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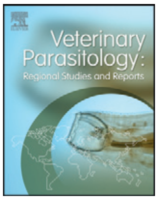

Molecular investigation of tick-borne haemoparasite infections among transhumant zebu cattle in Karamoja Region, Uganda

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ABSTRACT

Tick-borne diseases (TBDs) are a major constraint to cattle production in pastoral areas in Africa. Although information on tick-borne infections is important to prioritise control approaches, it is limited for transhumant zebu cattle in Karamoja, Uganda. We conducted a study to determine the occurrence and level of tick-borne infections among cattle in Karamoja Region. A total of 240 cattle were selected for blood collection using systematic sampling in 20 randomly-selected herds in two districts. The hypervariable V4 region of the 18S rRNA gene for *Theileria*/*Babesia* and the V1 region of the 16S rRNA gene for *Ehrlichia*/*Anaplasma* were amplified and hybridised to genus- and species-specific oligonucleotide probes on a reverse line blot (RLB) membrane. A duplex quantitative real-time polymerase chain reaction (qPCR) assay based on *msp1β* and *groEL* genes was used for the detection of *Anaplasma marginale* and *A. centrale*, while monoplex qPCR assays were used for the detection of *Ehrlichia ruminantium* (226 bp fragment of the pCS20 region) and *Theileria parva* (18S rRNA gene). The RLB hybridisation assay demonstrated the presence of tick-borne haemoparasites in all but one sample (99.6%), mostly as mixed infections (97.5%). The most frequently detected species were *Theileria mutans* (88.3%, 95% confidence interval: 84.6–91.7%), *A. marginale* (73.8%: 68.3–78.8%), *Theileria velifera* (71.3%: 65.8–76.7%) and *Anaplasma* sp. Omatjenne (63.3%: 57.5–68.8%). Other virulent pathogens, namely *Babesia bigemina* (5.0%) and *T. parva* (2.9%), were also detected with RLB, but not *E. ruminantium*. The proportions of qPCR positive samples were 82.9% (*A. marginale*), 12.1% (*A. centrale*), 3.3% (*T. parva*), and 1.7% (*E. ruminantium*). The full-length 18S rRNA genes from 6 out of 47 samples that were positive on RLB for the *Babesia* genus-specific probe and not for any of the *Babesia* species-specific probes were amplified, cloned and sequenced. The sequences were used to construct phylogenetic trees. Variations (5 to 9 nucleotides) in the 18S rRNA gene sequences of *B. bigemina* were identified, when compared with *B. bigemina* sequences from other parts of the world. Three nucleotide differences in the *B. bigemina* probe region may explain the failure of the RLB hybridisation assay to detect *B. bigemina* in some samples. *T. mutans* and *B. bigemina* sequences grouped in separate clades from previously published sequences. In conclusion, this study demonstrated high and widespread occurrence, and sequence variation of tick-borne haemoparasites among cattle in the pastoral area of Karamoja, which is useful for diagnosis and control of TBDs.

1. Introduction

In pastoral areas of sub-Saharan Africa, cattle are regularly exposed to tick-borne haemoparasites of the genera *Theileria*, *Anaplasma*, *Babesia* and *Ehrlichia* (Gachohi et al., 2010; Kasozi et al., 2014). Tick-borne diseases (TBDs) inflict significant economic losses on cattle production and resource use, thereby impacting the livelihoods of pastoralists (Kivaria, 2006; Ocaido et al., 2009a). Losses directly attributed to TBDs include mortality, production losses, and the costs of veterinary diagnosis, treatment and tick control (Kivaria, 2006). Previous studies in Uganda showed that 75.4% of losses in cattle were attributable to ticks and TBDs (Ocaido et al., 2009b), and the costs for controlling ticks and

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Abbreviations: bp, base pair; CI, confidence interval; ECF, East Coast fever; IBM SPSS, International Business machines — Statistical Package for the Social Sciences; IICD, Institute for International Cooperation and Development; MEGA, Molecular Evolutionary Genetics Analysis; OR, odds ratio; qPCR, quantitative real-time polymerase chain reaction; RLB, reverse line blot; TBD, tick-borne disease; UDG, Uracil-DNA Glycosylase.

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TBDs constituted 85% of the total costs for disease control in cattle (Ocaido et al., 2009a). Tick-borne diseases also constrain the improvement of the local breeds of cattle due to the high morbidity and mortality in exotic (*Bos taurus*) and crossbred cattle (Muhanguzi et al., 2010b).

The most pathogenic and economically significant tick-borne haemoparasites of cattle in East Africa are *Theileria parva*, *Anaplasma marginale*, *Babesia bigemina* and *Ehrlichia ruminantium*. *T. parva*, an apicomplexan protozoan parasite of cattle, is transmitted by *Rhipicephalus appendiculatus* and causes East Coast fever (ECF), an acute, usually fatal, lymphoproliferative disease (Norval et al., 1992). In Africa, bovine babesiosis is caused mainly by the intra-erythrocytic protozoan parasites, *B. bigemina* and *Babesia bovis* (Uilenberg, 2006). *B. bigemina* is reported to be the main cause of bovine babesiosis in Uganda and is transmitted by *Rhipicephalus decoloratus* (Magona et al., 2011b; Rubaire-Akiiki et al., 2004). *A. marginale*, an intra-erythrocytic rickettsia, is the main cause of bovine anaplasmosis, a tick-borne, haemolytic disease that is endemic across much of the globe (Aubry and Geale, 2011). In Uganda, *A. marginale* is transmitted by *R. decoloratus* (Magona et al., 2011a,b; Rubaire-Akiiki et al., 2006). Other forms of transmission for *A. marginale* include mechanical transfer of contaminated blood through haematophagous arthropods and fomites. Placental transmission during pregnancy can also occur (Costa et al., 2016). The obligate intracellular rickettsia, *E. ruminantium*, is transmitted by ticks of the genus *Amblyomma* and causes heartwater disease in ruminants (Allsopp, 2010). *A. variegatum* is the reported tick vector of heartwater in Uganda (Magona et al., 2011a).

