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Theileria infection in domestic ruminants in northern Ethiopia

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ABSTRACT

Piroplasmosis caused by different tick-borne hemoprotozoan parasites of the genera *Theileria* and *Babesia* is among the most economically important infections of domestic ruminants in sub-Saharan Africa. A survey for piroplasm infection was conducted in three locations in Northern Ethiopia. Of 525 domestic ruminants surveyed, 80% of the cattle, 94% of the sheep and 2% of the goats were positive for different *Theileria* spp. based on PCR of blood followed by DNA sequencing. Sheep had a significantly higher rate of infection compared with cattle ($P < 0.0003$) and both sheep and cattle had higher rates of infection compared to goats ($P < 0.0001$). Four species of *Theileria* were detected in cattle: *T. velifera*, *T. mutans*, *T. orientalis* complex and *T. annulata* with infection rates of 66, 8, 4, and 2%, respectively. This is the first report of *T. annulata*, the cause of Tropical Theileriosis in Ethiopia. Of the two *Theileria* spp. detected in small ruminants, *T. ovis* was highly prevalent (92%) in sheep and rare in goats (1.5%) whereas *T. seperata* was infrequent in sheep (2%) and rare in goats (0.4%). None of the animals were positive for *Babesia* spp.; however, *Sarcocystis capracanis* and *S. tenella* were detected in one goat and a sheep, respectively. The widespread distribution of *Theileria* spp. among cattle in northern Ethiopia including the virulent *T. annulata* and more mildly pathogenic *T. mutans* and *T. orientalis*, and the high infection rate in sheep with the usually sub-clinical *T. ovis* indicate extensive exposure to ticks and transmission of piroplasms with an important economic impact.

1. Introduction

Piroplasmosis caused by different tick-borne hemoprotozoan parasites of the genera *Theileria* and *Babesia* inflicts a major burden in domestic animal production and wildlife preservation in tropical and subtropical environments worldwide. Piroplasmosis is among the most economically important infection of domestic ruminants in sub-Saharan Africa. The disease is frequently characterized by fever, hemolytic anemia, high morbidity and death in severe cases (Schnittger et al., 2012; Uilenberg, 1995).

The most important *Theileria* and *Babesia* spp. which infect domestic ruminants are transmitted by ixodid ticks of the genera *Rhipicephalus, Amblyomma*, *Hyalomma* and *Haemaphysalis*. Several pathogenic, moderately pathogenic and non-pathogenic *Theileria* and *Babesia* spp. have been

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described to infect domestic ruminants. These include *Theileria lestoquardi*, *T. ovis*, *T. separata*, *Babesia ovis, B. motasi, T. uilenbergi, T. lewenshuni, Theileria* sp. MK, *Theileria* sp. OT1, *Theileria* sp. OT3 and *B. crassa*, which infect small ruminants (Ahmed et al., 2006; Altay et al., 2008; Heidarpour Bami et al., 2009; Inci et al., 2010; Li et al., 2011; Nagore et al., 2004; Yin et al., 2007), and *T. parva*, *T. annulata, T. mutans*, *T. velifera, T. tarurotragi, T. orientalis/T. sergenti/T. buffeli* (considered as belonging to the *T. orientalis* complex), *Babesia bovis, B. bigemina*, and *B. divergens*, which infect cattle (Devos and Geysen, 2004; Silva et al., 2009; Kivaria and Noordhuizen, 2010; Liu et al., 2010; Yusufmia et al., 2010; Altangerel et al., 2011; Kamau et al., 2011; Tomassone et al., 2012; Mbizeni et al., 2013; Sivakumar et al., 2013). Among these, the most important pathogenic *Theileria* spp. that infect cattle are *T. parva and T. annulata*, which cause East Coast Fever and Tropical Theileriosis, respectively (Schnittger et al., 2012; Criado-Fornelio et al., 2009).

