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ORIGINAL PAPER

Theileria parva genetic diversity and haemoparasite prevalence in cattle and wildlife in and around Lake Mburo National Park in Uganda

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Abstract Wildlife, especially Cape buffalo (*Syncerus caffer*), are thought to act as a reservoir for many of the important tick-borne pathogens of cattle. In this study, we have determined the prevalence of the most significant tick-borne haemoparasites in wildlife (buffalo, impala, eland and bushbuck) as well as in cattle grazing inside and neighbouring Lake Mburo National Park (LMNP) in Uganda. A high percentage of buffalo were carriers of *Theileria parva*, *Theileria mutans, Theileria velifera, Theileria buffeli* and *Theileria* sp. (buffalo) as well as *Anaplasma marginale* and *Anaplasma centrale*. The majority of impala sampled were carriers of *A. centrale,* and all were carriers of an unidentified *Babesia*/*Theileria* species. The eland and bushbuck sampled were all carriers of *Theileria taurotragi* and *Theileria buffeli,* and the majority were carriers of *T. mutans*. The bushbuck sampled were also carriers for *Erhlichia bovis*. There were some differences in the prevalence of haemoparasites between the calves sampled inside and neighbouring LMNP. In order to address the question of whether there is evidence for interbreeding between buffalo-associated and cattle-associated *T. parva* populations, multi-locus genotypes (MLGs) of *T. parva* (based on micro-satellite markers) from buffalo and from calves grazing inside and outside LMNP were compared, and the results revealed that buffalo and cattle gene pools were distinct, showing no evidence for transmission of buffalo-derived *T. parva* genotypes to the cattle population.

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Introduction

Cattle and wildlife in East Africa are exposed to a range of tick-borne pathogens of the genera *Theileria*, *Ehrlichia*, *Anaplasma* and *Babesia*. The most serious of these, *Theileria parva*, causes the disease East Coast fever (ECF) which is associated with high levels of mortality, primarily in exotic and crossbred cattle, but also in indigenous calves and adult cattle in endemically unstable areas (Perry and Young 1995). In this study, we have used a reverse line blot (RLB) assay (Bekker et al. 2002; Gubbels et al. 1999; Oura et al. 2004b) to compare the haemoparasite carrier prevalence in African Cape buffalo (*Syncerus caffer*), impala, eland and bushbuck from Lake Mburo National Park (LMNP) with cattle both co-grazing with wildlife and cattle from farms neighbouring LMNP that had no direct contact with wildlife.

African Cape buffalo are thought to be the major natural host of *T. parva* in which the parasite does not appear to cause clinical disease (Grootenhuis et al. 1987). It is believed that co-evolution of African Cape buffalo with *T. parva* populations occurred prior to the advent of the disease in cattle (Epstein 1971), and it is thought that ticks infected

with *T. parva* from buffalo first came into contact with *Bos indicus* cattle in Sub-Saharan Africa approximately 4,500 years ago (Epstein 1971). There is some controversy about the terminology used to describe *T. parva* infections in Sub-Saharan Africa. Initially, three different subspecies of *T. parva* were thought to occur that caused three clinically distinct diseases in cattle (Lawrence 1979), with *T. parva parva* causing ECF, *T. parva bovis* causing January disease and *T. parva lawrencei* causing Corridor disease. A more recent school of thought put forward the suggestion that *T. parva* parasites should be classified according to their host of origin, as cattle-derived or buffalo-derived (Norval et al. 1992), and in this paper, we use the cattle-derived/buffalo-derived terminology as descriptive terms.