The development of molecular biological diagnostic techniques has resulted in the improved detection, identification and genetic characterisation of many haemoparasites. Highly specific and sensitive species-specific quantitative real-time polymerase chain reaction (qPCR) and PCR-based reverse line blot (RLB) hybridisation assays have been developed and used for the detection of tick-borne infections (Bekker et al., 2002; Decaro et al., 2008; Gubbels et al., 1999; Muhanguzi et al., 2010a,b; Njiiri et al., 2015; Sibeko et al., 2008; Steyn et al., 2008). The RLB assay enables the identification of mixed infections and has proven to be a valuable tool in the identification of novel parasites (Nijhof et al., 2005; Oosthuizen et al., 2008). The RLB assay has previously been used to detect tick-borne haemoparasites in cattle samples from Uganda; the most common haemoparasites detected were: *T. parva*, *Theileria mutans*, *Theileria taurotragi*, *Theileria velifera*, *A. marginale*, *Anaplasma centrale*, *Anaplasma bovis* and *E. ruminantium* (Asiimwe et al., 2013; Muhanguzi et al., 2010a, 2010b; Oura et al., 2011).

Karamoja Region in north-eastern Uganda is characterised by a semi-arid climate, and communities rely on livestock as the main source of livelihood (Anderson and Robinson, 2009; IICD, 2010). Like other pastoral groups in eastern Africa, the Karamojong practice mobile livestock herding (Egeru et al., 2014). The major drivers of transhumant pastoralism are drought conditions, which result from climatic variability and consequent seasonal pasture and water scarcity, and animal diseases. The practice is therefore a key coping mechanism for pastoralists to avoid cattle losses and ensure livestock productivity (Anderson and Robinson, 2009; Egeru et al., 2014). Cattle in Karamoja Region are of the short-horned East African zebu type (*Bos indicus*) (Anderson and Robinson, 2009). Despite the social and economic importance of cattle in Karamoja communities, little is known about tick-borne haemoparasite infections in the Karamoja Region. A recent study showed that Karamojong pastoralists perceive TBDs to be the most important diseases impacting cattle production (Byaruhanga et al., 2015a). It is important to have knowledge of tick-borne infections in cattle populations from particular production systems so as to prioritise and develop appropriate control measures against TBDs.

The objectives of this study were to determine which tick-borne pathogens are present among cattle in the transhumant production system of Karamoja, Uganda, the prevalence of infections as indicated by RLB hybridisation and qPCR methods, and the molecular characteristics of these pathogens by sequencing and phylogenetic analyses.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Animal Ethics Committee of the University of Pretoria, South Africa (V026-14) and the National Agricultural Research Organisation, Uganda (no. 1416). Permission was obtained to do research in terms of Section 20 of the Animal Diseases Act, 1984 (Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa; reference number 12/11/1/1). Standard techniques were followed in collecting blood samples for laboratory examination.

2.2. Study area

This study was conducted in Moroto and Kotido Districts of Karamoja Region, north-eastern Uganda (Fig. 1), from November 2013 through January 2014. The region covers 27,511 km^2 (about 10% of Uganda) and lies between longitudes 33° 30′E to 35°E and latitudes 1° 30′N to 4°N. It is bordered by South Sudan to the north and Kenya to the east. The region is divided into seven administrative districts that are, in turn, divided into sub-counties containing a number of parishes and smaller settlement areas (*manyattas*). The region is mostly semi-arid with low rainfall (average 500–600 mm per year), with peak rainfall in April–May and July–September. The temperatures range from an average minimum of between 15 °C and 18 °C to an average maximum of between 28 °C and 33 °C (Anderson and Robinson, 2009).

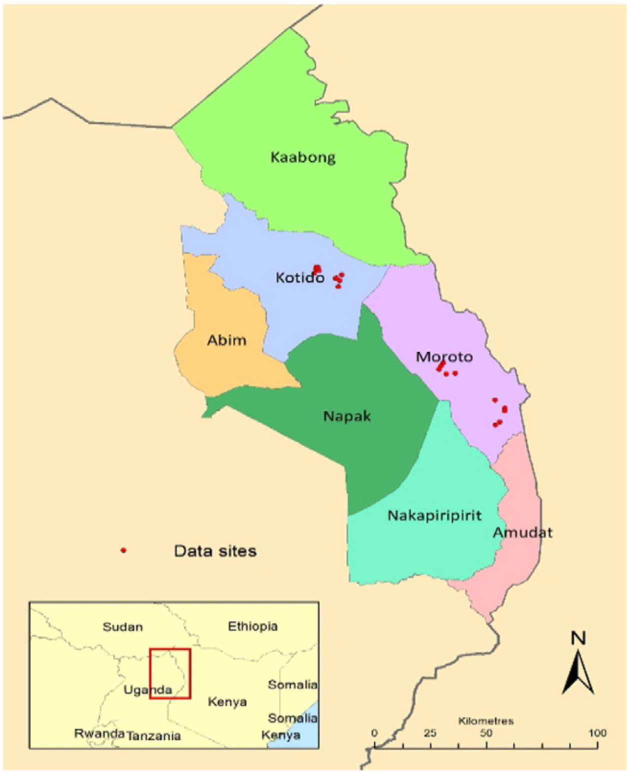

Fig. 1. Administrative map of Karamoja Region showing the study sites (red circles) in Moroto and Kotido Districts. Inset is the map of Uganda showing the location of Karamoja Region and the neighbouring countries.