Ethiopia is estimated to have the largest livestock population in Africa and is ranked 9th in the world in number of domestic livestock (Waret-Szkuta et al., 2008). According to the agricultural sample survey conducted during 2009, the number of domestic ruminants in Ethiopia is estimated to be 49 million cattle and 47 million small ruminants including sheep and goats. Despite this large population, Ethiopia's ruminant productivity is lower than the African average (Anon, 2009). One of the major constraints contributing to low productivity is prevailing animal infectious diseases. Among these, tick-borne diseases cause considerable losses to the livestock economy, ranking third among the prevalent parasitic diseases, after trypanosomiasis and endoparasitism with fascioliasis, nematodiasis and other gastro-intestinal tract parasites (Zeleke and Bekele, 2004). Tick-borne diseases are responsible for serious economic losses to the farmer, the tanning industry and the livestock industry in Ethiopia (MoARD, 2005).

The main tick genera described in Ethiopia are *Amblyomma, Haemaphysalis, Hyalomma* and *Rhipicephalus* with more than 60 spp. of ticks infesting both domestic and wild animals. Among these, about 37 spp. are widespread and transmit important parasites of livestock such as piroplasmosis but the significance of these infections in terms of mortality and production loss and the degree of enzootic stability are not thoroughly recognized (Tomassone et al., 2012). Limited previous studies confirmed that *B. bovis*, *T. mutans, T. velifera*, and *T. orientalis* infect cattle in western, eastern, and southern Ethiopia (Becerra et al., 1983; Sileshi, 1996; Solomon et al., 1998; Sileshi et al., 2011; Tomassone et al., 2012). Bovine tropical theileriosis or East Coast Fever have not been reported in Ethiopia to date (Sileshi et al., 2011).

The reported information on piroplasmosis in Ethiopian cattle is based mostly on morphological examination, presence of clinical signs and serological prevalence (Becerra et al., 1983; Solomon et al., 1998; Sileshi et al., 2011). These diagnostic methods are reliable for the detection of acute cases but have limited value for chronic infections, where only low parasitemia exists and a significant degree of expertise is needed to differentiate the various piroplasm species. Furthermore, several studies have revealed

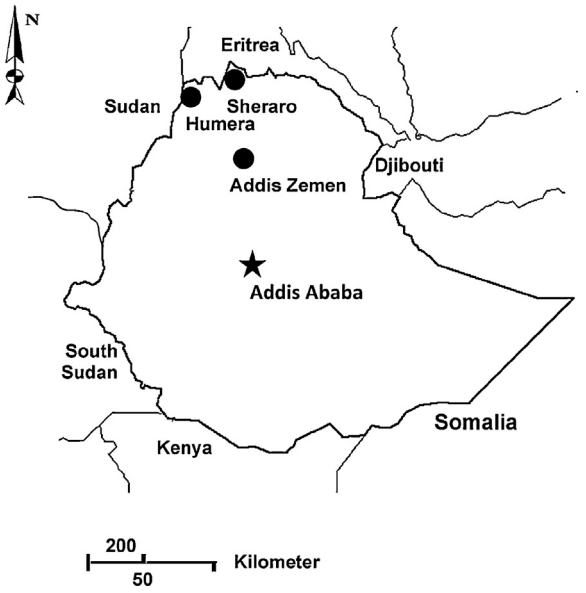

Fig. 1. Map of Ethiopia indicating the localities from which samples were collected (filled circles), international boundaries (continuous lines) and the capital of Ethiopia (filled star).

that these methods do not definitively discriminate species of domestic ruminant piroplasms (Schnittger et al., 2004; Aktas et al., 2007). The detection of parasite DNA by PCR and sequencing of the product to verify its identity is a highly sensitive and specific method able to detect infection and define the infecting pathogen spp. (Nagore et al., 2004; Schnittger et al., 2004; Aktas et al., 2005; Altay et al., 2005; Altay et al., 2008).

Almost no information has been published on molecular detection of piroplasmosis and its pathogenesis in domestic ruminants in Ethiopia except for one report in cattle in eastern Ethiopia (Tomassone et al., 2012). Therefore, the main purposes of the present study were to detect infection with piroplasms in cattle, goats and sheep in northern Ethiopia by PCR and to define the spp. infecting these ruminants and their prevalence.

