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Analysis of host genetic factors influencing African trypanosome species infection in a cohort of Tanzanian *Bos indicus* cattle

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ABSTRACT

Trypanosomosis caused by infection with protozoan parasites of the genus *Trypanosoma* is a major health constraint to cattle production in many African countries. One hundred and seventy one *Bos indicus* cattle from traditional pastoral Maasai (87) and more intensively managed Boran (84) animals in Tanzania were screened by PCR for the presence of African animal trypanosomes (*Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei*), using blood samples archived on FTA cards. All cattle screened for trypanosomes were also genotyped at the highly polymorphic major histocompatibility complex (MHC) class II *DRB3* locus to investigate possible associations between host MHC and trypanosome infection. Overall, 23.4% of the 171 cattle tested positive for at least one of the three trypanosome species. The prevalence of individual trypanosome species was 8.8% (*T. congolense*), 4.7% (*T. vivax*) and 15.8% (*T. brucei*). The high prevalence of *T. brucei* compared with *T. congolense* and *T. vivax* was unexpected as this species has previously been considered to be of lesser importance in terms of African bovine trypanosomosis. Significantly higher numbers of Maasai cattle were infected with *T. brucei* (23.0%, $p = 0.009$) and *T. congolense* (13.8%, $p = 0.019$) compared with Boran cattle (8.3% and 3.6%, respectively). Analysis of *BoLA-DRB3* diversity in this cohort identified extensive allelic diversity. Thirty-three *BoLA-DRB3* PCR-RFLP defined alleles were identified. One allele (*DRB3**15) was significantly associated with an increased risk (odds ratio, OR = 2.71, $p = 0.034$) of *T. brucei* infection and three alleles (*DRB3**35, *16 and *23) were associated with increased risk of *T. congolense* infection. While further work is required to dissect the role of these alleles in susceptibility to *T. brucei* and *T. congolense* infections, this study demonstrates the utility of FTA archived blood samples in combined molecular analyses of both host and pathogen.

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1. Introduction

Trypanosomosis is a disease caused by infection with protozoan parasites of the genus *Trypanosoma*. African animal trypanosomosis (AAT) is caused by *Trypanosoma congolense*, *Trypanosoma vivax* and to a lesser extent *Trypanosoma brucei* (Blood et al., 2007). Although AAT is mainly transmitted by tsetse flies, mechanical transmission by haematophagous flies can also be associated with transmission of infection, particularly in the case of *T. vivax* (Sewell and Brocklesby, 1990).

Trypanosomosis is a major health constraint to livestock production in many African countries. Although AAT is often regarded as purely a cattle disease, with

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an estimated susceptible population of 60 million cattle (Delespaux et al., 2008) and causing approximately 1.2 billion US$ of loss to the agricultural sector annually (Shaw, 2004), it also affects other domestic livestock species, including pigs, camels, goats, sheep and horses (Faye et al., 2001; Masiga et al., 2002). Trypanosomosis in cattle is characterised by a range of clinical signs including anaemia, intermittent fever, oedema and weight loss. It is commonly considered that *T. congolense* causes chronic disease, *T. vivax* a more acute disease and *T. brucei* a mild to chronic disease in cattle (Blood et al., 2007).

Routine diagnosis of trypanosomosis in the field is based mainly on clinical examination and microscopic inspection of Giemsa-stained thick and thin blood smears (Picozzi et al., 2002; Nonga and Kambarage, 2009). However, a number of serological and molecular-based diagnostic techniques have been developed and these are recommended in epidemiological studies due to their higher sensitivity and specificity (Connor and Halliwell, 1987; Faye et al., 2001; Picozzi et al., 2002; Thekisoe et al., 2007; Pinchbeck et al., 2008). In this study we set out to determine the prevalence of *T. congolense*, *T. vivax* and *T. brucei* in a cohort of *Bos indicus* cattle, using whole blood archived on FTA cards as the template in a species-specific polymerase chain reaction (PCR). We were also able to collect data on two variables that could potentially be risk factors for acquisition of trypanosome infections, namely geographical location and cattle breed.

