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Original article

Occurrence of tick-borne haemoparasites in cattle in the Mungwi District, Northern Province, Zambia

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ABSTRACT

Little is known about the occurrence of haemoparasites in cattle in communal grazing areas of Mungwi District of Northern Province, Zambia. Clinical signs and post mortem lesions are pathognomonic of mixed tick-borne infections especially babesiosis, anaplasmosis and East Coast fever. The main objective of this study was to screen selected communal herds of cattle for tick-borne haemoparasites, and identify the tick vectors associated with the high cattle mortalities due to suspected tick-borne diseases in the local breeds of cattle grazing along the banks of the Chambeshi River in Mungwi District, Northern Province, Zambia. A total of 299 cattle blood samples were collected from July to September 2010 from Kapamba (n = 50), Chifulo (n = 102), Chisanga (n = 38), Kowa (n = 95) and Mungwi central (n = 14) in the Mungwi District. A total of 5288 ticks were also collected from the sampled cattle from April to July 2011. DNA was extracted from the cattle blood and the hypervariable region of the parasite small subunit rRNA gene was amplified and subjected to the reverse line blot (RLB) hybridization assay. The results of the RLB assay revealed the presence of tick-borne haemoparasites in 259 (86.6%) cattle blood samples occurring either as single (11.0%) or mixed (75.6%) infections. The most prevalent species present were the benign *Theileria mutans* (54.5%) and *T. velifera* (51.5%). *Anaplasma marginale* (25.7%), *Babesia bovis* (7.7%) and *B. bigemina* (3.3%) DNA were also detected in the samples. Only one sample (from Kapamba) tested positive for the presence of *T. parva*. This was an unexpected finding; also because the tick vector, *Rhipicephalus appendiculatus*, was identified on animals from Kowa (14.0%), Chisanga (8.5%), Chifulo (6.0%) and Kapamba (1.4%). One sample (from Kapamba) tested positive for the presence of *Ehrlichia ruminantium* even though *Amblyomma variegatum* ticks were identified from 52.9% of the sampled animals from all study areas. There was significant positive association between *T. mutans* and *T. velifera* ($p < 0.001$) infections, and between *A. marginale* and *B. bovis* ($p = 0.005$). The presence of *R. microplus* tick vectors on cattle was significantly associated with *B. bovis* (odds ratio, OR = 28.4, $p < 0.001$) and *A. marginale* (OR = 42.0, $p < 0.001$) infections, while *A. variegatum* presence was significantly associated with *T. mutans* (OR = 213.0, $p < 0.001$) and *T. velifera* (OR = 459.0, $p < 0.001$) infections. *Rhipicephalus decoloratus* was significantly associated with *B. bigemina* (OR = 21.6, $p = 0.004$) and *A. marginale* (OR = 28.5, $p < 0.001$). Multivariable analysis showed a significant association between location and tick-borne pathogen status for *A. marginale* ($p < 0.001$), *T. mutans* ($p = 0.004$), *T. velifera* ($p = 0.003$) and *T. taurotragi* ($p = 0.005$). The results of our study suggest that the cause of cattle mortalities in Mungwi during the winter outbreaks is mainly due to *A. marginale*, *B. bovis* and *B. bigemina* infections. This was confirmed by the clinical manifestation of the disease in the affected cattle and the tick species identified on the animals. The relatively low prevalence of *T. parva, B. bigemina, B. bovis* and *E. ruminantium* could indicate the existence of endemic instability with a pool of susceptible cattle and the occurrence of disease outbreaks.

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1. Introduction

Tick-borne diseases (TBDs) are among the most important constraints to livestock production in developing countries (Kivaria, 2006). The most important TBDs of cattle in sub-Saharan Africa are theileriosis caused by *Theileria parva*, babesiosis caused by *Babesia bovis* and *B. bigemina*, anaplasmosis caused by *Anaplasma marginale* and heartwater caused by *Ehrlichia ruminantium* (Makala et al., 2003).

Theileriosis poses a major constraint to the Zambian livestock industry with losses of about 10 000 cattle per annum (Nambota et al., 1994). While East Coast fever (ECF) occurs in the Eastern and Northern provinces of Zambia, Corridor disease is widespread in the Southern, Central and Lusaka provinces and has been reported in the Copper-belt province (Makala et al., 2003). However, ECF is moving towards the Southern Province; 683 clinical cases were recorded in 2002 (Chisembele, 2005) and 377 cases and deaths of 87 head of cattle were reported in 2017 (Zambia Farmers Hub, 2017). The highest number of ECF cases occurs from January to March in the Northern and Eastern provinces, and the highest number of Corridor disease cases are recorded during the month of January in the Southern province (Samui, 1987). The epidemiology is complicated by, among other factors, the wide distribution of the tick vector, *Rhipicephalus appendiculatus*, which is found all over the country (Nambota et al., 1994).

Bovine babesiosis is an economically important TBD of cattle in tropical and subtropical regions of the world (McCosker, 1981). *Babesia bovis* and *B. bigemina* are present in all the Zambian provinces (Jongejan et al., 1988; McCosker, 1981) and are recognized as being of economic importance in cattle and small ruminants (Luguru, 1985; Pegram et al., 1989; Pegram and Banda, 1990). In Africa, *Rhipicephalus microplus*, *R. annulatus* and perhaps *R. geigyi* transmit *B. bigemina* and *B. bovis*. In addition, *B. bigemina* is transmitted by *R. decoloratus* and *R. evertsi evertsi* (Bock et al., 2004; Walker et al., 2003). *Babesia bovis* and *B. bigemina* are transovarially transmitted (Bock et al., 2004; Chauvin et al., 2009).

