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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.

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.

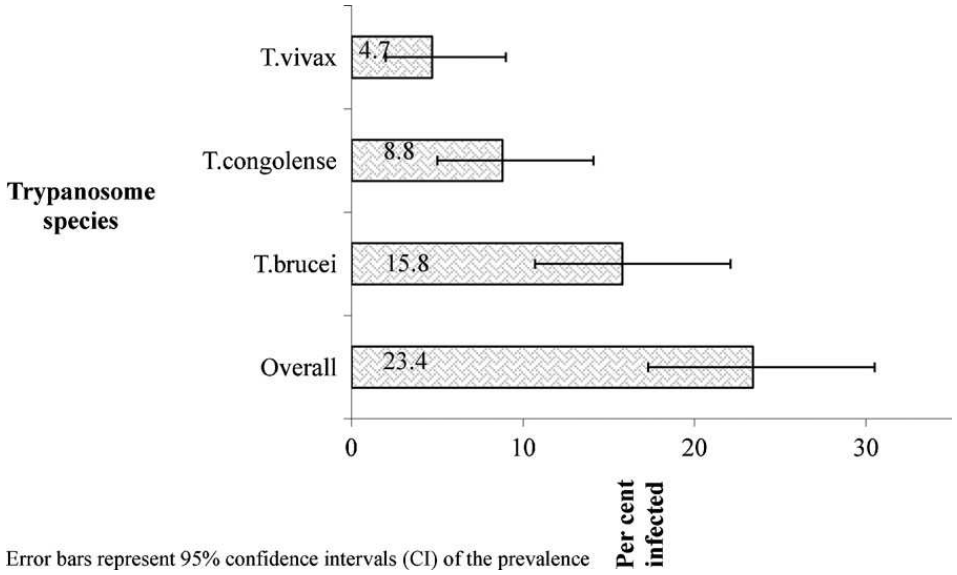

Fig. 1. African animal trypanosome infection rates in Tanzanian *Bos indicus* cattle. Error bars represent 95% confidence intervals (CIs) of the prevalence.

2.4. Genotyping of animals

Allelic diversity at the *BoLA-DRB3* locus was determined by PCR-RFLP according to the method described by van Eijk et al. (1992). Individual alleles are defined according to the patterns generated from exon 2 PCR products cut individually with restriction enzymes *Rsa*1, *BstY*1 and *Hae*III. Samples with the same pattern from different animals and samples that were considered inconclusive and difficult to type by PCR-RFLP were subjected to direct DNA sequencing of PCR products in order to validate and confirm their *DRB3* genotypes.

2.5. Data analysis

Descriptive statistics for the data collected were computed using Epi Info statistical programme (CDC, 2008). The units of analysis were individual *B. indicus* cattle screened for trypanosome infection. The proportions of categorical variables were compared using a Chi-squared test at the critical probability of $p < 0.05$. Associations between outcome (i.e. trypanosome infection status) and explanatory variables (i.e. geographical origin, cattle breed or *DRB3* allele) were investigated in two steps by logistic regression analysis employing the Epi Info programme. The most common *DRB3* alleles (frequency >4%) were used in logistic regression analysis so as to avoid convergence failures associated with low frequency terms. In the first step, relationships between each explanatory and outcome variable were individually investigated by univariate analysis. The second step involved fitting multivariate models which included any explanatory variables that were associated with outcome variable at the $p < 0.25$ level. By employing forward and backward substitution procedures, a final model for each outcome variable was reached when explanatory variables remained significant at the $p < 0.05$ level. The presence/absence of selected *DRB3* alleles was compared between the Maasai and Boran cattle using Chi-squared statistics.

3. Results

3.1. Trypanosome prevalence

The prevalence of the three trypanosome species in the 171 cattle analysed is shown in Fig. 1. Out of 171 cattle screened, 40 (23.4%) were found to be positive for at least one of the three trypanosome species. Breaking this down to individual species, the prevalence of *T. brucei*, *T. congolense* and *T. vivax* was 15.8%, 8.8% and 4.7%, respectively. As each animal was screened for all three trypanosome species, some animals were identified with multiple infections. Of the 27 animals identified with *T. brucei* infection, 9 (33.3%) of these had *T. congolense* and one was infected with all three parasite species.

