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A molecular study of tick-borne haemoprotozoan parasites (*Theileria* and *Babesia*) in small ruminants in Northern Tunisia

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ABSTRACT

In this study, the frequency of *Theileria* and *Babesia* species in sheep and goats was assessed via reverse line blotting (RLB). A total of 263 apparently healthy sheep and goats, from 16 randomly selected flocks located in 9 localities situated in 3 bioclimatic zones in Tunisia, were investigated for the blood protozoans. RLB hybridization with polymerase chain reaction detected only *Theileria ovis* in sheep and goats, accounting for 22.4% (95% confidence interval [CI]: 17.6–27.1%) positive samples. The infection rate in sheep (28.1%; 95% CI: 23.8–32.3%) was higher than in goats (4.7%; 95% CI: −10.9 to 20.4%). Neither *Babesia* nor mixed infections were detected. Only two Ixodid tick species (*Rhipicephalus turanicus* and *Rhipicephalus bursa*) were collected from the examined sheep and goats in 5 localities. *R. turanicus* was the dominant species (95.5%) collected mainly in the humid zone, while apparently rare in the sub-humid zone. *R. bursa* was the only species collected in the semi-arid area. RLB analysis identified six different piroplasms in ticks, with an overall prevalence of 31.5% (95% CI: 28.1–34.9%). Twenty percent (95% CI: 14.4–25.5%) of the collected ticks tested positive for *Theileria* spp., 3% (95% CI: −5.6 to 11.6%) for *Babesia* spp. and 0.9% (95% CI: −8.1 to 9.9%) of the ticks harbored both genera; several of these species are not known to occur in small ruminants. This is the first report on the detection of *Theileria* and *Babesia* species DNA in small ruminants and ticks in Tunisia.

1. Introduction

Piroplasm species are tick-borne parasitic protozoa that are differentiated into the genera *Theileria* and *Babesia*. Some of these protozoa are highly pathogenic for cattle, sheep, and goats, causing theileriosis and babesiosis, diseases that are widely distributed in tropical, subtropical, and temperate countries, where they are endemic. The economic impact of these diseases can be significant. Several named and as yet unnamed *Theileria* species cause ovine theileriosis (Preston, 2001); *Theileria lestoquardi*, *Theileria luwenshuni* and *Theileria uilenbergi* are pathogenic for sheep and goats, and recently, *Theileria* sp. OT3, and *Theileria* sp. MK have been described in sheep and goats, but there is no reliable information about their pathogenicity (Preston, 2001; Nagore et al., 2004a; Ahmed et al., 2006; Altay et al., 2007b; Yin et al., 2007; Duh et al., 2008). *Babesia ovis*, *Babesia motasi* and *Babesia crassa* are recognized as the species causing ovine babesiosis: *B. ovis* is highly pathogenic to sheep and goats while the other two species are non-pathogenic or less pathogenic (Uilenberg, 2001).

In Tunisia, theileriosis and babesiosis are the two main tick-borne haemoparasitic diseases occurring in cattle and

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small ruminants. They have been extensively studied in cattle (Bouattour et al., 1994; M'ghirbi et al., 2008), but a paucity of information exists concerning ovine theileriosis and babesiosis. The causative agents of piroplasmosis, their actual geographic distribution and their vectors are important components of the epidemiology of these diseases that need to be studied to evaluate the impact and implementation of successful control programs that include effective treatment of malignant theileriosis and/or pathogenic babesiosis. The laboratory diagnosis of small ruminant piroplasmosis was based on the microscopic detection of piroplasms in Giemsa-stained blood smears. However, species identification by microscopy is difficult because different parasites share a similar morphology, making identification particularly difficult if mixed infections occur. In addition, identification can be difficult in carrier animals where the presence of parasites is low and even in acute cases at the onset of the disease. In recent decades, molecular techniques with high sensitivity and specificity, such as species-specific polymerase chain reaction (PCR) and PCR-based Reverse Line Blot (RLB) hybridization have been used for detection and discrimination of ovine *Theileria* and *Babesia* species (Nagore et al., 2004a; Schnittger et al., 2004; Aktaş et al., 2005; Alhassan et al., 2005; Altay et al., 2007b).