2. Materials and methods

2.1. Animals and samples

Blood samples from domestic cattle, sheep and goats were collected between October and November 2010 from three localities in northern Ethiopia: Addis Zemen (12° 7′ 11″N, 37° 46′ 48″E), Sheraro (14° 24′ 0″N, 37° 56′ 0″E) and Humera (14°16′20″N 36°38′24″E) (Fig. 1). The field sites were selected because they are foci of human visceral leishmaniasis in the framework of a survey on this disease (to be published separately). Blood was taken from the jugular vein and collected into EDTA tubes. Blood samples were transported to the Hebrew University of Jerusalem, School of Veterinary Medicine, in Israel in a cold pack and stored at −80 °C until DNA extraction.

The animals were categorized as young or adult according to onsite observation and information from their

owners. Animals older than 2 years of age were considered as adult.

2.2. DNA extraction and PCR amplification

DNA extraction was performed essentially as previously described (Baneth et al., 2013). DNA was extracted successfully from 300 μl of blood using the illustra blood genomicPrep Mini Spin Kit (GE Health care, Buckinghamshire, UK), following the manufacturer's instructions.

Primers piroplasmid-F (5′-CCAGCAGCCGCGGTAATTC-3′) and piroplasmid-*R* (5′-CTTTCGCAGTAGTTYGTCTTTAACAAATCT-3′) were used for the amplification of an approximately 400 bp fragment of the 18S rRNA gene of *Theileria* and *Babesia* spp. as described previously (Tabar et al., 2008). PCR was conducted in a total volume of 25 μl composed of 1 μl (10 μM) of each primer, 3 μl genomic DNA and 20 μl ultra pure water using the Syntezza PCR-Ready high specificity kit (Syntezza Bioscience, Israel). DNA from the blood of a laboratory-bred piroplasmid- free dog and from a dog naturally infected with *B. vogeli* (1.5 μl DNA) were used as negative and positive controls, respectively. A non-template control (NTC) was also run with each PCR. This PCR protocol was used for all of the samples included in the study. The thermal cycling profile was 94 °C for 3 min followed by 40 cycles at 94 °C for 30 s, 64 °C for 45 s and 72 °C for 30 s with a final extension step of 72 °C for 7 min by a hold step at 4 °C. Amplified DNA was subjected to electrophoresis in a 1.5% agarose gel (95 V, 22 min), pre-staininig with ethidium-bromide and photographing under UV light. All positive PCR products detectable by gel electrophoresis were sequenced using the BigDye Terminator v.3.1 Cycle Sequencing kit (PerkinElmer, Applied Biosystems Divisions, Foster City, CA) and ABI PRISM 3100 Genetic Analyzer (Applied Biosystems Divisions, Foster City, CA). DNA sequencing was performed at the Center for Genomics Technologies, Hebrew University of Jerusalem, Israel. DNA sequences were evaluated using the Chromas Lite software version 2.01 and compared to sequence data available from GenBank using the BLAST 2.2.9 program (http://www.ncbi.nlm.nih.gov/BLAST/).

A second set of species-specific primers, Tams1-forward (5′-CAA ATT CGA GAC CTA CTA CGA TG-3′) and Tams1-reverse (5′-CCACTT RTC GTC CTT AAG CTC G-3′), which amplify an approximately 319 bp segment of the *T. annulata* merozoite surface antigen 1 (Tams1) encoding gene (Santos et al., 2013) were used on all samples positive for *Theileria* spp. other than *T. annulata* in order to detect possible co-infections of other *Theileria* spp. Furthermore, in order to test the ability of Tams primers to amplify *T. annulata* in animals co-infected with other *Theileria* spp., 1.5 μl DNA from a cow positive by PCR and sequencing for *T. annulata* was mixed with the same amount of DNA from one sample positive for each of the other *Theileria* spp. detected in this study, and PCR was performed on these mixtures using the Tams primers. Each PCR product resulting from these reactions was sequenced to verify that *T. annulata* DNA was amplified. The Tams PCR was performed in a thermal cycler at 94 °C for 10 min followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s and a final extension step of 72 °C for 10 min. A *T. annulata* control sample (1.5 μl DNA) from a naturally infected cow positive by PCR and sequencing, and DNA extracted from the blood of a piroplasmid- free cow were used as positive and negative controls, respectively. A non-template control (NTC) was also run with each PCR.

