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Short Communication

PCR-based detection of blood parasites in cattle and adult *Rhipicephalus appendiculatus* ticks

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Abstract

To ascertain the infection rate for tick-borne pathogens in Zambia, an epidemiological survey of *Theileria parva*, *Babesia bigemina* and *Anaplasma marginal*e in traditionally managed Sanga cattle was conducted using PCR. Of the 71 native Zambian cattle, 28 (39.4%) were positive for *T. parva*, 16 (22.5%) for *B. bigemina* and 34 (47.9%) for *A. marginale.* The mixed infection rate in cattle was 8.5% (6/71), 16.9% (12/71), 7.0% (5/71) and 2.8% (2/71) for *T. parva*/*B. bigemina*, *T. parva*/*A. marginale*, *B. bigemina*/*A. marginale* and *T. parva*/*B. bigemina*/*A. marginale*, respectively.

To predict the risk for transmission of tick-borne pathogens from ticks to cattle, a total of 74 *Rhipicephalus appendiculatus* ticks were collected from a location where cattle had been found positive for *T. parva*. Of the ticks collected, 10 (13.5%) were found to be PCR-positive for *T. parva*. The results suggest that the infection rate for tick-borne pathogens was relatively high in Sanga cattle and that adult *R. appendiculatus* ticks were highly infected with *T. parva*.

Keywords: *Theileria parva*; *Babesia bigemina*; *Anaplasma marginale*; *Rhipicephalus appendiculatus*; Cattle; Zambia

Tick-borne diseases (TBDs) result in major economic losses in cattle in many parts of Eastern, Southern and Central Africa, where they cause high morbidity and mortality as well as decreased meat and milk production, and they are an obstacle both to the upgrading of indigenous breeds and to the introduction of more exotic breeds.

Many cattle diseases are transmitted by ticks in Zambia, of which the most significant are theileriosis, babesiosis and anaplasmosis. Five *Theileria* species are known to be distributed in the country, and *Theileria parva,* a causative agent of East Coast fever (ECF), transmitted by the brown ear tick (*Rhipicephalus appendiculatus*), is the most economically important. *T. parva* sporozoites from the tick salivary gland invade bovine lymphocytes at the time of tick feeding and become schizonts that cause a severe febrile infection. Bovine babesiosis and anaplasmosis feature as part of a complex disease process transmitted by ixodid ticks and in many cases occur as a mixed infection (Jongejan et al., 1988). As with *T. parva*, the economic impact of *Babesia bigemina* and *Anaplasma marginale* is significant in terms of mortality, loss of production, cost of control and restrictions on the movement of animals.

Conventional microscopic and serological methods have shown that *Theileria*, *Babesia* and *Anaplasma* spp. are present in cattle in Zambia (Nambota et al., 1994). Although microscopic techniques for blood examination remain the most appropriate means of diagnosing haemoparasites, low sensitivity restricts its use in epidemiological studies, which are required to identify carrier animals, and it is not possible to discriminate pathogenic from non-pathogenic species. On the other hand, serological methods

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standardised for the diagnosis of blood parasites have been extensively employed in epidemiological field studies, and previous serological work has shown that *B. bigemina* and *A. marginale* are widely distributed in Zambia (Jongejan et al., 1988). However, serology cannot discriminate between previous exposure and current infection as it relies on the detection of antibodies, and there is a practical need for a sensitive and more efficient method to assist in epidemiological field investigations.

In this study, we determined the prevalence of three tick-borne pathogens, *T. parva*, *B. bigemina* and *A. marginale,* in Zambian cattle using polymerase chain reaction (PCR). We also examined the prevalence of *T. parva* in host-seeking and attached feeding *R. appendiculatus* ticks taken from cattle in the field. PCR has been used for surveys to determine the prevalence of tick-borne pathogens in cattle and ticks in several African countries.

Field sampling was conducted during the rainy season (March 2006) in the Shibuyunji area of Lusaka, Zambia. For DNA extraction, venous blood samples were collected from 71 Sanga cattle with no history of vaccination against ECF. Host-seeking ticks were collected by dragging vegetation with a cotton flannel in the same location where the blood samples were collected. The species of the collected ticks were identified based on their morphology, and maintained individually in methanol until DNA extraction.

Bovine genomic DNA samples were obtained from 0.5 mL whole blood samples using the Wizard Genomic DNA Purification kit (Promega), according to the manufacturer's instructions. Methanol-fixed individual ticks (17 host-seeking *R. appendiculatus* ticks and 57 infesting *R. appendiculatus* ticks on *T. parva*-uninfected cattle) were homogenised and lysed in the DNA extraction buffer (10 mM Tris–HCl [pH 7.6], 10 mM EDTA, 1M NaCl, 0.5% SDS) with 100 μg/mL Proteinase K at 55 °C for 24 h. DNA was then extracted from these samples using phenol and chloroform followed by the precipitation with ethanol as previously described (Konnai et al., 2006).

