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Systematic review

Molecular prevalence and distribution of tick-borne bacterial and protozoan pathogens of sheep and goats in Africa: A systematic review and meta-analysis

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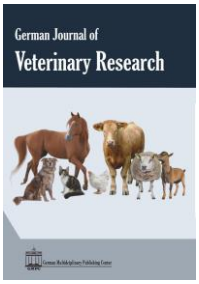

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Abstract

Tick-borne pathogens (TBPs) are a major impediment to the health, welfare, and production of small ruminants across the world, including Africa. Comprehensive information about the epidemiology of TBPs infecting sheep and goats across Africa is lacking. Therefore, this study was undertaken to determine the prevalence through a meta-analysis of selected TBPs in blood DNA from domestic sheep and goats in Africa obtained using molecular-based methods. The literature review was done according to Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines using five English electronic databases (PubMed, Science Direct, Springer Link, Web of Science, and African Journals Online (AJOL). The search was performed with no restriction in time through to 18th January 2023. Of the 63 full-text articles subjected to eligibility, only 30 articles met the eligibility criteria and were included in the review. The overall pooled prevalence of selected TBPs varied considerably between host species (sheep vs. goats), with *Anaplasma ovis* (44.50 vs. 48.40%), *Ehrlichia ruminantium* (5.50 vs. 2.00%), *Coxiella burnetii* (4.40 vs. 1.70%), *Borrelia theileri* in sheep (5.20%), *Babesia ovis* (1.70% vs. 1.90%), *Theileria ovis* (40.50% vs 10.00%), *T. separata* (1.00% vs 1.00%) and *T. lestoquardi* in sheep (8.40%). However, the prevalence of the selected TBPs was generally higher in sheep compared to goats. Several genetic loci were targeted in the characterization of tick-borne pathogens, such as *16S rDNA, groEL,* and *msp4* for *Anaplasma ovis*, *pCS20* for *Ehrlichia ruminantium*, Insertion Sequence (IS1111) for *Coxiella burnetii*, *flaB* (flagellin) and *16S rRNA* for *Borrelia theileri*, 5.8S rRNA and *18S rRNA* for *Babesia/ Theileria*, as well as the utilization of numerous PCR variants including conventional polymerase chain reaction (PCR), nested-PCR, qPCR, Loop-mediated isothermal amplification (LAMP), and reverse line blotting (RLB). In conclusion, *A. ovis* was the most widely distributed and prevalent TBP affecting small ruminants within the continent. Hence, this warrants adequate attention towards early diagnosis and treatment of infected animals as well as the control of the tick vectors involved in their transmission.

Keywords: Tick-borne, *Babesia*, *Theileria*, *Anaplasma*, Small ruminants, Genes, PCR

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Introduction

Small ruminants, especially sheep and goats, are exposed to a wide array of health problems attributed to infectious pathogens that challenge their productivity. Vector-borne pathogens transmitted by arthropod vectors epitomize a large group of these infectious diseases (Baneth, 2014; Chala and Hamde, 2021). The first arthropods to be established as vectors of pathogens were ticks (Dantas-Torres et al., 2012). Ticks and tick-borne diseases are regarded as major obstacles to livestock production in many world countries, including Africa (Ahmed et al., 2011; Onyiche et al., 2023). Next to mosquitoes, ticks are considered the most important vectors of pathogens, and more than 900 species (707

hard ticks and 210 soft ticks) have been described worldwide (Tijsse-Klasen et al., 2014).

Anaplasma and *Ehrlichia* belong to the family *Anaplasmataceae* and are transmitted by ixodid ticks. Infections with either of these bacterial infections can cause the death of their host, especially if they are co-infected with other pathogens (Dahmani et al., 2019). *Anaplasma ovis*, the causative agent of ovine anaplasmosis, is an intra-erythrocytic bacterial pathogen that parasitizes red blood cells. Although infection is often subclinical, it can occasionally be severe in debilitated small ruminants (Aubry and Geale 2011). Within Africa, *A. ovis* has been widely reported in both sheep and goats across the continent (Aouadi et al., 2017; Lee et al., 2018; Ringo et al., 2018; Dahmani et al., 2019). *Ehrlichia ruminantium*, an obligate intracellular bacterium transmitted by ticks in the genus *Amblyomma,* causes heartwater disease (Faburay et al., 2008). Small ruminants are at risk of the disease, and infections have been widely reported in Sub-Saharan Africa with varying prevalence (Djiba et al., 2013; Lee et al., 2018; Ringo et al., 2019).

Borreliosis caused by *Borrelia theileri*, a spirochaete, is an infectious disease of various domestic mammals, including small ruminants in tropical and subtropical Africa, and transmitted by hard ticks. Ticks of the genus *Rhipicephalus* (*Boophilus*) are recognized as vectors (Uilenberg et al., 1988). In addition, Q fever, caused by *Coxiella burnetii*, is an intracellular bacterium that is capable of infecting various species of domestic mammals, including small ruminants (Mares-Guia et al., 2014). Stillbirth and abortion are the two most notable clinical clinical signs of *C. burnietii* infection in small ruminants (Attia et al., 2024). Both *Bo. theileri* and *C. burnetii* are transmitted by hard ticks as these pathogens have been detected in some species of hard ticks (McCoy et al., 2014; Abdullah et al., 2021).

Babesia and *Theileria* species are responsible for babesiosis and theileriosis, respectively. They are the two most notable tick-borne piroplasmids responsible for economically important hemoparasitic diseases in ruminants (Uilenberg, 1995). Some *Theileria* species of importance in sheep and goats include *T. ovis, T. lestoquardi*, and *T. separata* (El Imam et al., 2016). *T. lestoquardi* is the causative agent of malignant ovine theileriosis, a fatal disease of sheep that has been predominantly reported in northern Africa (Hassan et al., 2019; Taha et al., 2013; Salih et al., 2012). Other species of *Theileria* (*T. ovis* and *T. separata*) cause mild forms of theileriosis (El Imam et al., 2016; Hassan et al., 2019). All these species have been reported in Africa infecting sheep and goats with prevalence varying from 0 - 88.0% (El Imam et al., 2016; Gebrekidan et al., 2014; Lee et al., 2018). *Babesia* species infecting sheep and goats include *B. ovis* and *B. motasi* (El Imam et al., 2016; Uilenberg, 2006). *Babesia* requires two hosts to complete its life cycle. Thus, it is referred to as a heterogeneous pathogen, and depending on the immune status of the host, the infection can be subclinical, clinical, or fatal (Abdelbaky et al., 2021). Of these two, *B. ovis* is highly pathogenic and has been reported widely (Schnittger et al., 2003). The molecular prevalence of *B. ovis* has been observed to be very low or non-existent (Rjeibi et al., 2014; Hussein et al., 2017).

Recently, interest in tick-borne diseases of small ruminants has increased due to the socio-economic impact in many livestock-producing countries of the world (Yin et al., 2004). Control of TBPs is dependent on the availability of accurate and timely data on the pathogen epidemiology in both the vertebrate and invertebrate hosts. Tick-borne pathogens comprising bacterial and protozoans have been recently reviewed in domestic animals, including ruminants in certain parts of Africa (Tawana et al., 2022; Defaye et al., 2022; El-Alfy et al., 2022). However, no attempt has been made to put together information on the epidemiology of some selected TBPs of small ruminants in Africa as a whole. To better understand the epidemiology of some tick-borne protozoan and bacterial pathogens of sheep and goats in Africa, a systematic review and meta-analysis was undertaken to determine the prevalence and distribution of TBPs of sheep and goats in Africa that were ascertained using molecular-based techniques.

Materials and methods

Search strategy

The protocol used for this systematic review was in line with the guidelines published by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) in carrying out this systematic review and meta-analysis (Moher et al., 2015; Page et al., 2021). Researches

published on tick-borne diseases of sheep and goats within Africa were searched on various databases such as Science Direct, AJOL, Springer Link, Web of Science, and PubMed. Additional articles were obtained through a grey literature search. Citations were searched with no restriction in time through to 31st July 2020. An additional search was conducted on 18th January 2023 to update the list of eligible studies. The search strategy involved the use of key terms "tick-borne pathogens", "ticks", "small ruminants", "sheep", goats, "Africa", "molecular", and "country name in Africa". The key terms were used either individually or in combination with the operators "and" and/or "or". Titles and their corresponding abstracts were scanned, and citations relevant in line with the aim of the study were subsequently downloaded.