Heartwater, caused by *E. ruminantium*, is a rickettsial disease that affects domestic and wild ruminants in Zambia (Jongejan et al., 1988; Makala et al., 2003). In the agricultural areas of Zambia, *Amblyomma variegatum* is the main vector of heartwater (Makala et al., 2003). In Zambia, heartwater is mainly a disease of cattle, although outbreaks in sheep and goats have been reported and recorded. Records from the Central Veterinary Research Institute (CVRI) for the period 1986–1997 revealed that the disease occurred throughout Zambia (Makala et al., 2003; Mangani, 1997). Heartwater is believed to be responsible for numerous deaths occurring throughout the year, but especially during the rainy season from March to September.

Bovine anaplasmosis (formerly known as gall sickness), caused by *A. marginale*, is an infectious but noncontagious disease that occurs in tropical and subtropical regions worldwide (Aubry and Geale, 2011). *Anaplasma centrale*, a less pathogenic but closely related organism, is used as a live vaccine for cattle in Israel, South Africa, South America and Australia (de la Fuente et al., 2005). Notably, there are strains of *A. centrale* with intermediate morphology (approximately half of the organisms touching the edge of the red blood cells, instead of some 70% in typical *A. marginale* and some 30% in *A. centrale*), and such strains are not particularly mild (FAO, 1994). *Anaplasma marginale* is present in all the provinces of Zambia (Jongejan et al., 1988); it is regarded as the only *Anaplasma* species of importance to cattle in Zambia (McCosker, 1981). Transmission experiments have listed up to 19 different ticks as capable of transmitting *A. marginale* (Kocan et al., 2004).

Despite the importance of TBDs, little is known about the occurrence and prevalence of haemoparasites in cattle in the communal grazing areas of Mungwi District of Northern Province, Zambia (Marufu et al., 2010). Mungwi District is located in an area of Zambia where ECF is thought to be present, and vector control using acaricides has proved to be very costly for the small-scale farmers. Also, Mungwi experiences increased cattle mortalities from December to March and from May through to July. All age groups of cattle are affected. This study was conducted to screen selected communal herds of cattle for tick-borne haemoparasites, and identify the tick vectors associated with the high cattle mortalities due to suspected TBDs in the local breeds of cattle grazing along the banks of the Chambeshi River in the Mungwi District, Northern Province, Zambia.

2. Materials and methods

2.1. Blood samples

Bovine blood samples were collected from July to September of 2010. The sampling areas were all in Mungwi District of Northern Province, Zambia. Kapamba, Chifulo, Chisanga and Kowa are located along the Chambeshi flood plains while Mungwi central is on the upland (Fig. 1). Although Mungwi District experiences increased mortalities in cattle due to TBDs between December to March and May to July, samples could not be collected during this time period as farmers are busy with agricultural activities (i.e., land preparation, planting, weeding and fertilizer application from December to March). Also, some cattle are used for draught power and are, therefore, not available for sampling during this time of cultivation. However, we did manage to collect ticks from April to July. Information on the owner, age, sex and color of each animal sampled was captured. Cattle of different age groups were sampled and all were indigenous breeds (Angoni). The sampled cattle included 84 males and 215 females. The animals were restrained in a crush pen during routine deworming and treatment. Blood samples were collected from the ear vein and 250 µl was spotted onto Whatman® filter paper (Merck, Darmstadt, Germany). The filter papers containing the dry blood spots were stored in silica gel before being transported to the Molecular Biology Laboratory, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa under Department of Agriculture, Forestry & Fisheries Veterinary Import Permit number 13/1/1/30/0/8-124.

2.2. Tick counts and identification

Half body tick counts were conducted on all the 299 cattle that had been sampled for blood. To ensure that the same animals be sampled during re-visitations, animals were ear-tagged, and individual identification records were kept. The objectives, time frame and benefits from the study were also discussed with the farmers. Regular visits were also made to the study areas to conduct routine animal husbandry practices to avoid drop out of the animals from the study.

Ticks were identified to genus and species level using a stereomicroscope and dichotomous identification keys of ticks as illustrated by Walker et al. (2003). The identified ticks were compared to species descriptions and distribution (Walker et al., 2003). The tick counts and identifications were done once on each animal from April to July 2011.

2.3. DNA extraction

DNA was extracted from dried blood spots using the QIAamp® DNA Mini kit (QIAGEN, Southern Cross Biotechnology [Pty] Ltd, Cape Town, South Africa) following the manufacturer's instructions. Extracted DNA was eluted in 100 µl elution buffer and stored at −20 °C until further analysis.

2.4. PCR amplification and reverse line blot (RLB) hybridization assay

Separate PCR master mixes were prepared for the amplification of *Theileria/Babesia* species (Nijhof et al., 2003, 2005) and *Ehrlichia/Anaplasma* species (Bekker et al., 2002). *Theileria* and *Babesia* group-specific forward primer, RLB F2 [5′-GAC ACA GGG AGG TAG TGA CAA G-3′] and biotin-labelled reverse primer, RLB R2 [5′-Biotin-CTA AGA ATT TCA CCT CTA ACA GT-3′] were used to amplify the V4