3.2. Factors associated with trypanosome prevalence

The analysis of the two factors tested for association with trypanosome infection is shown in Table 1. Overall, there was no statistical difference in the proportions of cattle types infected with the parasites. Considering individual trypanosome species, a significantly ($p = 0.009$) higher proportion of the Maasai cattle (23.0%) was infected with *T. brucei* than the Boran cattle (8.3%). Similarly, a significantly ($p = 0.019$) higher proportion of the Maasai cattle (13.8%) was infected with *T. congolense* than the Boran cattle (3.6%). There was no significant difference in the proportion of the Maasai (3.4%) and Boran (6.0) infected with *T. vivax* ($p = 0.440$).

3.3. Trypanosome infection and BoLA-DRB3 allelic diversity

A total of 33 *BoLA-DRB3* alleles were identified in the 171 cattle screened for the presence of trypanosome DNA (Table 2). Analysis of associations between the presence of the nine most frequent *DRB3* alleles and trypanosome infection (i.e. a combination of the three *Trypanosoma*

Table 1
Distribution of overall trypanosome infection by region and cattle type.

Variable factor	Category	Samples analysed (*n*)	Proportion infected	95% CI
Region	Pwani	82	19.5	11.6–29.7
	Morogoro	89	27.0	18.1–37.4
Cattle type	Boran	84	17.9	10.4–27.7
	Maasai	87	28.7	19.5–39.4

Table 2
Frequency of *BoLA-DRB3* alleles in 171 Tanzanian *Bos indicus* Maasai and Boran cattle arranged in descending order.

SN	*Rsa*1–*BstY*1–*Hae*III pattern of the allele	PCR-RFLP number of the allele	Frequency in Boran cattle (*n* = 84), counts (%)	Frequency in Maasai cattle (*n* = 87), counts (%)	Overall frequency (*n* = 171), counts (%)
1	iba	*15	27 (16.1)	17 (9.8)	44 (12.9)
2	cbb	*35	13 (7.7)	22 (12.6)	35 (10.2)
3	daa	*06	17 (10.1)	14 (8.0)	31 (9.1)
4	fba	*10	13 (7.7)	14 (8.0)	27 (7.9)
5	maa	*32	12 (7.1)	5 (2.9)	17 (5.0)
6	jbd	*16	4 (2.4)	12 (6.9)	16 (4.7)
7	obb	*28	7 (4.2)	9 (5.2)	16 (4.7)
8	nba	*23	6 (3.6)	9 (5.2)	15 (4.4)
9	oba	*37	5 (3.0)	9 (5.2)	14 (4.1)
10	mba	*22	6 (3.6)	6 (3.4)	12 (3.5)
11	bbb	*03	2 (1.2)	9 (5.2)	11 (3.2)
12	ibf	*31	10 (6.0)	1 (0.6)	11 (3.2)
13	lab	*34	8 (4.8)	2 (1.1)	10 (2.9)
14	sbb	*19	2 (1.2)	8 (4.6)	10 (2.9)
15	faa	*08	2 (1.2)	7 (4.0)	9 (2.6)
16	bba	*02	3 (1.8)	5 (2.9)	8 (2.3)
17	hba	*13	5 (3.0)	3 (1.7)	8 (2.3)
18	gea	*11	4 (2.4)	3 (1.7)	7 (2.0)
19	kbi	*44	3 (1.8)	4 (2.3)	7 (2.0)
20	lbb	*20	3 (1.8)	4 (2.3)	7 (2.0)
21	fda	*09	1 (0.6)	2 (1.1)	3 (0.9)
22	haa	*12	2 (1.2)	1 (0.6)	3 (0.9)
23	lba	*36	1 (0.6)	2 (1.1)	3 (0.9)
24	lbf	*18	2 (1.2)	1 (0.6)	3 (0.9)
25	oab	*26	1 (0.6)	2 (1.1)	3 (0.9)
26	obf	*27	2 (1.2)	1 (0.6)	3 (0.9)
27	aaa	*01	1 (0.6)	1 (0.6)	2 (0.6)
28	nbf	*33	2 (1.2)	0 (0.0)	2 (0.6)
29	ecc	*07	1 (0.6)	0 (0.0)	1 (0.3)
30	hbf	*42	1 (0.6)	0 (0.0)	1 (0.3)
31	kbb	*17	1 (0.6)	0 (0.0)	1 (0.3)
32	kbj	-	1 (0.6)	0 (0.0)	1 (0.3)
33	oaa	*25	0 (0.0)	1 (0.6)	1 (0.3)

Table 3a
Univariate association between risk factors, including the most common *DRB3* alleles, and *Trypanosoma brucei* infection in *Bos indicus* cattle (*n* = 171).