Inclusion and exclusion criteria

All articles included were original research papers published in the English language and meet the following criteria: (i) tick-borne pathogens belonging to the genera *Anaplasma* (*A. ovis*), *Coxiella* (*C. burnetii*), *Ehrlichia* (*E. ruminantium*), *Borrelia* (*Bo. theileiri*) *Babesia* (*B. ovis*) and *Theileria* (*T. ovis, T. separata,* and *T. lestoquardi)* with focus on species that are specific to sheep and/or goats (ii) molecular-based techniques were utilized in the study/investigation (iii) the targeted gene used for the amplification of the tick-borne pathogen must be stated (iv) the study must be within Africa, (v) the total number of small ruminants screened was clearly stated and the number of cases (vi) the pathogen must have been detected in the blood only and not just ticks, (vii) the sample size must be at least 50, (viii) if both sheep and goats were screened in the study, the results must be separated. Published literature involving experimental studies, case reports, book chapters, editorials, letters to the editors, and reviews were excluded. Also, studies of small ruminants with discrepancies in the results were removed, as well as those involving large ruminants such as cattle.

Data extraction

Only studies meeting the inclusion criteria were cataloged, and all relevant data was extracted to a Microsoft Excel® spreadsheet. Data extracted from the eligible studies includes variables such as the authors' names, country, molecular diagnostic technique, animal species (sheep/goats), the total number of animals sampled, and the total number of positives for each tick-borne species.

Data analysis

The pooled prevalence and their corresponding 95% confidence interval (CI) were computed using Comprehensive Meta-analysis (CMA) Version 3.0 unless otherwise stated. When the pooled analysis was computed, each logit event estimate underwent a transformation in the CMA software into proportions, giving a weighted overall outcome. Cochran's heterogeneity (Q) of all the included studies, including the percentage variation (I^2), was calculated using the Cochrane Q test. If I^2 was ≤ 25%, 50%, or ≥ 75%, then heterogeneity was described as low, moderate, or high (substantial), respectively (Higgins and Thompson, 2002). Secondary outcome was analyzed using the meta-regression involving Odds ratios (ORs) and 95% CI. Forest plots were generated to visualize the data for each logit event. All pooled estimates were arrived at using the random-effects model. Funnel plots and Eggers test were used for assessing the publication bias.

Results

Search results and eligible studies

In total, 831 relevant articles were retrieved following a search on all five electronic databases, including grey literature, using the PRISMA guidelines enumerated in Figure 1. Exactly 227 articles were preceded for further review after the removal of duplicates. After that, a careful review of titles and abstracts was carried out, and 164 articles were excluded as unlikely. A total of 63 full-text articles were downloaded for detailed review for potential eligibility for inclusion. A total of 33 articles were excluded for various reasons: (i) studies that failed to identify pathogen to species level (n=13), (ii) studies with sample size below 50 (n=12), and lastly (iii) studies with inconsistent data (n=8). In total, 30 studies were further subjected to quantitative synthesis and hence were included in the study.

Characteristics of eligible studies

Table 1 shows the characteristics of all the eligible studies used for the systematic review and meta-analysis. In total, 5,631 domestic small ruminants were studied across Africa, with sheep

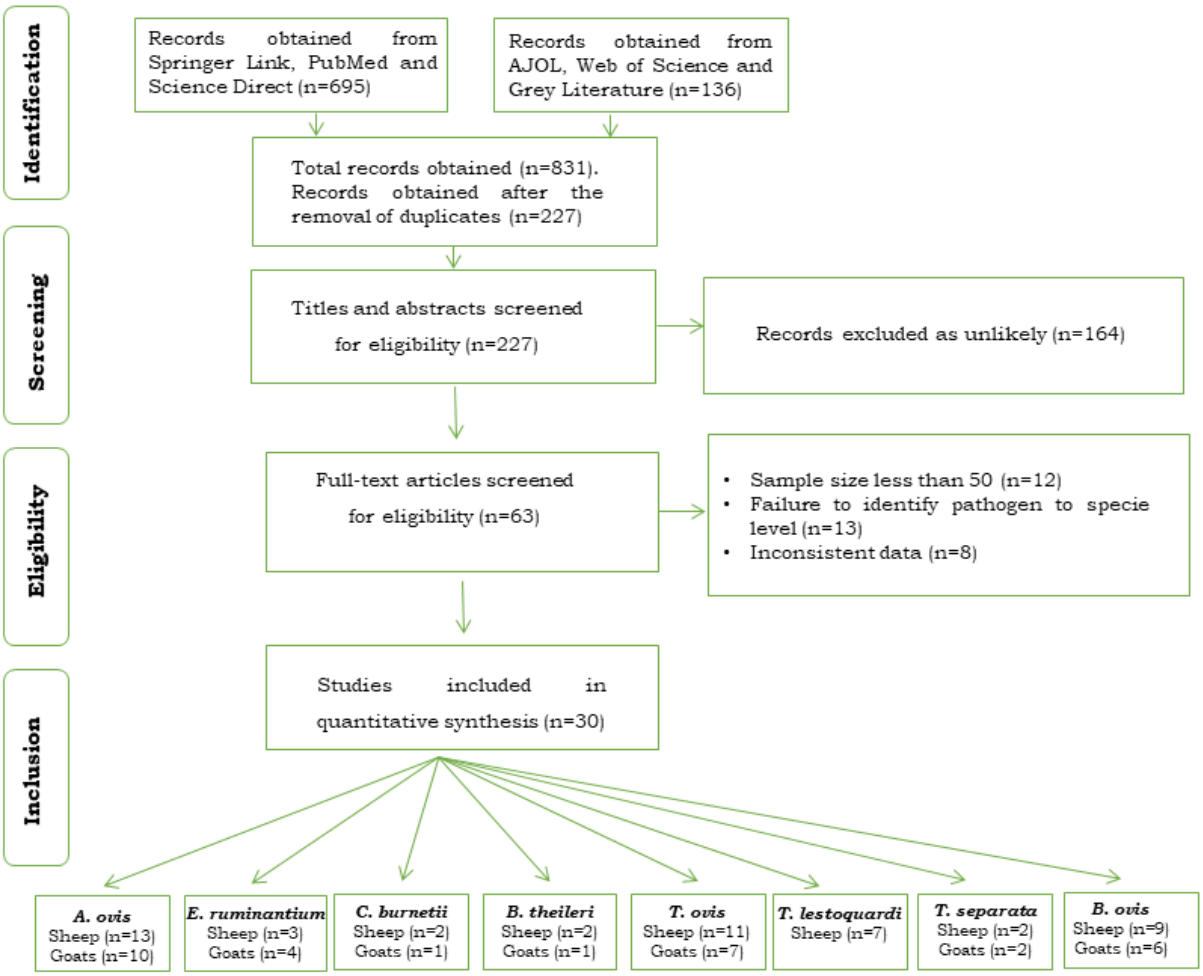

Figure 1: PRISMA flowchart used in the study for identification of eligible studies.

making the bulk of these animals, with a total of 3,634 (65.53%) live heads and goats making up the rest, with 1,997 (35.46%) live heads. The eligible studies cut across every region of the continent of Africa with northern Africa comprising of eighteen studies (Salih et al., 2012; M'ghirbi et al., 2013; Taha et al., 2013; Belkahia et al., 2014; Rjeibi et al., 2014; Ben Said et al., 2015; Rjeibi et al., 2016a; Rjeibi et al., 2016b; El Imam et al., 2016; Lee et al., 2018; Aouadi et al., 2017; Hassan et al., 2019; Tumwebaze et al., 2020a; Eisawi et al., 2020; Abdullah et al., 2021; Ben Said et al., 2022; M'ghirbi et al., 2022; ElHamdi et al., 2022), followed by southern Africa with four studies (Ringo et al., 2018; Berthelsson et al., 2020; Chatanga et al., 2021; Sili et al., 2021), eastern Africa with four studies (Gebrekidan et al., 2014; Ringo et al., 2019; Tumwebaze et al., 2020b; Kasozi et al., 2021) and western Africa with four studies (Djiba et al., 2013; Dahmani et al., 2019; Onyiche et al., 2022; Adewumi et al., 2022).

Exactly eight different tick-borne pathogens were selected, comprising four bacterial and four protozoal infections, which are known to infect small ruminants from all the eligible studies. These pathogens included *Anaplasma ovis*, *Ehrlichia ruminantium*, *Coxiella burnetii,* and *Borrelia theileri* for tick-borne bacterials and *Babesia ovis*, *Theileria ovis*, *T. separate* and *T. lestoquardi* for tick-borne protozoans. Of all the eight pathogens, *A. ovis* and *B. ovis* were the most and least common pathogens, respectively, of small ruminants. Twenty-five and fifteen studies, respectively, for sheep and goats reported the detection of at least one tick-borne pathogen. Furthermore, exactly six studies for sheep and goats reported the detection of more than three tick-borne pathogens (Table 1).