The species identity of sequences obtained from the two PCR assays described above was determined according to the closest BLAST match with an identity of 97%–100% to a GenBank accession.

2.3. Statistical analysis

Pearson's chi-square (χ^2) contingency table analysis, Fisher's Exact Test and descriptive statistics were used to statistically analyze the prevalence of pathogen spp. detected for correlated proportions and various parameters. Statistical calculations were performed using the JMP v.9.0 software (SAS Institute Inc., Cary, NC, USA). A P value<0.05 was considered as statistically significant.

3. Results

3.1. Overall infection rates

A total of 525 domestic ruminants including 100 cattle, 160 sheep, and 265 goats from three localities in northern Ethiopia were included in the study (Table 1). Eighty (80%) of the cattle, 150 (93.8%) of the sheep and 5 (1.9%) of the goats, in total 235 (44.8%) of the ruminants, were positive for infection with at least one species of *Theileria* based on PCR followed by DNA sequencing. Sheep had a significantly higher rate of infection with *Theileria* spp. when compared with cattle ($P < 0.0003$) and both sheep and cattle had significantly higher rates of infection ($P < 0.0001$) compared to goats (Table 1).

Identification of *Theileria* spp. by the Piroplasmid primers PCR using the molecular size of the product on agarose gel electrophoresis was not possible due to the similar size of PCR product found for all the different *Theileria* spp. Therefore, identification was based on sequencing of the PCR product. None of the animals were positive for *Babesia* spp. However, *Sarcocystis capracanis* and *S. tenella* DNA were detected by the Piroplasmid primers in the blood of one goat (1/265; 0.4%) and one sheep (1/160; 0.6%), respectively. Sequences from each piroplasm and *Sarcocystis* spp. detected in cattle, sheep and goats (i.e. 17 sequence accessions) were deposited in GenBank (Table 2).

3.2. Infection by sex and age

Of the cattle studied, gender was known for 99 (99%) of which 76 were females (76.8%) and 23 were males (23.2%) and it was not found to be significantly associated with positivity for *Theileria* infection. Association of infection with age was not available as age was not recorded for a sufficient number of cattle.

Of the sheep studied, gender was known for 134 (83.8%) of which 83 were females (61.9%) and 51 were males (38.1%). Similarly, age was recorded for 133 of the 160 (83.1%) sheep of which 106 were adults (79.7%) and 27

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Table 1
Infection rates with *Theileria* spp. in cattle, sheep and goats in three study sites in Northern Ethiopia evaluated by PCR and DNA sequencing.

Animal species	No. of animals in study sites			Total no. of animals	Infection rate *n* (%)
	Addis Zemen	Sheraro	Humera		
Cattle	59	21	20	100	80 (80)[a]
Sheep	23	5	132	160	150 (93.8)[b]
Goat	–	128	137	265	5 (1.9)[c]
Total	82	154	289	525	235 (44.8)

Unlike superscript letters (a, b, c) denote significantly different ($P < 0.05$) infection rates.

were young (20.3%). No significant association was found between gender or age and *Theileria* positivity.

Of the goats studied, gender was known for 137 (51.7%) of which 108 were females (78.8%) and 29 were males (21.2%). Age was recorded for 132 (49.8%) of which 118 were adults (89.4%) and 14 were young (10.6%). Both gender and age were not significantly associated with positivity for *Theileria* infection in the surveyed goats.

3.3. Infection with each Theileria spp. according to animal species

Four species of *Theileria* were detected in cattle: *T. velifera, T. mutans, T. orientalis* complex and *T. annulata* with infection rates of 66, 8, 4, and 2%, respectively (Table 3). *Theileria velifera*, the most prevalent sp. in cattle, was found to have a significantly higher rate of infection when compared with the other *Theileria* spp. ($P < 0.0001$). Similarly, *T. mutans* was found to have significantly higher rate of infection compared to the least prevalent *Theileria* sp. (*T. annulata*) ($P < 0.046$), which did not differ statistically compared to *T. orientalis* complex. Furthermore, infection rates with *T. orientalis* complex and *T. annulata* were not significantly different.