Here, we conducted a cross-sectional study to detect and differentiate *Theileria* and *Babesia* species in small ruminants and ticks in three different bioclimatic zones of Tunisia based on PCR amplification associated with RLB species-specific hybridization.

2. Materials and methods

2.1. Farm location and small ruminant populations

This cross-sectional study was carried out in 9 localities located in three different bioclimatic zones (humid, sub-humid and semi-arid) in northern Tunisia where piroplasmosis is endemic (Fig. 1). All sites have a Mediterranean climate, with cool, moist winters, and dry, hot summers. A total of 263 small ruminants were randomly chosen following recommendations of the State Veterinary Office as representative of the local management system, which was generally traditional, that is, small flocks grazing on permanent pastures or bush.

The studied population of small ruminants ($n = 263$) was composed of 199 Barbarine sheep ($n = 193$ females and $n = 6$ males) and 64 Arbi (local breed) goats (females) from 16 randomly selected flocks (Table 1). Among these animals, 42 were younger than one year ($n = 26$ lambs, $n = 16$ kids) and there were 221 adults ($n = 173$ sheep, $n = 48$ goats). The distribution of sheep and goats has no relation to the bioclimatic zone; their breeding reflects regional habits and available pastures.

2.2. Collection of blood and tick samples

Animals were bled once between April and June 2010, the period when they graze in pastures and are exposed to ticks. Blood samples taken in ethylenediamine tetraacetic acid (EDTA) containing tubes were used for subsequent DNA extraction and hybridization analyses.

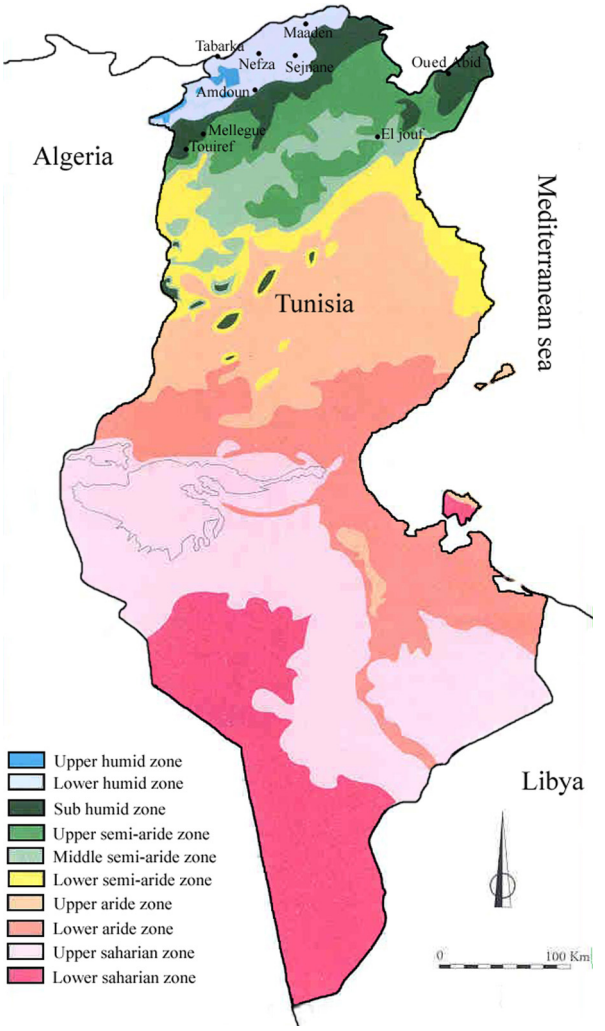

Fig. 1. Map of Tunisia showing the location of sites where blood and tick samples were collected.

In addition, the entire body of each of the 263 animals was inspected for ticks, particularly on the ears and neck. Ticks were removed manually from the host body, placed in bottles with 70% ethanol and labeled. They were identified using published taxonomic keys (Bouattour, 2002).

2.3. DNA extraction

DNA from whole-blood samples and semi-engorged adult ticks was extracted using the Invitrogen (CA, USA) Kit for DNA purification. DNA was eluted in the elution buffer provided with the kit. DNA yields were determined with a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, DE, USA).