Pooled prevalence estimates of tick-borne pathogens of small ruminants in Africa

The results of the pooled prevalence estimates (PPE) and their heterogeneities for the different tick-borne pathogens are presented in Table 2.

Table 1: List and characteristics of 30 eligible studies included in the meta-analysis.

Country of study	Molecular Diagnostic Technique	Animal species	Sample size	Tick-borne bacterial (%)				Tick-borne protozoans (%)				Reference
				A. ovis	*E. ruminantium*	*C. burnetii*	*Bo. theileri*	*T. ovis*	*T. lestoquardi*	*T. separata*	*B. ovis*	
Egypt	PCR	Sheep	58	1 (1.7)	-	1 (1.7)	2 (3.4)	5 (8.6)	-	-	-	Abdullahi et al. (2021)
Nigeria	PCR	Sheep	198	73 (36.9)	-	-	-	-	-	-	-	Adewumi et al. (2022)
Algeria	PCR	Sheep	120	74 (61.6)	-	7 (5.83)	7 (5.8)	64 (53.3)	-	-	0 (0)	Aouadi et al. (2017)
	PCR	Goats	120	65 (54.2)	-	2 (1.67)	13 (10.8)	25 (20.3)	-	-	0 (0)	
Tunisia	LAMP	Sheep	204	143 (70.1)	-	-	-	-	-	-	-	Belkahia et al. (2014)
Tunisia	LAMP	Sheep	260	198 (76.2)	-	-	-	-	-	-	-	Ben Said et al. (2015)
	LAMP	Goats	303	244 (80.5)	-	-	-	-	-	-	-	
Egypt	PCR	Sheep	355	55 (15.5)	-	-	-	-	-	-	-	Ben Said et al. (2022)
Botswana	PCR	Goats	100	76 (76.0)	-	-	-	-	-	-	-	Berthelsson et al. (2020)
Malawi	PCR	Goats	99	61 (61.6)	1 (1.0)	-	-	48 (48.5)	-	2 (2.0)	-	Chatanga et al. (2021)
Senegal	PCR	Sheep	136	76 (55.8)	-	-	-	-	-	-	-	Dahmani et al. (2019)
Senegal	PCR	Sheep	120	38 (31.7)	-	-	-	-	-	-	-	Djiba et al. (2013)
	PCR	Goats	120	-	6 (5.0)	-	-	-	-	-	-	
Sudan	PCR	Sheep	200	65 (32.5)	-	-	-	-	-	-	-	Eisawi et al. (2020)
	PCR	Goats	198	71 (35.9)	-	-	-	-	-	-	-	
Tunisia	PCR	Sheep	116	51 (43.9)	-	-	-	-	-	-	-	ElHamdi et al. (2022)
Sudan	PCR	Sheep	219	-	-	-	-	74 (33.8)	0 (0)	0 (0)	-	El imam et al. (2016)
Ethiopia	PCR	Sheep	160	-	-	-	-	147 (91.9)	-	3 (1.9)	0 (0)	Gebrekidan et al. (2014)
	PCR	Goats	265	-	-	-	-	4 (1.5)	-	1 (0.4)	0 (0)	
Sudan	RLB	Sheep	393	-	-	-	-	-	116 (29.5)	-	0 (0)	Hassan et al. (2019)
Uganda	PCR	Goats	666	169 (25.4)	-	-	-	-	-	-	-	Kasozi et al. (2021)
Sudan	PCR	Sheep	62	52 (83.9)	0 (0)	-	-	23 (37.1)	0 (0)	-	0 (0)	Lee et al. (2018)
	PCR	Goats	116	52 (47.4)	1 (0.9)	-	-	0 (0)	0 (0)	-	0 (0)	

Tunisia	PCR	Sheep	199	-	-	-	-	56 (28.1)	-	-	-	M'ghirbi et al. (2013)
	PCR	Goats	64	-	-	-	-	3 (4.7)	-	-	-	
Tunisia	PCR	Sheep	199	160 (80.4)	-	-	-	-	-	-	-	M'ghirbi et al. (2022)
	PCR	Goats	64	45 (70.3)	-	-	-	-	-	-	-	
Nigeria	PCR	Sheep	162	-	-	-	-	-	-	-	0 (0)	Onyiche et al. (2022)
	PCR	Goats	184	-	-	-	-	-	-	-	0 (0)	
Tunisia	PCR	Sheep	270	-	-		-	44 (16.3)	-	-	-	Rjeibi et al. (2016a)
Tunisia	PCR	Sheep	166	-	-	-	-	-	2 (1.2)	-	-	Rjeibi et al. (2016b)
Tunisia	PCR	Sheep	172	-	-	-	-	10 (5.8)	-	-	30 (17.4)	Rjeibi et al. (2014)
	PCR	Goats	166	-	-	-	-	0 (0)	-	-	15 (9.0)	
South Africa	PCR	Goats	61	28 (45.9)	12 (19.6)	-	-	54 (88.5)	-	-	-	Ringo et al. (2018)
Kenya	PCR	Sheep	76	26 (34.2)	6 (7.9)	-	-	39 (51.3)	0 (0)	-	0 (0)	Ringo et al. (2019)
Sudan	PCR	Sheep	100	-	-	-	-	-	24 (24.0)	-	-	Salih et al. (2012)
Angola	PCR	Sheep	85	-	-	-	-	68 (80.0)	-	-	-	Sili et al. (2021)
Sudan	PCR	Sheep	51	-	-	-	-	-	15 (29.4)	-	-	Taha et al. (2013)
Egypt	PCR	Sheep	66	6 (9.1)	-	-	-	-	-	-	-	Tumwebaze et al. (2020a)
Uganda	PCR	Goats	201	11 (5.5)	1 (0.5)	-	-	-	-	-	11 (5.5)	Tumwebaze et al. (2020b)

PCR: polymerase chain reaction; RLB: reverse line blotting; LAMP: loop-mediated isothermal amplification

Table 2: Meta-analysis of molecular detection of tick-borne pathogens of sheep and goats in Africa.

Subgroup	Number of studies	Pooled prevalence estimates			Measure of heterogeneity			Meta-regression	
		Sample size	No. of positives	Prevalence 95% CI (%)	*Q*	I^2	*Q-p*	*p*-value	OR (95% CI)
A. ovis									
Sheep	13	1972	945	44.50 (30.00 - 60.10)	414.73	97.11	*p*<0.000	0.0014	a
Goats	10	1928	825	48.40 (31.90 - 65.30)	364.69	97.53	*p*<0.000		1.23 (1.08 – 1.39)
E. ruminantium									
Sheep	3	258	12	5.50 (2.70 – 10.90)	2.81	28.84	*p*<0.000	0.3098	a
Goats	4	477	15	2.00 (0.20 – 17.30)	27.66	89.15	*p*<0.000		1.50 (0.69 – 3.26)
C.burnetii									
Sheep	2	178	8	4.40 (1.60 -11.60)	1.36	26.54	*p*=0.243	0.3254	a
Goats	1	120	2	1.7 (0.40 - 6.40)	0.00	0.00	*p*=1.000		2.78 (0.58 - 13.31)
Bo. theileri									
Sheep	2	178	9	5.2 (2.70 – 9.70)	0.45	0.00	*p* = 0.501	0.0724	a
Goats	1	120	13	10.80 (6.40 – 17.80)	0.00	0.00	*p* = 1.000		0.44 (0.18 - 1.06)
T. ovis									
Sheep	11	1429	533	40.50 (24.30 – 59.10)	291.87	96.57	*p*<0.000	*p*<0.0001	a
Goats	7	891	134	10.0 (2.40 – 33.60)	151.49	96.04	*p*<0.000		3.36 (2.72-4.16)
T. lestoquardi									
Sheep	7	1067	157	8.40 (3.60 – 18.30)	52.99	88.68	*p*<0.000	-	-
T. separata									
Sheep	2	379	3	1.00 (0.10 - 6.30)	1.93	48.18	*p* = 0.165	*p*=1.000	a
Goats	2	364	3	1.00 (0.20 - 5.00)	1.89	47.26	*p*= 0.169		0.96 (0.19 - 4.79)
B. ovis									
Sheep	9	1562	103	1.70 (0.50 – 5.60)	93.49	91.44	*p*<0.000	*p*<0.0001	a
Goats	6	1052	26	1.90 (0.60 – 5.60)	21.25	76.47	*p*<0.001		2.79 (1.79 - 4.32)

[a] denotes reference value; I^2: inverse variance; Q: measure of heterogeneity; Q-p: Cochran's; OR: Odd ratio: CI: confidence interval

Anaplasma ovis

The overall PPE due to *A. ovis* in the goats was 48.4% (95% CI: 31.9 - 65.3), while that of sheep was 44.5% (95% CI: 30 - 60.1). No statistically significant difference was observed between species (p>0.05).