Two *Theileria* spp. were detected in sheep, *T. ovis* and *T. separata* (Table 4). The infection rates with *T. ovis* and *T. separata* were 91.9% (147/160) and 1.9% (3/160), respectively, with a significantly higher *T. ovis* infection rate ($P < 0.0001$). *Theileria ovis* and *T. separata* were also the only *Theileria* spp. detected in goats. However, the infection rates of *T. ovis* and *T. separata* were much lower than those found in sheep, 1.5% (4/265) and 0.4% (1/265), respectively, and did not differ statistically. Sheep were found to have significantly higher rates of infection with *T. ovis* when compared with goats ($P < 0.0001$).

No co-infection with *T. annulata* was detected in any sample positive for the other *Theileria* spp. in all the screened ruminants. Furthermore, the five samples positive for other *Theileria* spp. and mixed with *T. annulata* DNA were positive with the Tams PCR and sequencing yielded a product compatible with *T. annulata* and not with any other species confirming the specificity of these primers.

3.4. Infection by geographic locations

The infection rates of cattle and sheep in Addis Zemen were 81.4% (48/59) and 78.3% (18/23), respectively, with no significant difference in prevalence between them. No goats were present in Addis Zemen. In Sheraro, infection rates were 85.7% (18/21), 80% (4/5) and 2.4% (3/128) in cattle, sheep and goats, respectively, and did not differ between cattle and sheep, however, they were significantly higher compared to goats ($P < 0.0001$). In Humera, the infection rates were 70% (14/20), 97.7% (129/132) and 2.2% (3/137) in cattle, sheep and goats, respectively. Infection rates were significantly higher in sheep compared to cattle ($P < 0.0001$) and in cattle and sheep compared to goats ($P < 0.0001$). Infection rates with *Theileria* spp. in goats from Sheraro and Humera were almost negligible when compared to those of sheep and cattle.

Theileria velifera was the most prevalent piroplasm sp. in cattle in all locations ($P < 0.0001$). Its prevalence was 81% (17/21), 64.4% (38/59) and 55% (11/20) in Sheraro, Addis Zemen and Humera, respectively. No significant difference in infection rates with *T. velifera* was found among the locations. Likewise, the infection rate of *T. mutans* was 10.2% (6/59), 5% (1/20) and 4.7% (1/21) in Addis Zemen, Humera and Sheraro, respectively, and no significant difference in infection rate was found among the locations.

Three *Theileria* species were detected in cattle in Addis Zemen, i.e. *T. velifera*, *T. mutans*, and *T. orientalis* complex (Table 3). The prevalence of *T. velifera* was significantly higher than the other two species ($P < 0.0001$), but the infection rates with *T. mutans* and *T. orientalis* complex

Table 2
DNA sequences deposited in GenBank from each *Theileria* and *Sarcocystis* species detected in cattle, sheep and goats from Northern Ethiopia.

Animal species	Pathogen	Target gene	GenBank accession number
Cattle	*T. velifera*	18S rRNA	KF557889, KF557890
	T. mutans	18S rRNA	KF557887, KF557888
	T. orientalis complex	18S rRNA	KF557878, KF557879
	T. annulata	Tams1	KF632589, KF632590
Sheep	*T. ovis*	18S rRNA	KF557885, KF557886
	T. separata	18S rRNA	KF557883, KF557884
	S. tenella	18S rRNA	KF557882
Goats	*T. ovis*	18S rRNA	KF557876, KF557877
	T. separata	18S rRNA	KF557880
	S. capracanis	18S rRNA	KF557881

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Table 3
Distribution of *Theileria* spp. infections in cattle in Northern Ethiopia as detected by PCR followed by DNA sequencing.