An advantage of using FTA cards for detecting blood-borne parasite diversity is that genetic material from both pathogen and host is present allowing the analysis of both. Due to their extended co-evolution, indigenous African herbivores are generally tolerant to African pathogens. The zebu or humped cattle of east Africa represent a genetically diverse population which derive from *B. indicus* cattle introduced from the middle east crossed with indigenous African *Bos taurus* animals (Bradley et al., 1996). More recently, genetically improved European *B. taurus* cattle have also been introduced and crossed with the indigenous breeds in an attempt to increase productivity while maintaining a degree of resistance to endemic disease. We are interested in identifying genes in African cattle that are associated with the prevalence of African cattle trypanosomes. We have targeted the cattle major histocompatibility complex (MHC), also known as the bovine leucocyte antigen (*BoLA*) complex, which includes polymorphic protein-encoding loci with central roles in determining the specificity of both innate and adaptive immune responses. Diversity within the MHC of cattle has previously been analysed in studies of the genetic basis of resistance and susceptibility to disease (Xu et al., 1993; Sharif et al., 1998; Ballingall et al., 2004). These studies have focused on the second exon of the principal polymorphic class II locus within the MHC of cattle *BoLA-DRB3*. Here we have combined the analysis of parasite prevalence with host MHC diversity to investigate whether the host MHC genotype may have an influence upon the prevalence of African trypanosome species in *B. indicus* cattle.

2. Materials and methods

2.1. Study design and animals

This study used 171 *B. indicus* cattle that were part of a mastitis study project in Tanzania. The study animals were all adult lactating cows of either Maasai or Boran types. The Maasai cattle were from traditionally managed pastoralist herds ($n = 87$), and the Boran cattle were from intensively managed parastatal ranches ($n = 67$) and livestock multiplication units (LMU, $n = 17$). The study animals were from the eastern coastal regions: Morogoro and Pwani, which experience a hot and humid climate.

2.2. Sample collection, storage and processing

Blood samples were collected from study animals by jugular venipuncture between December 2006 and March 2007. For each sample, 150 μl of whole blood was applied to an FTA card (Whatman® Bioscience Ltd., Abington, UK), air-dried, stored at room temperature and then transported to the UK for further analysis.

FTA blood cards provided genetic material for PCR-based molecular analyses of trypanosome prevalence and host MHC genotype. Using a Harris 1.2 mm micropunch (Sigma–Aldrich Ltd., Dorset, UK), discs were punched from each card for use as template in PCR reactions; a separate disc was used for each PCR reaction. FTA discs were washed firstly with FTA purification reagent (Whatman® Bioscience Ltd., Abington, UK) and then in Tris EDTA buffer pH 8.0, according to manufacturer's instructions. The discs were then air-dried at 56 °C before performing PCR.

2.3. Polymerase chain reaction detection of trypanosome species

Detection of the three species of African animal trypanosome was carried out by PCR, using species-specific primer pairs directed against multi-copy satellite repeats (Masiga et al., 1992). The following reaction conditions were used; PCR buffer (45 mM Tris–HCl, pH 8.8), 11 mM $(NH_4)_2SO_4$, 4.5 mM $MgCl_2$, 6.7 mM 2-mercaptoethanol, 4.4 μM EDTA, 113 μg/ml BSA, 1 mM of each 4 deoxyribonucleotide triphosphates (dNTPs), 1 μM of each oligonucleotide primer and 1 unit of *Taq* polymerase per 20 μl. Amplification was carried out using 1 disc in a final volume of 20 μl, and cycling conditions as described in Masiga et al. (1992). PCR products were resolved by electrophoresis on a 2% Seakem (Lonza, Basel, Switzerland) agarose gel and were stained with 0.2 μg/ml ethidium bromide to allow visualisation under UV light. Positive controls included in each set of reactions were genomic DNA from TREU 927 *T. brucei*, ILRAD V-34 *T. vivax* and GAM 2 *T. congolense* (Savannah strain), and negative control reactions included distilled H_2O. It should be noted that animals were not checked for the presence of *T. congolense* Forest or Kilifi strains.

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