Ehrlichia ruminantium

The PPE due to *E. ruminantium* was higher in the sheep (5.50%, 95% CI: 2.7 – 10.9) compared to the goats (2.0%, 95% CI: 0.2 – 17.3). The difference was not statistically significant (p>0.05).

Coxiella burnetii

In the goats, the PPE attributed to *C. burnetii* was 1.7% (95% CI: 0.4 – 6.4), which was lower compared to 4.4% (95% CI: 1.6 – 11.6) obtained in the sheep. The difference was not statistically significant (p>0.05).

Borrelia theileri

The infection of goats with *Bo. theileri* was moderate with a PPE of 10.8% (95% CI: 6.4 – 17.8) compared with sheep with a PPE of 5.30% (95% CI: 2.7 – 9.7). The difference was not statistically significant (p>0.05).

Theileria ovis

The PPE in the sheep was 40.5% (95% CI: 24.3 - 59.1) higher than that of the goats, 10.% (95% CI: 2.4 – 33.6). A significant difference was observed with regards to animal species (p<0.0001), and the odds of positivity was 3.36 times (OR=3.36; 95% CI: 2.72 - 4.16) more likely in the sheep compared to the goats.

Theileria lestoquardi

The PPE in the sheep was 8.4% (95% CI: 3.6 – 18.3, Q-p<0.000).

Theileria separata

The PPE was exactly 1.0% (95% CI: 0.1 - 6.3) in sheep and 1.0% (95% CI: 0.2 - 5.0) in the goats. No significant difference was observed between animal species (p>0.05).

Babesia ovis

With regards to goats, the PPE was 1.9% (95% CI: 0.6 - 5.6), higher than that of the sheep, which was 1.7% (95% CI: 0.5 - 5.6). Interestingly, the likelihood of infection was 2.79 times greater (OR: 2.79, 95% CI: 1.79 – 4.32) in the goats compared to sheep. A statistically significant difference (p<0.0001) was observed between species.

Publication bias

The funnel plots and their corresponding Egger's coefficient indicate no significant bias with regard to *A. ovis* in the sheep (b: -3.02; p =0.28) and goats (b: 5.29; p=0.17). Similarly, for *E. ruminantium* in the sheep (b: -1.97; p=0.07) and in the goats (b: -4.13; p=0.16). Also, for *T. ovis* in sheep (b:4.82; p=0.28) while in the goats (b: -3.70; p=0.19). Significant bias was observed in *B. ovis* in the sheep (b: -3.94; p=0.00011) and goats (b:-2.91; p=0.00072) as well as in *T. lestoquardi* in sheep (b: -3.55; p=0.002).

Target genes and molecular diagnostic techniques for the detection of tick-borne bacterial pathogens

Of the seventeen eligible studies that screened for *A. ovis*, thirteen targeted the *msp*4 gene, while seven utilized the *16S rDNA* gene (Table 3). Other genes targeted include *gro*EL, with three eligible studies, and lastly, two studies used the *23S rRNA* gene as their target locus. Molecular diagnostic techniques employed for the screening of *A. ovis* include conventional PCR, nested PCR, qPCR, and LAMP (Table 3).

Three eligible studies utilized the *pCS20* gene, while two studies utilized 16S rRNA and *gro*EL as their preferred targeted genes for the amplification of *E. ruminantium* (Table 4). Both conventional and nested PCR were employed as diagnostic assays for screening *E. ruminantium* in sheep and goat blood DNA.

With regards to *C. burnetii*, the insertion sequence (*IS1111*) gene was widely utilized as the preferred target for the amplification of *C. burnetii*, while the *fla*B (flagellin) and *16S rRNA* genes were targeted for the amplification of *Bo. theileri* (Table 4).

Target genes and molecular diagnostic techniques for the detection of tick-borne protozoan pathogens

The *18S rRNA* was the most preferred target gene for detecting piroplasmids (*Theileria* and *Babesia*). Different PCR variants, including conventional PCR, reverse line blotting (RLB), nested-PCR, qPCR, and restriction fragment length polymorphism (RFLP)-PCR, have been registered and used for screening blood DNA (Table 5).

Table 3: Target gene(s) and molecular diagnostic techniques employed from eligible studies in the detection of *Anaplasma ovis*.

Target gene(s)	Molecular diagnostic technique/variant	Reference
23S rRNA and Ana-*rpoB*	qPCR and conventional PCR	Abdullah et al. (2021)
23S rRNA	qPCR	Aouadi et al. (2017)
msp4	LAMP	Belkahia et al. (2014)
msp4	LAMP	Ben Said et al. (2015)
msp4	Conventional PCR	Ben Said et al. (2022)
msp4	Conventional PCR	Berthelsson et al. (2020)
16S rDNA, groEL and *msp4*	Conventional PCR	Chatanga et al. (2021)
16S rRNA and *23S* rRNA	Conventional PCR	Dahmani et al. (2019)
16S rRNA and *groEL*	Conventional PCR	Djiba et al. (2013)
16S rRNA and *msp4*	Conventional PCR	Eisawi et al. (2020)
16S rRNA and *msp4*	Conventional PCR	ElHamdi et al. (2022)
16S rRNA and *msp4*	Conventional PCR	Kasozi et al. (2021)
16S rRNA, msp4 and *groEL*	nPCR	Lee et al. (2018)
msp4	Conventional PCR	M'ghirbi et al. (2022)
msp4	Conventional PCR	Ringo et al. (2018)
msp4	Conventional PCR	Ringo et al. (2019)
msp4	Conventional PCR	Tumwebaze et al. (2020a)

qPCR: real-time polymerase chain reaction; LAMP: loop-mediated isothermal amplification; nPCR: nested PCR

Table 4: Target gene(s) and molecular diagnostic techniques employed from eligible studies in the detection of *Ehrlichia ruminantium*, *Coxiella burnetii,* and *Borrelia theileri*.

Target gene(s)	Molecular diagnostic technique/variant	Reference
Ehrlichia ruminantium		
16S rRNA and *groEL*	Conventional PCR	Chatanga et al. (2021)
16S rRNA and groEL	Conventional PCR	Djiba et al. (2013)
pCS20	Semi-nested PCR	Lee et al. (2018)
pCS20	Conventional PCR and nPCR	Ringo et al. (2019)
pCS20	Conventional PCR	Tumwebaze et al. (2020b)
Coxiella burnetii		
Insertion Sequence (*IS1111*) gene spacer and *IS30a* spacer	qPCR	Aouadi et al. (2017)
IS1111 and intergenic spacers (Cox2, Cox5 and Cox18)	qPCR and MST	Abdullah et al. (2021)
Borrelia theileri		
rrs, flaB (flagellin), and *16S rRNA*	qPCR and conventional PCR	Aouadi et al. (2017)
Internal transcribed spacer *16S RNA*	qPCR	Abdullah et al. (2021)

qPCR: real-time polymerase chain reaction; nPCR: Nested PCR; MST: multispacer typing

Discussion

In this systematic review and meta-analysis, we estimated the pooled prevalence of tick-borne pathogens, including *A. ovis*, *E. ruminantium*, *Bo. theileri*, *C. burnetii T. ovis*, *T. lestoquardi*, *T. separate* and *B. ovis* of sheep and goats in Africa. The results from this study indicate that the PPE of these bacterial and protozoan pathogens analyzed varied considerably.