Study site	No. of animals	PCR+ *n* (%)	*T. velifera n* (%)	*T. mutans n* (%)	*T. orientalis* complex *n* (%)	*T. annulata n* (%)
Addis Zemen	59	48 (81.4)[a]	38 (64.4)[a]	6 (10.2)[b]	4 (6.8)[b]	–
Sheraro	21	18 (85.7)[a]	17 (81)[a]	1 (4.8)[b]	–	–
Humera	20	14 (70)[a]	11 (55)[a]	1 (5)[b]	–	2 (10)[b]
Total	100	80 (80)	66 (66)[a]	8 (8)[b]	4 (4)[bc]	2 (2)[c]

Unlike superscript letters (a, b, c) denote significantly different ($P < 0.05$) infection rates. Comparisons were made within rows for the different *Theileria* spp. and within columns for the geographic locations.

were not significantly different. In Sheraro, two *Theileria* species were detected, i.e. *T. velifera* and *T. mutans*. The prevalence of *T. velifera* was significantly higher than *T. mutans* ($P < 0.0001$). Of the three *Theileria* secies detected in cattle in Humera, i.e. *T. velifera*, *T. annulata*, and *T. mutans* (Table 3), the prevalence of *T. velifera* was significantly higher than the other two species ($P < 0.0001$).

Of the two *Theileria* spp. detected in sheep and goats (i.e. *T. ovis and T. separata*), the prevalence of sheep *T. ovis* infection in all locations was significantly higher ($P < 0.0001$) compared to *T. separata* (Table 4). In Humera, 95.4% of the sheep were infected with *T. ovis*, followed by 80% in Sheraro and 73.9% in Addis Zemen. Infection rates with *T. ovis* in sheep were significantly higher in Humera when compared to Addis Zemen ($P < 0.003$), but did not differ when comparing Humera to Sheraro and Sheraro to Addis Zemen. *Theileria separata* was only detected in Humera and Sheraro and infection rates were low, 2.3% in sheep and 0.8% in goats, with no significant differences among these ruminant species or among locations (Table 4).

4. Discussion

This is the first study in small ruminants and second study in cattle in which molecular diagnostic techniques were used to investigate piroplasm infections in Ethiopia. It provides new information on the diversity, distribution, and extent of *Theileria* spp. infecting domestic ruminants in northern Ethiopia. Furthermore, we report infection with *T. annulata* in cattle for the first time in Ethiopia. Although the rate of infection with this highly pathogenic species was low, the overall infection rates with *Theileira* spp. was high in both cattle and sheep, with most of the cattle and almost all of the sheep included in the study infected with *Theileria* spp. of which some are considered mildly or non-pathogenic, whereas others are potentially responsible for severe disease.

The differences in *Theileria* sp. infection rates between cattle, sheep and goats, and particularly between sheep and goats, which typically share the same pasture and management practices in northern Ethiopia, are striking. Almost no infection was detected in goats whereas the vast majority of sheep were infected. This is different from a study carried out in Turkey where 34.6% of the sheep and 10% of the goats surveyed were positive for *Theileria* spp. by reverse line blot (RLB) (Aydin et al., 2013). The variation in infection rates of domestic ruminants with tick-borne pathogens is related to several factors including the presence and abundance of tick species which act as vectors for specific pathogens, genetic variation among animals and breeds in resistance, and the presence of wild-life reservoirs. Although the African buffalo (*Syncerus caffer*) is a wildlife reservoir of *T. mutans* and *T. velifara* found in this study (Chaisi et al., 2013), wild buffaloes are not common in northern Ethiopia and therefore other wild animals may serve as reservoirs, or infection might be circling only in domestic animals.

Gender and age were not found to be significantly associated with positivity for *Theileria* infection in the surveyed animals. This is in agreement with other studies on *Theileria* spp. infections in small ruminants and cattle (Flach and Ouhelli, 1992; Razmi et al., 2002; Weir et al., 2011).

The presence of co-infection with several piroplasmid spp. in the same host was not evaluated in this study, except for co-infection with *T. annulata*, as the DNA amplified by PCR probably represented the most abundant species present in the host's blood. Therefore, the study presents a general picture of the *Theileria* species that infect domestic ruminants in northern Ethiopia, but not the absolute infection rates with each one of the species, except for the

Table 4
Distributions and *Theileria* infections in sheep and goats in Northern Ethiopia as detected by PCR followed by DNA sequencing.

