27 (87.1%)	38 (79.2%)	10 (37.0%)		12 (31.6%)		11 (40.7%)		17 (44.7%)	
Location	Seno province	11 (35.5%)	26 (54.2%)	5 (45.5%)	0.93	11 (42.3%)	0.22	6 (54.6%)	0.48	14 (53.9%)	0.04*
	Oudalan province	20 (64.5%)	22 (45.8%)	8 (40.0%)		5 (22.7%)		8 (40.0%)		5 (22.7%)	

*: Denote the significant differences ($p < 0.05$) between infected and uninfected sheep and goats among the identified variables
n: Denote the number of examined animals

Infection rates based on location

Seno province showed higher infection rate 24/37 (64.9%) than Oudalan province which had the infection rate of 16/42 (38.1%). Furthermore, the animals infected by *T. ovis* and *A. ovis* in Seno province were 16/37 (43.2%) and 20/37 (54.1%) respectively. In Oudalan province *T. ovis* and *A. ovis* infections were 13/42 (30.9%) and 13/42 (30.9%) respectively (Table 3). Remarkably, the infection rate reported in Seno province was significantly higher than Oudalan province (Table 3).

Infection rates based on sex

The infection rates based on sex revealed that 8/14 (57.1%) male animals were infected. Among male animals 7/14 (50.0%) were infected by *T. ovis* while 5/14 (35.7%) was infected by *A. ovis*. Female animals had infection rate of 32/65 (49.2%) (Table 3). Among the females, 22/65 (33.9%) were infected with *T. ovis* while 28/65 (43.1%) were infected with *A. ovis*.

Infection rates based on age

The infection rates of the sampled animals based on age showed that adult animals had a prevalence of 33/62 (50.8%) while young animals had 7/17 (41.2%). Moreover, young animals showed a prevalence of 5/17 (29.4%) and 6/17 (35.3%) for *T. ovis* and *A. ovis* respectively. Meanwhile, adult animals had a prevalence of 24/62 (38.7%) and 27/62 (43.6%) for *T. ovis* and *A. ovis* respectively (Table 3).

Mixed infection

In this study, 22/79 (27.9%) animals were co-infected by *T. ovis* and *A. ovis* at the time of sampling. Notably, each of the two hosts had 11 animals co-infected by the two pathogens (Table 2). However, Seno province had 12/37 (32.4%) of the total co-infection while Oudalan province had 10/42 (23.8%) co-infections (Table 4). Moreover, based on age the co-infection in young animals were 4/17 (23.5%) and adult animals were 18/62 (29.0%), whereas based on sex 4/14 (28.6%) male and 18/65 (27.7%) female animals were co-infected by *T. ovis* and *A. ovis* (Table 4).

Table. 4. Univariate analysis of co-infection detected in sheep and goats.

Variables	Categories	Examined animals (n)		Co-infection			
		Sheep	Goats	Sheep	*p*-value	Goats	*p*-value
Age	Young	9 (29.1%)	8 (16.7%)	3 (33.3%)	0.79	1 (12.5%)	0.66
	Adult	22 (70.9%)	40 (83.3%)	8 (36.4%)		10 (25.0%)	
Sex	Males	4 (12.9%)	10 (20.8%)	2 (50.0%)	0.6	2 (20.0%)	0.86
	Females	27 (87.1%)	38 (79.2%)	9 (33.3%)		9 (23.7%)	
Location	Seno province	11 (35.5%)	26 (54.2%)	5 (45.5%)	0.45	7 (26.9%)	0.52
	Oudalan province	20 (64.5%)	22 (45.8%)	6 (30.0%)		4 (18.2%)	

*: Denote the significant differences ($p < 0.05$) between the co-infected sheep and goats among the identified variables
n; Denote the number of examined animals

Comparative sequence analysis of AoMSP4 and SSU rRNA genes

The percentage nucleotide identity of all five *A. ovis* AoMSP4 gene sequences (MF945969 – MF945973) was 100% among themselves. Moreover, they showed 100% identity with sequences MF360028 and MF360029 from Kenya and KY659323 from Tunisia. Nevertheless, these sequences showed 99.71% identity value with sequence OM127898 from Turkey.