Term	Odds ratio	95% CI	β (SE)	Wald statistic	*p*-Value
(a) *DRB3* alleles					
iba (*15)	2.24	0.93–5.38	0.80 (0.45)	1.79	0.073
cbb (*35)	1.59	0.61–4.15	0.46 (0.49)	0.95	0.345
daa (*06)	1.58	0.57–4.37	0.46 (0.52)	0.89	0.374
fba (*10)	1.33	0.45–3.90	0.29 (0.55)	0.52	0.602
maa (*32)	0.33	0.04–2.61	−1.11 (1.05)	−1.05	0.294
jbd (*16)	3.48	0.78–15.50	1.25 (0.76)	1.63	0.103
nba (*23)	1.38	0.36–5.24	0.32 (0.68)	0.47	0.641
obb (*28)	0.33	0.004–2.61	−1.11 (1.05)	−1.05	0.294
oba (*37)	0.88	0.19–4.17	−0.13 (0.79)	−0.16	0.872
(b) Other factors					
Cattle type: Maasai/Boran	3.28	1.31–8.25	1.19 (0.47)	2.53	0.011
Region: Morogoro/Pwani	2.06	0.87–4.88	0.72 (0.44)	1.64	0.102

Table 3b
Univariate association between risk factors, including the most common *DRB3* alleles, and *Trypanosoma congolense* infection in *Bos indicus* cattle ($n = 171$).

Term	Odds ratio	95% CI	β (SE)	Wald statistic	p-Value
(a) *DRB3* alleles					
iba (*15)	1.21	0.36–4.04	0.19 (0.61)	0.31	0.754
cbb (*35)	4.38	1.46–13.12	1.48 (0.56)	2.63	0.008
daa (*06)	0.34	0.04–2.70	−1.08 (1.06)	−1.02	0.309
fba (*10)	0.85	0.18–4.00	−0.17 (0.79)	−0.21	0.833
maa (*32)	0.67	0.08–5.46	−0.40 (1.07)	−0.37	0.710
jbd (*16)	3.85	0.70–21.00	1.35 (0.87)	1.56	0.120
nba (*23)	3.00	0.74–12.11	1.10 (0.71)	1.54	0.123
obb (*28)	0.00	0.00–0.00	−13.25 (349.94)	−0.04	0.970
oba (*37)	0.79	0.10–6.46	−0.24 (1.07)	−0.22	0.823
(b) Other factors					
Cattle type: Maasai/Boran	4.32	1.17–15.90	1.46 (0.66)	2.20	0.028
Region: Morogoro/Pwani	1.43	0.48–4.19	0.35 (0.55)	0.64	0.520

Table 4a
Final model for the association between PCR-based *Trypanosoma brucei* infection rate and risk factors.

Term	Odds ratio	95% CI	β (SE)	Wald statistic	p-Value
Cattle type: Maasai/Boran	3.73	1.45–9.62	1.32 (0.48)	2.72	0.006
DRB3 allele *15	2.71	1.07–6.82	1.00 (0.47)	2.12	0.034
Constant	–	–	−2.76 (0.45)	−6.10	0.000

species) was unable to identify any significant associations. However, univariate analysis of *T. brucei* infected animals identified a significantly increased risk of infection in the Maasai cattle. Other variables that qualified for inclusion in the multivariate analysis ($p < 0.25$) were *DRB3* alleles *15 and *16 and; geographical location of animal defined by the region (Table 3a). During multivariate analysis, two factors that were retained in the final model which were significantly associated with increased risk of *T. brucei* infection in *B. indicus* cattle were the Maasai (rather than Boran) cattle (OR = 3.73) and presence of *DRB3* allele *15 (OR = 2.71) (Table 4a). Similarly, univariate analysis of *T. congolense* infection identified a significantly increased risk of infection with the Maasai cattle ($p = 0.028$) as well as *DRB3* allele *35 ($p = 0.008$) (Table 3b). Other variables that qualified for inclusion in the multivariate analysis ($p < 0.25$) were *DRB3* allele *16 and *23. The final model for the *T. congolense* infection included three *DRB3* alleles *35, *16 and *23 which were still significantly associated with the increased risk of *T. congolense* infection (Table 4b).