Study site	Animal species	No. of samples	PCR+	*T. ovis*	*T. separata*
			n (%)	*n* (%)	*n* (%)
Addis Zemen	Sheep	23	17 (73.9)[a]	17 (73.9)[a]	–
Sheraro		5	4 (80)[ab]	4 (80)[ab]	–
Humera		132	129 (97.7)[b]	126 (95.4)[b]	3 (2.3)[c]
Total		160	150 (93.8)	147 (91.9)[a]	3 (1.9)[b]
	Goat				
Sheraro		128	2 (1.6)[a]	1 (0.8)[a]	1 (0.8)[a]
Humera		137	3 (2.2)[a]	3 (2.2)[a]	–
Total		265	5 (1.9)	4 (1.5)[a]	1 (0.4)[a]

Unlike superscript letters (a, b, c) denote significantly different ($P < 0.05$) infection rates. Comparisons were made within rows for the different *Theileria* spp. and within columns for the geographic locations.

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most pathogenic *Theileria* sp. detected in the study, *T. annulata.* Furthermore, the PCR protocol with the Piroplasmid primers used in the study (Tabar et al., 2008) was found to detect not only piroplasms, but also *Sarcocystis* spp. This highlights the necessity to further analyze the PCR products of these non-specific primers by DNA sequencing or restriction fragment length polymorphism (RFLP) to identify the pathogen detected.

High infection rates with *T. annulata* were reported in many tropical and sub-tropical countries worldwide including Africa (Salih et al., 2007; Branco et al., 2010; Sevgili et al., 2010; Atif et al., 2012; Hussein et al., 2012; Taha et al., 2013). The low infection rate with *T. annulata* found in northern Ethiopia could be explained by the presence of mostly local African cattle breeds in the surveyed areas. The resistance of indigenous breeds to tropical theileriosis is well known and the disease mostly affects exotic dairy breeds and their crosses with indigenous breeds (Brown, 1990). An additional factor that may have influenced the prevalence of *T. annulata* is the abundance of its tick vectors, *Hyalomma* spp., which has not been studied. The low prevalence of *T. annulata*, which was detected only in Humera close to the Sudanese border, may also explain why mixed infections of *T. annulata* with other *Theileria* spp. were not detected in the study.

Cattle infection with the *T. orientalis* complex was only detected in four cows from one location, Addis Zemen. Although the *T. orientalis* complex agents transmitted by *Haemaphysalis* spp. ticks are benign and non-proliferative, they can be responsible for clinical disease representing a threat to livestock (Altangerel et al., 2011; Kamau et al., 2011). Disease outbreaks and subsequent financial losses caused by infection with *T. orientalis* complex have been reported from India, New Zealand and Australia (Aparna et al., 2011; Kamau et al., 2011; McFadden et al., 2011; Cufos et al., 2012). Almost no information has been published on *T. orientalis* complex in Ethiopia except for one report in cattle from western Ethiopia with detection by morphological and serological (indirect fluorescent antibody test) techniques (Becerra et al., 1983). The prevalence of *T. orientalis* complex (4%) by PCR in the present study was lower than the 20% seropositivity previously reported in western Ethiopia (Becerra et al., 1983), but higher than the 0.5% found by PCR in South Sudan (Salih et al., 2007). Nevertheless, its pathogenesis, subsequent financial losses and local vector in cattle remain unknown in Ethiopia.