Similarly, the percentage identity value of all four *T. ovis* SSU rRNA gene sequences of this study, was 100% among themselves. Moreover, they had 100% identity with sequence MG333458 from Sudan, MF360022 from Kenya, MN625886 from Egypt and MG203886 from South Africa.

Phylogenetic analysis

In this study, phylogenetic trees were constructed based on AoMSP4 and SSU rRNA gene sequences of *A. ovis* and *T. ovis,* respectively against sequences extracted from the NCBI GenBank. The phylogenetic analysis of SSU rRNA gene sequences of *T. ovis* showed that OP811308, OP811309, OP811310 and OP811311 sequences clustered together in a clade of *T. ovis* isolates (Fig. 2). The phylogenetic analysis of AoMSP4 gene showed that all five sequences of *A. ovis* from this study were clustered together in a clade that also contained sequences from Tunisia, Kenya, South Africa, Malawi, Sudan, Turkey, Portugal and Palestine (Fig. 3).

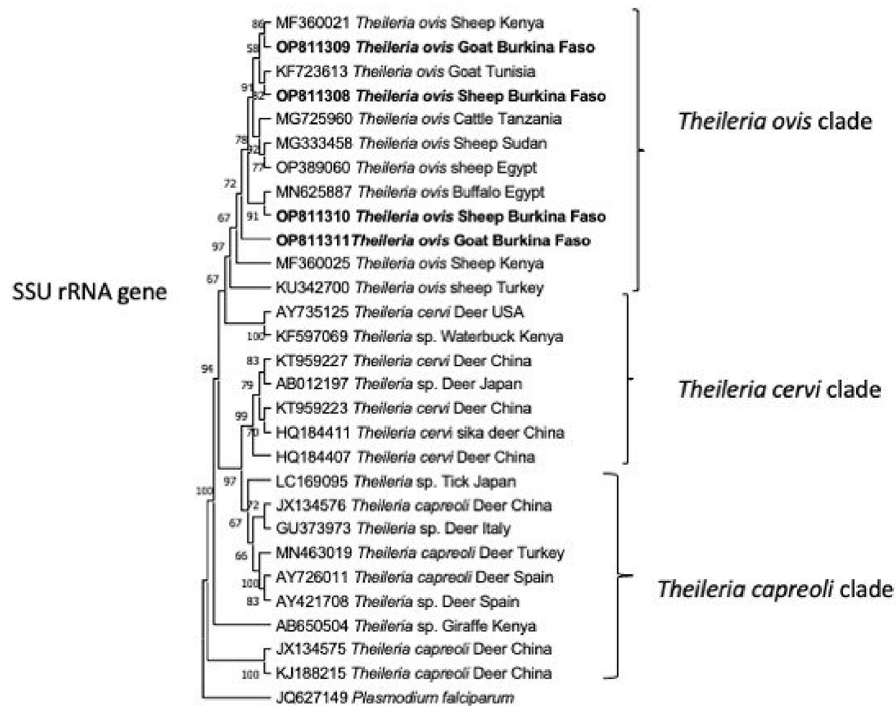

Fig. 2. Phylogenetic analysis by maximum likelihood method based on *Theileria ovis* SSU rRNA gene sequences. Numbers at the nodes represent the percentage occurrence of a clade in 1000 bootstrap replications of data. Sequences in bold font are the sequences obtained in this study. The 18S rRNA gene of *Plasmodium falciparum* (JQ627149) was used as an outgroup.