Anaplasma ovis, the agent of small ruminant anaplasmosis has been widely reported across every region in Africa (Ben Said et al., 2015; Dahmani et al., 2019; Ringo et al., 2019; Berthelsson et al., 2020; Tumwebaze et al., 2020a; Tumwebaze et al., 2020b; Kasozi et al., 2021; Chatanga et al., 2021; Ben Said et al., 2022). Overall, the PPE was slightly higher in the goats compared to sheep. On the contrary, it has been documented that sheep appear to be more sensitive than goats to infection with *A. ovis,* as observed in comparative studies undertaken in Sudan (Lee et al., 2018) and Algeria (Aouadi et al., 2017). Individually, the prevalence in small ruminants ranges from 1.72% - 83.87% and higher prevalence above 70% has been reported in Tunisia, Algeria, Botswana, and Sudan in both sheep and goats (Aouadi et al., 2017; Belkahia et al., 2014; Ben Said et al., 2015; Berthelsson et al., 2020).

Table 5: Target gene(s) and molecular diagnostic techniques employed from eligible studies in the detection of *Babesia*/ *Theileria*.

Target gene(s)	Molecular diagnostic technique/variant	Reference
***Theileria* species**		
5.8S rRNA and *18S rRNA*	qPCR and Conventional PCR	Abdullah et al. (2021)
18S rRNA, 28S rRNA,	qPCR and Conventional PCR	Aouadi et al. (2017)
18S rRNA	RLB and nPCR	Chatanga et al. (2021)
18S rRNA	RLB	El Imam et al. (2016)
18S rRNA	Conventional PCR	Gebrekidan et al. (2014)
18S rRNA	RLB and nPCR	Hassan et al. (2019)
Immunodominant antigen	Conventional PCR	Lee et al. (2018)
18S rRNA	Conventional PCR	M'ghirbi et al. (2013)
18S rRNA	nPCR and Conventional PCR	Ringo et al. (2018)
18S rRNA	nPCR and Conventional PCR	Ringo et al. (2019)
18S rRNA	RLB, nPCR, and RFLP	Rjeibi et al. (2014)
18S rRNA	Nested-PCR, RFLP	Rjeibi et al. (2016a), Rjeibi et al. (2016b)
18S rRNA	LAMP	Salih et al. (2012)
18S rRNA	RLB	Sili et al. (2021)
Babesia ovis		
ssu rRNA	Conventional PCR	Adewumi et al. (2022)
18S rRNA, 28S rRNA,	qPCR and Conventional PCR	Aouadi et al. (2017)
18S rRNA	RLB	El Imam et al. (2016)
18S rRNA	Conventional PCR	Gebrekidan et al. (2014)
18S rRNA	RLB and nPCR	Hassan et al. (2019)
18S rRNA	Conventional PCR	Lee et al. (2018)
18S rRNA	Conventional PCR	Onyiche et al. (2022)
18S rRNA	Conventional PCR	Ringo et al. (2019)
18S rRNA	RLB and Conventional PCR	Rjeibi et al. (2014)
ssu rRNA	Conventional PCR	Tumwebaze et al. (2020b)

qPCR: real-time polymerase chain reaction; nPCR: nested-polymerase chain reaction; RLB: reverse line blotting; LAMP: loop-mediated isothermal amplification; restriction fragment length polymorphism (RFLP)

On the other hand, a lower prevalence below 10% has been sparingly reported, as observed in Egypt (Tumwebaze et al., 2020a; Abdullah et al., 2021). Differences in farm management and husbandry, tick control programs, wildlife reservoir hosts, and other abiotic factors may be responsible for the discrepancies in prevalence values (Belkahia et al., 2014). The most targeted genes for *Anaplasma* species (including *A. ovis*) from published literature in Africa were those of *16S rRNA*, major surface protein *(msp*4), and heat shock protein (*gro*EL) (Djiba et al., 2013; Lee et al., 2018; Chatanga et al., 2021; Kasozi et al., 2021; ElHamdi et al., 2022). On the whole, most of the eligible literature utilized the *msp*4 gene locus for the molecular screening of biological samples for *A. ovis* (Belkahia et al., 2014; Ben Said et al., 2015; Ringo et al., 2019; Ben Said et al., 2022; M'ghirbi et al., 2022). The multicopy *msp* approaches are preferred over single-copy genes for the molecular screening of biological samples, while for phylogenetic inferences and database crossmatch, the *groEL* gene is regarded as the best choice (Silaghi et al., 2017). This is true as we observed that 12 out of the 17 eligible studies dealing with *A. ovis* utilized the *msp4* gene locus for the molecular detection of *A. ovis* from blood DNA from sheep and goats. Both *msp4* and *gro*EL gene loci were widely used for the phylogenetic analysis of *A. ovis*. The multilocus approach was also used in several eligible studies for the confirmation of new strain/variant species, as this has been strongly encouraged (Silaghi et al., 2017). Conventional PCR was the most notable and convenient molecular assay variant for the molecular detection of *A. ovis* in small ruminants across the African continent.

Ehrlichia ruminantium is the causative agent of heartwater disease in ruminants and is transmitted by ticks belonging to the genus *Amblyomma*. The overall prevalence estimates were higher in sheep compared with goats. The prevalence estimate in sheep is similar to the 5.0% reported in Senegal (Djiba et al., 2013). On the other hand, the prevalence estimates for goats in three out of four eligible studies were lower or equal to 1.0% (Lee et al., 2018; Tumwebaze et al., 2020b; Chatanga et al., 2021). The reason for the difference in prevalence between sheep and goats is unknown. However, we speculate that immunity may play a role as goats are generally known to be somewhat resistant to infectious diseases compared to sheep. On the other hand, *Bo. theileri* is the aetiological agent of bovine and ovine borreliosis. Our systematic review documented this pathogen's existence in sheep and goats within the continent (Aouadi et al.,

2017; Abdullah et al., 2021). It is transmitted by hard ticks, mainly *Rhipicephalus* species, and has been detected in *Rh. geigyi* in Mali (McCoy et al., 2014). *Bo. theileri* has previously been detected in *Rh. annulata* ticks in Egypt (Hassan et al., 2017). Several genes or targeted regions have been utilized for the molecular detection of *E. ruminantium,* including *pCS*20 (Steyn et al., 2008; Van Heerden et al., 2004), *map1* gene (Allsopp et al., 2001; Faburay et al., 2008) and the 16S ribonucleic acid (RNA) gene (Allsopp et al., 1997). From published studies, the *pCS*20 gene was the most utilized for the molecular detection and characterization of *E. ruminantium* in studies across the continent. This could be attributed to a great extent to the high degree of conservation of the *pCS*20 target nucleotide sequence in *E. ruminantium* as observed in a previous study (Faburay et al., 2007) compared with either *map1* or 16S rRNA genes. Sequence polymorphisms of the *map1* gene of *E. ruminantium* isolates have been reported (Reddy et al., 1996; Allsopp et al., 2001), leading to low PCR detection rates. Furthermore, nested-PCR or conventional PCR molecular diagnostic variants targeting the *pCS*20 gene locus exhibited higher sensitivity and detection rates. Thus, all the eligible studies included in this review utilized either of the two molecular diagnostic techniques for the detection of *E. ruminantium* in small ruminants across Africa.

Molecular evidence of *C. burnetii* in the blood of small ruminants, as observed in this study, was low across Africa. The low prevalence probably suggests a low load of the bacteria in animal blood. This organism is responsible for Q fever and is known to infect a wide range of animals, including small ruminants (Mares-Guia et al., 2014). This pathogen is known to be associated with abortion and has been registered from the vaginal discharge and placenta of dairy goats in Egypt (Abdel-Moein and Hamza, 2017). The transposon-like repetitive region (*IS1111*) is one of the most reliable targets for the molecular characterization of *C. burnetii* and has been proven to be highly specific and sensitive for the detection of this bacterium in clinical samples (Vaidya et al., 2008; Vaidya et al., 2010).