To check that the associations of infection with certain alleles were not caused by asymmetric distribution of these alleles between Maasai and Boran cattle, the distributions of these alleles were compared in the two breeds using Chi-square. Except for the *DRB3* allele *16 which was significantly ($p = 0.048$) more frequent in the Maasai than in Boran cattle, other alleles (*15, *23 and *35) were uniformly distributed in the Maasai and Boran cattle (Table 2).

4. Discussion

Using PCR-based molecular diagnostics, this study shows a prevalence of trypanosome infection (23.4%) in the selected cohort of *B. indicus* cattle, which is higher than previously reported in Tanzanian cattle by Connor and Halliwell (1987) and Nonga and Kambarage (2009). In these studies prevalence ranged between 2.3% and 16.0%. The difference is likely to be due to the current study having employed a more sensitive PCR-based technique, while previous studies employed either microscopic or serological techniques to estimate the prevalence. Previous studies in Tanzania and neighbouring countries have also reported a higher prevalence of *T. congolense* or *T. vivax* compared to *T. brucei*, in contrast to the results presented here (Tarimo-Nesbitt et al., 1999; Waiswa and Katunguka-Rwakishaya, 2004; Nonga and Kambarage, 2009). The current study identified a higher prevalence of *T. brucei* infection compared with the other two species, although the sample size is relatively limited and confidence intervals are large, and the apparent differences need interpretation with some circumspection. However, it should also be noted that due to the inherent limitations of the FTA technology (Cox et al., 2010), the trypanosome species

Table 4b
Final model for the association between PCR-based *Trypanosoma congolense* infection rate and risk factors.

Term	Odds ratio	95% CI	β (SE)	Wald statistic	p-Value
DRB3 allele *35	6.25	1.85–21.12	1.83 (0.62)	2.95	0.003
DRB3 allele *16	6.71	1.04–43.21	1.90 (0.95)	2.00	0.045
DRB3 allele *23	5.68	1.200–26.89	1.74 (0.79)	2.19	0.029
Constant	–	–	−3.30 (0.48)	−6.92	0.000

prevalence we report are by definition a minimum prevalence, and therefore likely to be an underestimate in the population concerned. The variation in the prevalence of the different species of *Trypanosoma* observed between different studies may partly be explained by failure of techniques such as microscopic examination to discriminate between parasites at the species level. In addition, infections with *T. brucei* in livestock (and particularly in cattle) may present with parasitaemias that are lower than the detection level for microscopy, but within the limit of detection by PCR (Pinchbeck et al., 2008). Our findings would suggest that the hypothesis of PCR-based diagnostic approaches being able to more accurately determine the infection status of cattle is correct. Another potential explanation is that we have sampled lactating adult cows which might not be representative of the population as a whole. While cattle sex differences in susceptibility to trypanosome infections appear to be negligible, a strong correlation of prevalence with age would be expected in areas where trypanosomes are endemic (Simukoko et al., 2007). It is clear that we are limited in the conclusions that can be drawn from the current sample set, and in order to fully incorporate analysis of both the genetic and the epidemiological aspects discussed above a fully and appropriately designed cross-sectional study is required.

The data on mixed infections are interesting in that, if *T. brucei* and *T. congolense* infections were distributed independently in the population sampled, the expected number of mixed infections would be 2.4 [i.e. $(0.088 \times 0.158) \times 171 = 2.4$], much lower than the observed number of 9, and implying that infections of these two species are not independent. Mixed trypanosome species with aggregated distributions in tsetse have been observed previously (Woolhouse et al., 1996). Whether *T. vivax* infections are distributed independently of the other two species cannot be discerned from these data because of the low prevalence of *T. vivax*. The aggregated distribution of *T. brucei* and *T. congolense* infections could potentially arise from three sources. Firstly, cattle with increased resistance/susceptibility to infection with one species have similar responses to the other. Second, species could be co-distributed in tsetse such that when a host is infected by fly bite, there is a high probability of infection with two species rather than only one. Our observation that infections with the two trypanosome species associate with different *DRB3* alleles suggest that perhaps the second of these explanations is the more likely. An additional third potential explanation is that trypanosomes interact during co-infections, impacting upon the resulting prevalence at population levels. Interactions between trypanosome species or strains, or between trypanosomes and other endemic pathogens has not to date been intensively studied (Dwinger et al., 1989; Faye et al., 2001; Mattioli et al., 1999), but is an issue that may have significant consequent impact upon the epidemiology of the disease, particularly as potential interactions could be an explanation for our data, and others (Pinchbeck et al., 2008).