Theileria mutans infection transmitted by *Amblyomma* spp. ticks can result in mild clinical signs, but some pathogenic strains in Eastern Africa cause severe anemia and sometimes death in cattle (Yusufmia et al., 2010). In this study, it was present in all three survey localities and infected 8% of the total cattle evaluated. This is in agreement with previous review from Ethiopia (Sileshi, 1996), which reported that the local vector of *T. mutans* is *Amblyomma variegatum* and the geographic distribution of this infection is wide. In previous studies, infection with *T. mutans* in cattle was reported from southern and eastern Ethiopia based on blood smear microscopy, serology by ELISA and the RLB (Solomon et al., 1998; Tomassone et al., 2012). In the present study, the prevalence of *T. mutans* (8%) was lower than the 30.9% previously reported by ELISA in southern Ethiopia (Solomon et al., 1998), but similar to the 8% found by the RLB from Eastern Ethiopia (Tomassone et al., 2012). These variances in infection rates might be related to the differences in diagnostic techniques with serology indicating exposure whereas molecular biology techniques indicating present infection, or to true differences in the prevalence of infection in different geographic regions due to environmental, host, and tick vector variability (García-Sanmartín et al., 2006).

In the present study, *Theileria velifera* was the most prevalent species detected in cattle. Infection with *T. velifera*, which is transmitted by *Amblyomma* spp. ticks and considered apathogenic, has already been described by RLB from the Somali region in Eastern Ethiopia (Tomassone et al., 2012). The study from the Somali region reported a much lower infection rate of 4% with *T. velifera* in cattle whereas the current study revealed a 66% infection rate. These differences in infection rates are probably related to variations in vector tick distribution and abundance due to differences in climate conditions and vegetation (Estrada-Peña and Santos-Silva, 2005).

Theileria ovis and *T. separata* are reported in sheep and goats for the first time in Ethiopia in this study. *Theileria ovis* was highly prevalent (91.9%) in sheep and rare in goats (1.5%). Studies from other countries have also shown that infection in sheep is more common than in goats, however, to our best knowledge, no study has documented such as high prevalence of infection in sheep (Inci et al., 2010; Durrani et al., 2012; Alessandra and Santo, 2012; Altay et al., 2012). In contrast to the virulent *T. lestoquardi*, *T. ovis* and *T. separata* are considered as causing benign and mostly subclinical infections in small ruminants (Uilenberg, 1995).

Interestingly, *S. capracanis* and *S. tenella* were detected in one goat and a sheep, respectively. This is the first report of infection with these species in goats and sheep from Ethiopia. Moreover, to our best knowledge, it is also the first molecular report of *S. capracanis* and *S. tenella* from blood samples in goat and sheep worldwide. Most of the published reports of *S. capracanis* and *S. tenella* in goat and sheep were from muscle tissues (Morsy et al., 2011; da Silva et al., 2009), however, *S. tenella* has been associated with pneumonia and myelitis in lambs (Schock et al., 2012) and S. *capracanis* has also been shown to infect sheep and cause neurological disease (Formisano et al., 2013). Goats and sheep are considered the main intermediate hosts of *S. capracanis* and *S. tenella*, respectively, while canids are their definitive host (Tenter, 1995; Elsheikha and Mansfield, 2007) and the present study suggests that these parasites circulate between canines and small ruminants in northern Ethiopia. The presence of *S. capracanis* and *S. tenella* DNA in the blood may be due to migration of the parasite or phagocytosis of parts of it and dispersion of its remnants in the blood, or perhaps due to aspiration of muscle tissue or endothelial cells harboring the parasite into the syringe during venipuncture, as *S. tenella* and *Sarcocystis arieticanis* have been described in vascular endothelial cells and in small arterioles of mesenteric lymph nodes of experimentally infected lambs (O'Donoghue and Ford, 1984).

The lack of detection of babesiosis in the studied domestic ruminants suggests that this infection is not common in

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the surveyed area. *Babesia* parasites were also not detected in livestock and ticks from nomadic herds in the Somali region of eastern Ethiopia (Tomassone et al., 2012). However, further molecular studies targeting only *Babesia* sp. are needed to ascertain whether ruminant babesiosis is present in northern Ethiopia.

5. Conclusions

In summary, this study reports a widespread distribution of *Theileria* spp. among domestic ruminants in northern Ethiopia. *Theileria annulata* in cattle, *T. ovis, T. separata*, *S. capracanis* and *S. tenella* in small ruminants are reported for the first time in Ethiopia. Further studies covering larger geographic areas are required to estimate the prevalence and economic importance of these infections in Ethiopia.

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