Piroplasmosis in small ruminants caused by *Babesia ovis* has been seldom reported in sheep and goats across Africa. The PPE was very low at about 1.90% in goats and 1.70% in sheep. Nonetheless, it is believed that the signs of clinical infection are more frequently observed in sheep compared with goats. The majority of the studies reported the absence of infection with *B. ovis* in both sheep and goats (Gebrekidan et al., 2014; Lee et al., 2018; Aouadi et al., 2017; Hassan et al., 2019; Onyiche et al., 2022). So far, only three documented reports in Africa on the presence of *B. ovis* DNA in the blood of sheep and goats (Rjeibi et al., 2014; Tumwebaze et al., 2020b; Adewumi et al., 2022). However, the prevalence is closely associated with the distribution of the tick vector, *Rhipicephalus bursa,* common in the Palaearctic region, including the Mediterranean basin, with northern Africa to the south (Yeruham et al., 1998). Additionally, *B. ovis* piroplasmid has been amplified in *Rh. bursa* and *Rh. turanicus* ticks in Algeria (Aouadi et al., 2017). Theileriosis in small ruminants is caused by several *Theileria* species (Berggoetz et al., 2014). Initially, *T. ovis* was regarded as less virulent compared to *T. lestoquardi*, the causative agent of malignant ovine theileriosis. Both pathogens are present in the arid regions of Africa (Ahmed et al., 2013). In contrast to *T. lestoquardi,* which is more virulent, both *T. ovis* and *T. separata* are considered benign, and infections in small ruminants are subclinical (Uilenberg, 1995). We observed that infection with *T. ovis* was higher in sheep compared to goats. This observation is consistent with the majority of the studies in Africa (Gebrekidan et al., 2014; Aouadi et al., 2017; Lee et al., 2018). This may be unconnected with genetic variation among animals as well as the presence and abundance of tick species (Gebrekidan et al., 2014). The PPE of *T. lestoquardi* in sheep in this study is moderately low. Reports of infection with *T. lestoquardi* have so far been exclusively in sheep and mostly in northern Africa, precisely in Sudan and Tunisia (Salih et al., 2012; Taha et al., 2013; Rjeibi et al., 2014; Hassan et al., 2019). Previously, experimental infection of goats with *T. lestoquardi* was unsuccessful (El Hussein et al., 2004), but natural outbreaks in goats have been reported in Sudan (Taha et al., 2011). Reports on *T. separata* are limited with just two eligible studies, one each from Eastern (Gebrekidan et al., 2014) and northern Africa (El Imam et al., 2016). PCR-based studies for the molecular identification of *Babesia* and *Theileria* parasites in small ruminants across Africa have majorly targeted the *18S rRNA* gene locus. Piroplasmids have been identified using

different regions of the *18S rRNA* gene. The eukaryotic *18S rRNA* gene has both variable and conserved regions. Consequently, its high specificity and sequence conservation make it an ideal target as a universal biomarker to screen closely related species and biodiversity studies (Kumar et al., 2022). This was not different as this gene locus was the most utilized across the eligible studies for the detection of piroplasmids in small ruminants in Africa (M'ghirbi et al., 2013; Rjeibi et al., 2014; Gebrekidan et al., 2014; Hassan et al., 2019; Onyiche et al., 2022). Interestingly, different molecular techniques and their variants, including conventional PCR, nested PCR, reverse line blot, qPCR, and LAMP, have been utilized from the eligible studies.

In the majority of the TBPs, the prevalence was comparatively higher in sheep compared with goats. Two possible reasons were cautiously hypothesized to support this observation by previous authors. Firstly, the vast quantity of hair in sheep compared with goats could prevent the easy detection of ticks, as observed in native indigenous breeds in Ethiopia (Gebrekidan et al., 2014) and Tunisia (Rjeibi et al., 2014). Secondly, the natural differences in resistance against TBPs exist between animal species (sheep vs goats) (Lee et al., 2018). For example, experimental infection of *T. lestoquardi* was unsuccessful in goats despite the isolate having been collected from sheep (El Hussein et al., 2004).

Conclusions

Findings from this study indicate that small ruminants in Africa are infected with a diverse range of TBPs of veterinary and economic importance. With the exception of *T. lestoquardi*, all other TBPs, including bacterial (*A. ovis*, *E. ruminantium*, *C. burnetii,* and *Bo. theileri*) and protozoal (*B. ovis*, *T. ovis,* and *T. separata*) have been registered in both sheep and goats. Knowledge of their prevalence and distribution across several regions of Africa will require a robust control program against the tick vectors responsible for the transmission of these pathogens within the continent. Finally, several gene targets and PCR-based diagnostic variants have been extensively investigated for the molecular characterization of TBPs of small ruminants across the African continent.

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References

Abdel-Moein, K.A., Hamza, D.A., 2017. The burden of *Coxiella burnetii* among aborted dairy animals in Egypt and its public health implications. Acta Tropica 166, 92-95. https://doi.org/10.1016/j.actatropica.2016.11.011

Abdelbaky, H.H., Umeda, K., Nguyen, T., Mohamed, A.E.A., Fereig, R.M., 2021. A review on current knowledge of major zoonotic protozoan diseases affecting farm and pet animals. German Journal Veterinary Research 1, 61-76. https://doi.org/10.51585/gjvr.2021.2.0021

Abdullah, H.H., Amanzougaghene, N., Dahmana, H., Louni, M., Raoult, D., Mediannikov, O., 2021. Multiple vector-borne pathogens of domestic animals in Egypt. PLoS Neglected Tropical Disease 15, e0009767. https://doi.org/10.1371/journal.pntd.0009767

Adewumi, T.S., Takeet, M.I., Akande, F.A., Sonibare, A.O., Okpeku, M., 2022. Prevalence and molecular characterization of *Babesia ovis* infecting sheep in Nigeria. Sustainability 14, 16974. https://doi.org/10.3390/su142416974

Ahmed, B.M., Taha, K.M., Enan, K.A., Elfahal, A.M., El Hussein, A.R.M., 2013. Attenuation of *Theileria lestoquardi* infected cells and immunization of sheep against malignant ovine theileriosis. Vaccine 31, 4775-4781. https://doi.org/10.1016/j.vaccine.2013.08.004

Ahmed, J., Yin, H., Bakheit, M., Liu, Z., Mehlhorn, H., Seitzer, U. 2011. Small Ruminant Theileriosis. In: Mehlhorn, H. (eds) Progress in Parasitology. Parasitology Research Monographs, vol 2. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-21396-0_8

Allsopp, M.T.E.P., Dorfling, C.M., Maillard, J.C., Bensaid, A., Haydon, D.T., Van Heerden, H., 2001. *Ehrlichia ruminantium* major antigenic protein gene (map1) variants are not geographically constrained and show no evidence of having evolved under positive selection pressure. Journal of Clinical Microbiology 39, 4200–4203. https://doi.org/10.1128/JCM.39.11.4200-4203.2001

Allsopp, M.T.E.P., Visser, E.S., Du Plessis, J.L., Vogel, S.W. Allsopp, B.A., 1997. Different organisms associated with heartwater as shown by analysis of 16S ribosomal RNA gene sequences. Veterinary Parasitology 71, 283–300. https://doi.org/10.1128/JCM.39.11.4200-4203.2001

Aouadi, A., Leulmi, H., Boucheikhchoukh, M., Benakhla, A., Raoult, D., Parola, P., 2017. Molecular evidence of tick-borne hemoprotozoan-parasites (*Theileria ovis* and *Babesia ovis*) and bacteria in ticks and blood from small ruminants in Northern Algeria. Comparative Immunology, Microbiology and Infectious Disease 50, 34-39. https://doi.org/10.1016/j.cimid.2016.11.008

Attia, M.M.M., Mahmoud, H.Y.A.H., Ali, A.O., Fereig, R.M., 2024. *Coxiella burnetii* seroprevalence, risk factors, and health hazards in sheep and goats in Upper Egypt. German Journal Veterinary Research 4, 23-31. https://doi.org/10.51585/gjvr.2024.1.0069

Aubry, P., Geale, D.W., 2011. A review of bovine anaplasmosis. Transboundary and Emerging Disease 58, 1-30. https://doi.org/10.1111/j.1865-1682.2010.01173.x

Baneth, G., 2014. Tick-borne infections of animals and humans: A common ground. International Journal of Parasitology 44, 591-596. https://doi.org/10.1016/j.ijpara.2014.03.011

Belkahia, H., Said, M.B., El Hamdi, S., Yahiaoui, M., Gharbi, M., Daaloul-Jedidi, M. et al., 2014. First molecular identification and genetic characterization of *Anaplasma ovis* in sheep from Tunisia. Small Ruminant Research 121, 404-410. https://doi.org/10.1016/j.smallrumres.2014.07.009

Ben Said, M., Attia, K.A., Alsubki, R.A., Mohamed, A.A., Kimiko, I., Selim, A., 2022. Molecular epidemiological survey, genetic characterization and phylogenetic analysis of *Anaplasma ovis* infecting sheep in Northern Egypt. Acta Tropica 229, 106370. https://doi.org/10.1016/j.actatropica.2022.106370

Ben Said, M., Belkahia, H., Alberti, A., Zobba, R., Bousrih, M., Yahiaoui, M. et al., 2015. Molecular survey of *Anaplasma* species in small ruminants reveals the presence of novel strains closely related to *A. phagocytophilum* in Tunisia. Vector-Borne Zoonotic Diseases 15, 580-590. https://doi.org/10.1089/vbz.2015.1796.