Analysis of allelic diversity at the MHC class II *BoLA-DRB3* locus in 171 East African *B. indicus* identified a higher than expected level of genetic diversity (Ballingall et al., 2004). The PCR-RFLP typing method identified 33 alleles with frequencies ranging from 0.3 to 12.9%. This level of diversity is likely to reflect the traditional herd management practices of the pastoralist community in combination with environmental pressures relating to infectious disease and climate which act to maintain the genetic diversity of these cattle. This is in contrast to the dairy industry in developed countries which for many years have used artificial insemination with a limited number of bulls selected mainly for productivity, a practice likely to reduce genetic diversity over time.

An analysis of trypanosome infection and *BoLA-DRB3* diversity could only be applied to alleles that occurred at a frequency greater than 4%. A much larger sample size will be required to include those alleles that occur at lower frequencies. Analysis of the 9 most frequent alleles identified a significantly increased risk of *T. brucei* infection associated with PCR-RFLP allele *15 and an increased risk of *T. congolense* infection associated with alleles *35, *16 and *23. The logistic regression procedure specifically takes into account the variables 'region' and 'cattle type' thus improving confidence in these two associations. These findings suggest that *T. brucei* and *T. congolense* infections may be influenced by genetic diversity within the cattle MHC. Such associations could arise through various direct mechanisms associated with the roles of MHC genes in determining the specificity of innate or adaptive immune responses. Direct mechanisms focus on the role of class II MHC molecules in presentation of antigenic peptides to $CD4^+$ T cells. In addition to provision of help for the B cell responses observed in trypanosome infections, priming of specific T cells may have direct cytokine-mediated effects on the parasite. An alternative indirect explanation is that the *DRB3* locus is simply a genetic marker for a linked gene or genes that are involved in controlling the infection. The MHC is known to be the most gene dense region of the mammalian genome (Xie et al., 2003) and alleles *15, *16, *23 and *35 may be linked to other polymorphic loci that are in some way detrimental in the response to *T. congolense* and *T. brucei* infection. Previous studies into the genetic basis of the trypanotolerance trait in mouse identified a QTL on chromosome 17 in close proximity to the murine MHC (Iraqi et al., 2000). In contrast, a QTL identified on bovine chromosome 23 which includes the MHC has only a marginal effect compared to other QTL identified in cattle (Hanotte et al., 2003). However, these studies were carried out with *T. congolense* and parasite control mechanisms may be parasite species-dependent, which may also provide an explanation of why different *DRB3* alleles are associated with susceptibility to *T. congolense* and *T brucei* in our study. Many studies have also demonstrated that body odors are regulated by genes present within the MHC (Carroll et al., 2002). *BoLA-DRB3* alleles *15 and *35 may be associated with odors attractive to tsetse flies carrying either *T. brucei* or *T. congolense* parasites, although this would require further study.

The higher prevalence of trypanosome infection (*T. brucei* and *T. congolense*) in Maasai pastoral herds than in

intensively managed Boran cattle may well be because the Maasai community does not apply either chemoprophylaxis or tsetse control methods on a regular basis, or alternatively, that the two cattle breeds differ in their degree of trypanotolerance (Mwangi et al., 1998; Maichomo et al., 2005). As mentioned previously, however, it was not possible to distinguish between the contribution of management or breed with the current data set, and a larger sample set with better stratification would help to resolve all of the above issues.

In conclusion this study demonstrates the use of FTA-archived blood samples in order to apply molecular analyses to both blood borne pathogen diagnosis and host genetic diversity. The higher prevalence of *T. brucei* infection in *B. indicus* cattle reported here than in previous studies in Tanzania suggests that the use of PCR will be a crucial tool in identifying the true prevalence and impact of the different species of African trypanosomes in livestock. The role of the MHC haplotypes which includes *BoLA-DRB3**15, *16, *23 and *35 alleles requires further investigation, as this may be an important factor in the inherent tolerance to trypanosomes displayed by several breeds of cow. A cross-sectional study using a much larger cohort of cattle would allow both of these findings to be fully addressed.

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