PCR for the detection of *T. parva*, *B. bigemina* and *A. marginale* was performed as previously described (Bishop et al., 1992; Figueroa et al., 1992; Lew et al., 2002). Seventy-one DNA samples from Sanga cattle were examined by PCR using specific primers for the detection of *T. parva*, *B. bigemina* and *A. marginale*. To determine the presence of DNA in the samples, PCR amplification of the tick actin or bovine β-globin gene was also performed using primer sets specific for the tick actin gene, Act-108/Act-rev-A, or bovine β-globin gene, PCO3/PCO4, as described by Konnai et al. (2006). The PCR products obtained from each analysis were purified with the Geneclean III Kit (Q-BIOgene), and DNA sequencing was performed by the CEQ 2000 DNA analysis system (Beckman Coulter) to confirm specificity.

Expected 405 bp and 278 bp fragments of *T. parva* and *B. bigemina* DNA, respectively, were amplified. For the detection of *msp1α*-gene of *A. marginale*, the Am1733F/Am2957R primer set amplified a 789 bp product (Fig. 1A). Twenty-eight (39.4%) of the 71 bovine DNA samples showed a positive reaction for *T. parva*, 22.5% (16) for *B. bigemina* and 47.9% (34) for *A. marginale*. Among the samples, mixed infections were found in 25 (35.2%) of the investigated cattle as shown in Fig. 1. The mixed infection rate in cattle was 8.5% (6/71), 16.9% (12/71), 7.0% (5/71) and 2.8% (2/71) for *T. parva*/*B. bigemina*, *T. parva*/*A. marginale*, *B. bigemina*/*A. marginale* and *T. parva*/*B. bigemina*/*A. marginale*, respectively (Table 1A). The prevalence (22.5%) of *B. bigemina* in cattle in this study was similar to that (35%) observed by PCR-based detection in Zimbabwe (Smeenk et al., 2000).

Although we could not directly compare serological results with our findings, a high prevalence of *A. marginale* infection (47.9%) was observed as was found in previous serological work (38.6%) in Zambia (Jongejan et al., 1988). With regard to *T. parva* infection in Zambian cattle, the high prevalence (39.4%) was consistent with our previous survey (41.4%). While a high prevalence of TBD was

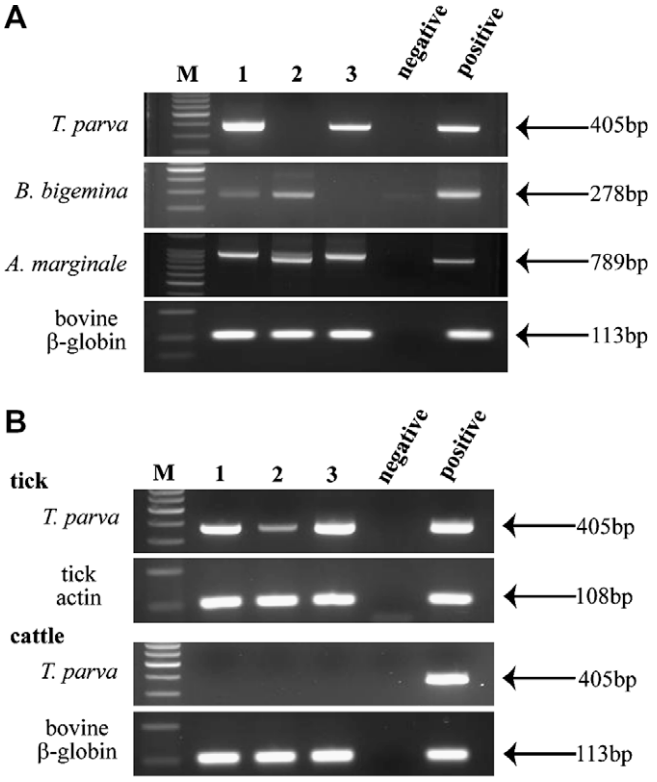

Fig. 1. (A) Detection of *T. parva*, *B. bigemina* and *A. marginale* in cattle. Lane M, 100 bp DNA ladder (Promega); lanes 1–3, individual cattle samples (animals 1–3). Lane 1: a triple-infection case, Lane 2: a co-infection case of *B. bigemina* and *A. marginale* and Lane 3: a co-infection case of *T. parva* and *A. marginale*. (B) Detection of *T. parva* in *R. appendiculatus* (attached feeding ticks) collected in the field. Lane M, 100 bp DNA ladder; lanes1–3, individual cattle or tick samples (animals 1–3 or ticks 1–3). Feeding ticks were collected from *T. parva*-negative cattle. Each sample number corresponds to the tick examined and the source of tick-infested cattle. The recombinant plasmid bearing a cloned PCR product was used as a PCR template for positive control, while non-infected cattle and tick samples were used for the negative template.