Berggoetz, M., Schmid, M., Ston, D., Wyss, V., Chevillon, C., Pretorius, A.M. et al., 2014. Tick-borne pathogens in the blood of wild and domestic ungulates in South Africa: Interplay of game and livestock. Ticks and Tick-borne Diseases 5, 166-175. https://doi.org/10.1016/j.ttbdis.2013.10.007

Berthelsson, J., Ramabu, S.S., Lysholm, S., Aspán, A., Wensman, J.J., 2020. *Anaplasma ovis* infection in goat flocks around Gaborone, Botswana. Comparative Clinical Pathology 29, 167-172. https://doi.org/10.1007/s00580-019-03044-4

Chala, B., Hamde, F., 2021. Emerging and re-emerging vector-borne infectious diseases and the challenges for control: a review. Frontiers in Public Health 9, 715759. https://doi.org/10.3389/fpubh.2021.715759

Chatanga, E., Kainga, H., Maganga, E., Hayashida, K., Katakura, K., Sugimoto, C. et al., 2021. Molecular identification and genetic characterization of tick-borne pathogens in sheep and goats at two farms in the central and southern regions of Malawi. Ticks and Tick-Borne Diseases 12, 101629. https://doi.org/10.1016/j.ttbdis.2020.101629

Dahmani, M., Davoust, B., Sambou, M., Bassene, H., Scandola, P., Ameur, T. et al., 2019. Molecular investigation and phylogeny of species of the Anaplasmataceae infecting animals and ticks in Senegal. Parasite and Vectors 12, 1-15. https://doi.org/10.1186/s13071-019-3742-y

Dantas-Torres, F., Chomel, B.B., Otranto, D., 2012. Ticks and tick-borne diseases: A One Health perspective. Trends in Parasitology 28, 437-446. https://doi.org/10.1016/j.pt.2012.07.003

Defaye, B., Moutailler, S., Pasqualini, V., Quilichini, Y., 2022. Distribution of tick-borne pathogens in domestic animals and their ticks in the countries of the Mediterranean basin between 2000 and 2021: A systematic review. Microorganisms 10, 1236. https://doi.org/10.3390/microorganisms10061236

Djiba, M.L., Mediannikov, O., Mbengue, M., Thiongane, Y., Molez, J.F., Seck, M.T. et al., 2013. Survey of Anaplasmataceae bacteria in sheep from Senegal. Tropical Animal Health and Production 45, 1557-1561. https://doi.org/10.1007/s11250-013-0399-y

Eisawi, N.M., El Hussein, A.R.M., Hassan, D.A., Musa, A.B., Hussien, M.O., Enan, K.A. et al., 2020. A molecular prevalence survey on Anaplasma infection among domestic ruminants in Khartoum State, Sudan. Tropical Animal Health and Production 52, 1845-1852. https://doi.org/10.1007/s11250-019-02176-7

ElHamdi, S., Mhadhbi, M., Ben Said, M., Mosbah, A., Gharbi, M., Klabi, I., Messadi, L. 2022. Anaplasma ovis prevalence assessment and cross-validation using multiparametric screening approach in sheep from central Tunisia. Pathogens, 11(11), 1358. https://doi.org/10.3390/pathogens11111358

El Hussein, A.M., Majid, A.M., Hassan, S.M., 2004. The present status of tick-borne diseases in the Sudan. Archive Institute de Pasteur de Tunis 81, 31-34.

El Imam, A.E., Hassan, S.M., Gameel, A.A., El Hussein, A.M., Taha, K.M., Oosthuizen, M.C., 2016. Molecular identification of different *Theileria* and *Babesia* species infecting sheep in Sudan. Annals of Parasitology 62. https://doi.org/10.17420/ap6201.31

El-Alfy, E.S., Abbas, I., Baghdadi, H.B., El-Sayed, S.A.E.S., Ji, S., Rizk, M.A., 2022. Molecular epidemiology and species diversity of tick-borne pathogens of animals in Egypt: A systematic review and meta-analysis. Pathogens 11, 912. https://doi.org/10.3390/pathogens11080912

Faburay, B., Jongejan, F., Taoufik, A., Ceesay, A., Geysen, D., 2008. Genetic diversity of *Ehrlichia ruminantium* in *Amblyomma variegatum* ticks and small ruminants in The Gambia determined by restriction fragment profile analysis. Veterinary Microbiology 126, 89–199. https://doi.org/10.1016/j.vetmic.2007.06.010

Gebrekidan, H., Hailu, A., Kassahun, A., Rohoušová, I., Maia, C., Talmi-Frank, D. et al., 2014. *Theileria* infection in domestic ruminants in northern Ethiopia. Veterinary Parasitology 200, 31-38. https://doi.org/10.1016/j.vetpar.2013.11.017

Hassan, M.I., Gabr, H.S.M., Abdel-Shafy, S., Hammad, K.M., Mokhtar, M.M., 2017. Prevalence of tick-vectors of *Theileria annulata* infesting the one-humped camels in Giza, Egypt. Journal of the Egyptian Society of Parasitology 47, 425–32. https://doi.org/10.21608/JESP.2017.77797

Hassan, S., Skilton, R.A., Pelle, R., Odongo, D., Bishop, R.P., Ahmed, J. et al., 2019. Assessment of the prevalence of *Theileria lestoquardi* in sheep from Sudan using serological and molecular methods. Preventive Veterinary Medicine 169, 104697. https://doi.org/10.1016/j.prevetmed.2019.104697

Hussein, N. M., Mohammed, E. S., Hassan, A. A., and El-Dakhly, K. M. 2017. Distribution Pattern of Babesia and *Theileria* species in sheep in Qena Province, Upper Egypt. Archives of Parasitology 1, 102.

Higgins, J. P., Thompson, S. G. 2002. Quantifying heterogeneity in a meta-analysis. Statistics in medicine, 21, 1539-1558.

Kasozi, K.I., Welburn, S.C., Batiha, G.E.S., Marraiki, N., Nalumenya, D.P., Namayanja, M. et al., 2021. Molecular epidemiology of anaplasmosis in small ruminants along a human-livestock-wildlife interface in Uganda. Heliyon 7, e05688. https://doi.org/10.1016/j.heliyon.2020.e05688

Kumar, B., Maharana, B.R., Thakre, B., Brahmbhatt, N.N., Joseph, J.P., 2022. 18S rRNA gene-based piroplasmid PCR: An assay for rapid and precise molecular screening of *Theileria* and *Babesia* Species in Animals. Acta Parasitologica 67, 1697-1707. https://doi.org/10.1007/s11686-022-00625-2

Lee, S.H., Mossaad, E., Ibrahim, A.M., Ismail, A.A., Moumouni, P.F.A., Liu, M. et al., 2018. Detection and molecular characterization of tick-borne pathogens infecting sheep and goats in the Blue Nile and West Kordofan state in Sudan. Ticks and Tick-Borne Diseases 9, 598-604. https://doi.org/10.1016/j.ttbdis.2018.01.014

M'ghirbi, Y., Oporto, B., Hurtado, A., Bouattour, A., 2022. First molecular evidence for the presence of *Anaplasma phagocytophilum* in naturally infected small ruminants in Tunisia and confirmation of *Anaplasma ovis* endemicity. Pathogens 11, 315. https://doi.org/10.3390/

pathogens11030315

M'ghirbi, Y., Ros-García, A., Iribar, P., Rhaim, A., Hurtado, A., Bouattour, A., 2013. A molecular study of tick-borne haemoprotozoan parasites (*Theileria* and *Babesia*) in small ruminants in Northern Tunisia. Veterinary Parasitology 198, 72-77. https://doi.org/10.1016/j.vetpar.2013.08.005

Mares-Guia, M.A.M.D.M., Rozental, T., Guterres, A., Gomes, R., Almeida, D.N.D., Moreira, N.S. et al., 2014. Molecular identification of the agent of Q fever–*Coxiella burnetii*–in domestic animals in the State of Rio de Janeiro, Brazil. Revista da Sociedade Brasileira de Medicina Tropical 47, 231-234. https://doi.org/10.1590/0037-8682-0076-2013

McCoy, B.N., Maïga, O., Schwan T.G., 2014. Detection of *Borrelia theileri* in *Rhipicephalus geigyi* from Mali. Ticks and Tick-Borne Diseases 5, 401–403. https://doi.org/10.1016/j.ttbdis.2014.01.007

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M. et al., 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Systematic Reviews 4, 1-9. https://doi.org/10.1186/2046-4053-4-1

Onyiche, T.E., Mofokeng, L.S., Thekisoe, O., MacLeod, E.T., 2022. Molecular survey for tick-borne pathogens and associated risk factors in sheep and goats in Kano Metropolis, Nigeria. Veterinary Parasitology: Regional Studies and Report 100753. https://doi.org/10.1016/j.vprsr.2022.100753

Onyiche, T.E., MacLeod, E.T., 2023. Hard ticks (Acari: Ixodidae) and tick-borne diseases of sheep and goats in Africa: A review. Ticks and Tick-Borne Diseases 14, 102232. https://doi.org/10.1016/j.ttbdis.2023.102232

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C.,Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., Chou, R. 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Systematic Reviews 10, 1–11.