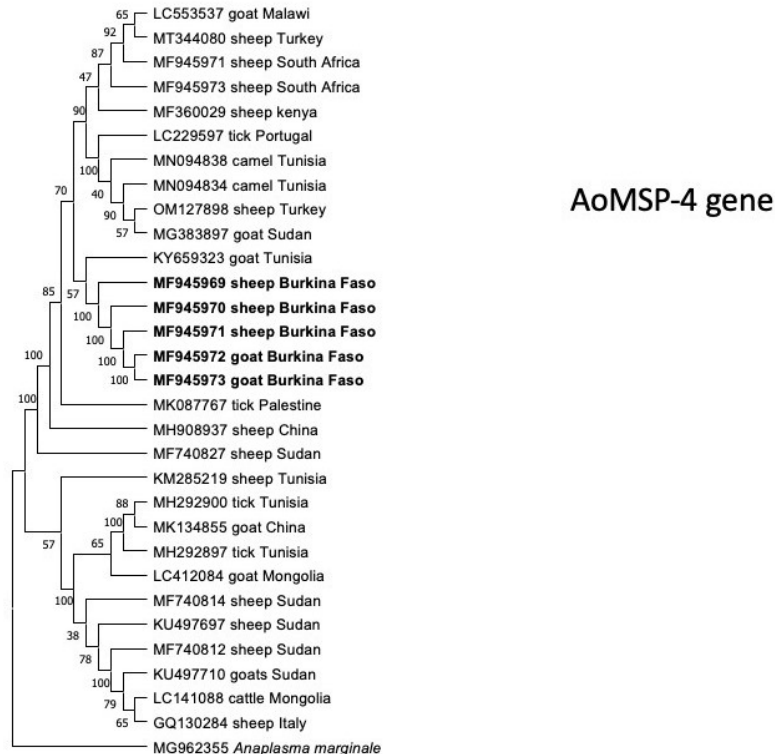

Fig. 3. Phylogenetic analysis by maximum likelihood method based on *Anaplasma ovis* AoMSP4 gene sequences. Numbers at the nodes represent the confidence of occurrence of a clade in 1000 bootstrap replications of data. Sequences in bold font are sequences obtained in this study. The MSP4 gene of *Anaplasma marginale* (MG962355) was used as an outgroup.

DISCUSSION

Ovine tick-borne pathogen infections affect small ruminants worldwide and are widely distributed in the tropical and sub-tropical regions (Ros-García et al., 2013). In sub-Saharan Africa little information is available on the presence and distribution of the tick-transmitted pathogens. Therefore, the present study aimed to investigate the presence and genetic composition of occurrence of *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp. and *Theileria* spp. in blood samples of sheep and goats collected from Seno and Oudalan provinces of Burkina Faso. The pathogens detected in this study were *Anaplasma ovis* and *Theileria ovis*.

In general, this study revealed that adult animals had higher infections of detected pathogens than the younger animals. This might be attributed to the fact that animals in the endemic areas are continuously exposed to the parasites as they grow older onto which they create immunity against those particular infections. These animals are able to carry the

pathogens in their entire lives without showing the clinical signs of a disease (Jonsson et al., 2012). However, young animals with short time in the pastures have lower chances of acquiring the infection.

Ovine anaplasmosis is a tick-borne infection of sheep and goats and is mainly caused by *A. ovis*. The disease causes sub-clinical infection in small ruminants (Yousefi et al., 2017). However, these animals when subjected to stressful condition such as heavy tick burden, long transportation, vaccination, worm infestation, climate change, poor health condition and pregnancy they develop serious disease characterized by respiratory distress, anaemia, increased heart rates, depression, abortion, decline in body weight and eventually death (Lagerkvist, 2017). Additionally, the disease results in severe jaundice in infected lambs leading to disposal of their carcasses on slaughtering (Lacasta et al., 2020). *A. ovis* in this study was the most frequently detected pathogen with an overall prevalence of 41.8%. The prevalence is higher compared to the previous study conducted in the neighboring country of Nigeria (Egbe-Nwiyi et al., 2018) but is lower compared to a study conducted in Niger (Dahmani et al., 2017). The mixed result could be due to the different management systems practiced in those west African countries. The higher prevalence of *A. ovis* in this study could be attributed to the fact that the pathogen can be transmitted by several vectors apart from its main vector *Rhipicephalus evertsi evertsi* (Ringo et al., 2019). Other tick species have been reported to transmit the pathogen (Belkahia et al., 2014). Moreover, the biting insects and contaminated fomites can transmit the pathogen mechanically (Woldehiwet, 2010).

For genetic characterization of *Anaplasma* spp., MSP4 gene is widely used (de La Fuente et al., 2005). The AoMSP4 gene sequences for *A. ovis* obtained in this study were highly conserved among the sheep and goat isolates. Phylogenetic analysis showed that all sequences obtained from this study appeared in the same clade. The results are consistent with the previous finding which reported that the low or high genetic diversity of the MSP4 gene is associated with the prevalence of the *A. ovis* (de La Fuente et al., 2007; Han et al., 2017). However, larger sample size is recommended in the study area to confirm this hypothesis.

