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The epidemiology of tick-borne haemoparasites as determined by the reverse line blot hybridization assay in an intensively studied cohort of calves in western Kenya

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ABSTRACT

The development of sensitive surveillance technologies using PCR-based detection of microbial DNA, such as the reverse line blot assay, can facilitate the gathering of epidemiological information on tick-borne diseases, which continue to hamper the productivity of livestock in many parts of Africa and elsewhere. We have employed a reverse line blot assay to detect the prevalence of tick-borne parasites in an intensively studied cohort of indigenous calves in western Kenya. The calves were recruited close to birth and monitored for the presence of infectious disease for up to 51 weeks. The final visit samples from 453 calves which survived for the study period were analyzed by RLB. The results indicated high prevalences of *Theileria mutans* (71.6%), *T. velifera* (62.8%), *Anaplasma* sp. Omatjenne (42.7%), *A. bovis* (39.9%), *Theileria* sp. (sable) (32.7%), *T. parva* (12.9%) and *T. taurotragi* (8.5%), with minor occurrences of eight other haemoparasites. The unexpectedly low prevalence of the pathogenic species *Ehrlichia ruminantium* was confirmed by a species-specific PCR targeting the pCS20 gene region. Coinfection analyses of the seven most prevalent haemoparasites indicated that they were present as coinfections in over 90% of the cases. The analyses revealed significant associations between several of the *Theileria* parasites, in particular *T. velifera* with *Theileria* sp. sable and *T. mutans*, and *T. parva* with *T. taurotragi*. There was very little coinfection of the two most common *Anaplasma* species, although they were commonly detected as coinfections with the *Theileria* parasites. The comparison of reverse line

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blot and serological results for four haemoparasites (*T. parva*, *T. mutans*, *A. marginale* and *B. bigemina*) indicated that, except for the mostly benign *T. mutans*, indigenous cattle seem capable of clearing infections of the three other, pathogenic parasites to below detectable levels. Although the study site was located across four agroecological zones, there was little restriction of the parasites to particular zones.

1. Introduction

Tick-borne infections in cattle in Africa are complex, with many tick species interacting with different hosts and transmitting a wide range of pathogenic and non-pathogenic organisms. The diseases caused by tick-borne pathogens cause substantial economic loss (Uilenberg, 1995) and the improvement of strategies to control the diseases caused by these organisms requires more detailed knowledge of their prevalence and how they interact with each other. In Kenya, the most important tick-borne diseases are theileriosis, anaplasmosis, babesiosis and heartwater (Wesonga et al., 2010), with East Coast fever (ECF), caused by the protozoan parasite *Theileria parva*, being of particular importance. ECF causes substantial production losses through mortality and decreased productivity (Mukhebi et al., 1992), and is a major constraint to keeping improved breeds of cattle in endemic areas (Conelly, 1998; Gitau et al., 2001). Currently, the only practical means of controlling the disease are regular application of acaricides or immunization using the infection and treatment method (Di Giulio et al., 2009).

The application of laboratory diagnostic assays to determine the prevalence of infections is well established. Microscopy is commonly used for diagnosis of tick-borne diseases, because it is both easy to carry out and cheap. However, the method is relatively insensitive and the organisms are difficult to find and identify to the level of species. Serological methods including indirect fluorescent antibody tests (IFAT) and enzyme-linked immunosorbent assays (ELISA) are used to detect antibodies in animals that have been exposed to infections, but these do not necessarily reveal current infections. Several PCR diagnostic techniques have been developed to detect single parasite species, such as the pCS20 quantitative real-time PCR (qPCR) assay for the detection of *Ehrlichia ruminantium* (Steyn et al., 2008) and the nested p104 PCR assay for the detection of *T. parva* (Skilton et al., 2002). A reverse line blot (RLB) hybridization assay has been developed to detect and differentiate between several parasite species simultaneously. The initial RLB assay was developed to detect all the *Theileria* and *Babesia* species that infect cattle (Gubbels et al., 1999). Bekker et al. (2002) described a further development of this assay that enabled simultaneous detection of all the *Anaplasma* and *Ehrlichia* species that infect ruminants. In eastern Africa, a combination of these techniques has been applied to field samples for the identification of tick-borne haemoparasites in an endemic region in Uganda (Oura et al., 2004). In their study, the RLB assay was assessed for the ability to detect the principal tick-transmitted protozoan and rickettsial cattle pathogens in indigenous and crossbred cattle and to identify the carrier states of the parasites. The assay was able to identify *T. parva* at a level comparable with previously developed PCR methods and well below conventional microscopic detection. More recently, Asiimwe et al. (2013) also used the RLB assay to determine the prevalence of haemoparasites and their infection kinetics in cattle on a single farm in central Uganda.

The aim of the study reported here was to gain further information on the prevalence of tick-borne parasites in an important farming system in eastern Africa through the use of the RLB assay. The samples were obtained from an intensively studied birth cohort of 548 calves in western Kenya (Bronsvoort et al., 2013). Apart from baseline prevalence, the results were used to determine the levels of coinfections in the calf population.

2. Materials and methods

2.1. Animals

The IDEAL project has been described in detail elsewhere (Bronsvoort et al., 2013). In brief, a cohort of 548 East African shorthorn calves was recruited into the project at birth and monitored for a period of one year. The calves were visited every 5 weeks, at which time they were subject to a complete physical examination and samples, including blood, were taken for further analysis. The calves were chosen from 20 randomly selected sub-locations in western Kenya, which were distributed across four agro-ecological zones (AEZs). The AEZs are defined according to climate, altitude and agricultural activities (Jaetzold and Schmidt, 1983). As shown in Fig. 1, the study region encompassed the AEZs Lower Midland 1 (LM1), Lower Midland 2 (two areas middle (LM2m) and south (LM2s) split by LM1), Lower Midland 3 (LM3) and Upper Midland 3 (UM3).

For the purposes of the current study, samples from 453 of the 455 IDEAL calves which survived until 51 weeks of age were analyzed, with samples from two calves being unavailable for analysis. There were 181 calves from LM1, 72 calves from LM2m, 70 calves from LM2s, 62 calves from LM3 and 68 from UM3.

2.2. Blood samples

Five milliliters of blood was collected at the final routine visit at 51 weeks before the calves left the study. The blood was collected into sterile vacutainer tubes containing EDTA as anticoagulant and stored at −80 °C. DNA was extracted from 250 μl of each blood sample using a blood DNA extraction kit (Invitrogen, Germany) according to the manufacturer's instructions, and eluted in 100 μl of elution buffer.