Reddy, G.R., Sulsona, C.R., Harrison, R.H., Mahan, S.M., Burridge, M.J. Barbet, A.F., 1996. Sequence heterogeneity of the major antigenic protein 1 genes from *Cowdria ruminantium* isolates from different geographical areas. Clinical and Diagnostic Laboratory Immunology 3, 417–422. https://doi.org/10.1128/cdli.3.4.417-422.1996

Ringo, A.E., Aboge, G.O., Moumouni, P.F.A., Lee, S.H., Jirapattharasate, C., Liu, M. et al., 2019. Molecular detection and genetic characterisation of pathogenic *Theileria, Anaplasma,* and *Ehrlichia* species among apparently healthy sheep in central and western Kenya. Onderstepoort Journal of Veterinary Research 86, 1-8. https://doi.org/10.4102/ojvr.v86i1.1630

Ringo, A.E., Moumouni, P.F.A., Taioe, M., Jirapattharasate, C., Liu, M., Wang, G. et al., 2018. Molecular analysis of tick-borne protozoan and rickettsial pathogens in small ruminants from two South African provinces. Parasitology International 67, 144-149. https://doi.org/10.1016/j.parint.2017.11.002

Rjeibi, M.R., Darghouth, M.A., Gharbi, M., 2016a. Prevalence of *Theileria* and *Babesia* species in Tunisian sheep. Onderstepoort Journal of Veterinary Research 83, 1-6. https://doi.org/10.4102/ojvr.v83i1.1040

Rjeibi, M.R., Darghouth, M.A., Rekik, M., Amor, B., Sassi, L., Gharbi, M., 2016b. First molecular identification and genetic characterization of *Theileria lestoquardi* in sheep of the Maghreb region. Transboundary and Emerging Disease 63, 278-284. https://doi.org/10.1111/tbed.12271

Rjeibi, M.R., Gharbi, M., Mhadhbi, M., Mabrouk, W., Ayari, B., Nasfi, I. et al., 2014. Prevalence of piroplasms in small ruminants in North-West Tunisia and the first genetic characterisation of *Babesia ovis* in Africa. Parasite 21. https://doi.org/10.1051/parasite/2014025

Salih, D.A., Ali, A.M., Liu, Z., Bakheit, M.A., Taha, K.M., El Imam, A.H. et al., 2012. Development of a loop-mediated isothermal amplification method for detection of *Theileria lestoquardi*. Parasitology Research 110, 533-538. https://doi.org/10.1007/s00436-011-2518-x

Schnittger, L., Yin, H., Gubbels, M.J., Beyer, D., Niemann, S., Jongejan, F. et al., 2003. Phylogeny of sheep and goat *Theileria* and *Babesia* parasites. Parasitology Research 91, 398-406. https://doi.org/10.1007/s00436-003-0979-2

Silaghi, C., Santos, A.S., Gomes, J., Christova, I., Matei, I.A., Walder, G. et al., 2017. Guidelines for the direct detection of *Anaplasma* spp. in diagnosis and epidemiological studies. Vector-Borne and Zoonotic Diseases 17, 12-22. https://doi.org/10.1089/vbz.2016.19

Sili, G., Byaruhanga, C., Horak, I., Steyn, H., Chaisi, M., Oosthuizen, M.C. et al., 2021. Ticks and tick-borne pathogens are infecting livestock and dogs in Tchicala-Tcholoanga, Huambo Province, Angola. Parasitology Research 120, 1097-1102. https://doi.org/10.1007/s00436-020-07009-3

Steyn, H.C., Pretorius, A., McCrindle, C.M.E., Steinmann, C.M.L., Van Kleef, M., 2008. A quantitative real-time PCR assay for *Ehrlichia ruminantium* using pCS20. Veterinary Microbiology 131, 258–265. https://doi.org/10.1016/j.vetmic.2008.04.002

Taha, K.M., Salih, D.A., Ali, A.M., Omer, R.A., El Hussein, A.M., 2013. Naturally occurring infections of cattle with *Theileria lestoquardi* and sheep with *Theileria annulata* in the Sudan. Veterinary Parasitology 191, 143-145. https://doi.org/10.1016/j.vetpar.2012.08.003

Tawana, M., Onyiche, T.E., Ramatla, T., Mtshali, S., Thekisoe, O., 2022. Epidemiology of ticks and tick-borne pathogens in domestic ruminants across southern african development community (SADC) region from 1980 until 2021: A systematic review and meta-analysis. Pathogens 11, 929. https://doi.org/10.3390/pathogens11080929

Tijsse-Klasen, E., Koopmans, M.P., Sprong, H., 2014. Tick-borne pathogen–reversed and conventional discovery of disease. Frontiers in Public Health 2, 73. https://doi.org/10.3389/fpubh.2014.00073

Tumwebaze, M.A., Lee, S.H., Moumouni, P.F.A., Mohammed-Geba, K., Sheir, S.K. et al., 2020a. First detection of *Anaplasma ovis* in sheep and *Anaplasma platys*-like variants from cattle in Menoufia governorate, Egypt. Parasitology International 102150. https://doi.org/10.1016/j.parint.2020.102150

Tumwebaze, M.A., Byamukama, B., Tayebwa, D.S., Byaruhanga, J., Angwe, M.K., Galon, E.M. et al., 2020b. First molecular detection of *Babesia ovis*, *Theileria* spp., *Anaplasma* spp., and *Ehrlichia ruminantium* in goats from western Uganda. Pathogens 9, 895. https://doi.org/10.3390/pathogens9110895

Uilenberg, G., 1995. International collaborative research: Significance of tick-borne hemoparasitic diseases to world animal health. Veterinary Parasitology 57, 19-41. https://doi.org/10.1016/0304-4017(94)03107-8

Uilenberg, G., 2006. Babesia—a historical overview. Veterinary Parasitology 138, 3-10. https://doi.org/10.1016/j.vetpar.2006.01.035

Uilenberg, G., Hinaidy, H.K., Perié, N.M., Feenstra, T., 1988. *Borrelia* infections of ruminants in Europe. Veterinary Quarterly 10, 63-67. https://doi.org/10.1080/01652176.1988.9694148

Vaidya, V.M., Malik, S.V., Bhilegaonkar, K.N., Rathore, R.S., Kaur, S. Barbuddhe, S.B., 2010. Prevalence of Q fever in domestic animals with reproductive disorders. Comparative Immunology Microbiology and Infectious Disease 33, 307–321. https://doi.org/10.1016/j.cimid.2008.10.006

Vaidya, V.M., Malik, S.V., Kaur, S., Kumar, S., Barbuddhe, S.B., 2008. Comparison of PCR, immunofluorescence assay, and pathogen isolation for diagnosis of Q fever in

humans with spontaneous abortions. Journal of Clinical Microbiology 46, 2038–2044. https://doi.org/10.1128/JCM.01874-07

Van Heerden, H., Steyn, H.C., Allsopp, M.T.P., Zweygarth, E., Josemans, A.I., Allsopp, B.A., 2004. Characterization of the pCS20 region of different *Ehrlichia ruminantium* isolates. Veterinary Microbiology 101, 279–291. https://doi.org/10.1016/j.vetmic.2004.02.015

Yeruham, I., Hadani, A., Galker, F., 1998. Some epizootiological and clinical aspects of ovine babesiosis caused by *Babesia ovis*—A review. Veterinary Parasitology 74, 153-163. https://doi.org/10.1016/S0304-4017(97)00143-X

Yin, H., Luo, J., Schnittger, L., Lu, B., Beyer, D., Ma, M. et al., 2004. Phylogenetic analysis of *Theileria* species transmitted by *Haemaphysalis qinghaiensis*. Parasitology Research 92, 36-42. https://doi.org/10.1007/s00436-003-0900-z