Ovine theileriosis in sheep and goats can be caused by several *Theileria* spp. (Berggoetz et al., 2014). The disease is an important tick-borne piroplasm infection and lead to economic losses in the small ruminant industry. The common clinical symptoms of ovine theileriosis include cough, fever, lymphadenopathy, lethargy and weight loss (Abid et al., 2021). *Theileria* ovis was the only *Theileria* spp. detected in this study. The infection rate of this pathogen was 36.7%, this is not consistent with the previous studies conducted in the neighboring countries of Nigeria (Useh et al., 2007) and Algeria (Aouadi et al., 2017) which reported lower prevalence of this pathogen. Elsewhere in Africa the studies in Sudan (Lee et al., 2018), Tunisia (Rjeibi et al., 2014) and Egypt (Al-Hosary et al., 2021) reported lower prevalence of this pathogen. The differences in infection rates of *T. ovis* in this study to the studies conducted previously in different locations of Africa might be due to several factors including climate of the area, variation in the susceptibilities of animals from different geographical areas, vector activities and the sample sizes. Further studies into the seasonal dynamics, vectors and the use of larger sample size will be valuable and provide more information of *T. ovis* in the study area.

The Small Sub-Unit ribosomal RNA (SSU rRNA) gene has been used extensively in phylogenetic tree analysis of eukaryotes (Vawter and Brown, 1993). In this study, the phylogenetic analysis showed that sequences of *T. ovis* SSU rRNA gene appeared in the same clade, the finding suggests that similar strains of this pathogen are circulating in the sampled animals, which can be explained by the fact that small sample size of the small ruminants was used in this study. Therefore, to confirm this finding we recommend a more extensive study in the study site, using a larger sample size.

Notably, this study reveals that Seno province had significantly higher infection rates compared to the Oudalan province, this might be due to the geographical location of Oudalan province which is located further north towards the Sahara Desert which is relatively drier and more arid. Therefore, presumably this suggest that Oudalan province could provide more adverse environment for the thriving of the ticks than Seno province. Normally, ticks do not survive well in the drier and more arid climate (Zeleke and Bekele, 2004).

In this study, *B. ovis*, *T. lestoquardi*, *E. ruminantium* and *A. phagocytophilum* were not detected in sheep and goat samples, suggesting that these parasites are not common in the study area. However, we recommend a more extensive study with larger sample size to analyze the distribution and economic importance of tick-borne pathogens in the study area.

Mixed infection of *T. ovis* and *A. ovis* was observed in this study among goats and sheep samples, the presence of mixed infection in this study can be attributed by the system of livestock management practice in the study area. Animals owned by the transhumance pastoralists are normally in movement from one location to the other in search of pasture and water, in so doing they are exposed to different species of ticks which carry different pathogens. Technically, the use of acaricide to control ticks in the pastoralist's community is not commonly practiced. Therefore, animal movement to different pastures without the use of acaricides to control ticks could be the contributing factor of mixed infection. The significance of co-infections in animals include altering susceptibility of the animal to other infections, altering the clinical symptom patterns of the diseases in the animal, but also might have effects on the transmission risks and infection duration (Andersson et al., 2017; Bursakov and Kovalchuk, 2019).

Conclusion

This study has revealed the presence of important tick-borne hemo-parasites, namely, *T. ovis* and *A. ovis* of small ruminants in Seno and Oudalan provinces of Burkina Faso. The fact that these ruminants were asymptomatic might mean that ovine theileriosis and anaplasmosis are endemic in the study area although further studies on large number of samples are required. Phylogenetic analysis of AoMSP4 and SSU rRNA genes of *A. ovis* and *T. ovis* respectively, are highly conserved in the study area. However, we recommend more extensive studies involving larger sample size for detailed information on the distribution and economic importance of tick-borne diseases in the area.

ACKNOWLEDGEMENTS

This study was supported by a research fellowship from the Japan Society for the Promotion of Science (JSPS) for the RONPAKU program, Japan and Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and a grant from the Japan Society for the Promotion of Science (JSPS) Core-to-Core program (26304036 and 17F17105). We thank the farmers for the support, and the State veterinarians for sample collection in the field.

CONFLICT OF INTEREST

The authors declare no conflict of interest associated with this manuscript.

SUBMISSION DECLARATION AND VERIFICATION

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