The epidemiology of theileriosis is complicated in some areas by buffalo-derived *T. parva* parasites. ECF is caused by *T. parva* parasites that can be transmitted between cattle by ticks, but a different clinical syndrome (Corridor disease) in cattle is caused by the transmission of *T. parva* directly from buffalo to cattle via infected ticks. Although Corridor disease is often fatal, it is thought to be self-limiting within cattle populations, as erythrocytic piroplasms are either absent or an insufficient level to infect new ticks (Norval et al. 1991). Onward transmission of buffalo-derived strains of *T. parva* between cattle has been demonstrated experimentally (Maritim et al. 1989a, b), and repeated passage in cattle leads to a change such that the disease becomes indistinguishable from classical ECF. However, it is unknown whether the *T. parva* parasite is genetically 'transformed' as it adapts to the bovine host or whether there is selection of an individual strain that causes ECF-like clinical signs from a mixed population of genotypes in the buffalo. The use of molecular characterisation tools has revealed a high level of diversity among buffalo-derived *T. parva* stocks compared to cattle-derived *T. parva* stocks (Bishop et al. 1994; Collins and Allsopp 1999; Geysen et al. 2004; Oura et al. 2004a). Thus, there is a growing body of evidence that only a limited subset of the total *T. parva* gene pool present within buffalo has become established in cattle. It is unknown whether the transfer of buffalo-derived strains to cattle resulting in ECF was a single event or whether there is a constant trickle of new strains transferring from buffalo to cattle. This has very important implications as it has been demonstrated that there is incomplete cross-protection between animals immunised with cattle-derived *T. parva* when challenged with parasites from buffalo (Young et al. 1973), which suggests that any live vaccination programme may not protect against Corridor disease in areas where cattle and buffalo co-graze.

An important question that we have addressed in this study is to what extent the gene pools of buffalo-associated and cattle-associated *T. parva* are separated. Data on ribosomal ITS sequences indicates that the two populations are substantially, but not completely, distinct. The mosaic nature of the ITS sequences suggests that limited genetic exchange may still occur through occasional sexual recombination between the two populations, indicating that they may not yet be fully reproductively isolated (Collins and Allsopp 1999).

The advent of new tools for the detection of different tick-borne pathogens, such as the reverse line blot (Gubbels et al. 1999) and new discriminatory methods for genotyping parasite isolates (Oura et al. 2003) allows a more detailed analysis of the role of wildlife in the transmission of tick-borne diseases. These developments have allowed us to specifically address a series of questions about the prevalence of pathogens in wildlife, the role of wildlife as a reservoir of infection for co-grazed cattle and the genetic relationship between *T. parva* in cattle and buffalo. In this paper, we report the results of a study analysing these questions in a discrete geographical area of Uganda.

Materials and methods

Animals

Four wildlife species (Cape buffalo (*S. caffer*), impala, bushbuck and eland) were blood sampled as part of the Pan African programme for the control of epizootics (PACE). In total, 19 adult buffalo, 12 adult impala, six adult eland and three adult bushbuck were sampled from LMNP in western Uganda.

Groups of indigenous *B. indicus* calves were sampled both from outside and inside LMNP in western Uganda. The calves sampled within the park regularly grazed in areas that were also grazed by buffalo and other wildlife. Two groups of calves were sampled from within the park: a group of 20 calves (group A) that were 1–3 months old and a group of 25 calves (group B) that were 3–12 months old. Ninety-nine 3–12-month-old calves were sampled from two farms neighbouring but separated from LMNP. These calves had never entered LMNP and so had no previous direct contact with wildlife species.

Haemoparasite detection

Blood samples were processed and analysed for the presence of haemoparasites by an RLB assay (Gubbels et al. 1999) with modifications previously described (Oura et al. 2004b).

Micro- and mini-satellite PCR assay

Sample preparation

Blood was collected in EDTA vacutainers (Becton Dickinson), aliquoted into 1.5 ml tubes and stored at −20°C.

DNA was purified from bovine blood samples spotted on to FTA filter paper (Whatman BioScience) according to a previously described protocol (Oura et al. 2005).

PCR amplification

A nested PCR reaction was carried out on DNA from the blood samples immobilised on FTA filter paper. The inner and outer nested primers were designed from sequences in the flanking regions of two micro- and ten mini-satellite repeats (ms5, ms7, MS 3, MS 7, MS 8, MS 16, MS 19, MS 21, MS 25, MS 27, MS 33 and MS 40). The copy number and consensus repeat sequences as well as the sequences of the inner nested primers have been described (Oura et al. 2003) as well as the sequences of the outer nested primers (Oura et al. 2004a, 2005). The conditions used in the nested PCR amplifications are as described previously (Oura et al. 2004a).

High-resolution amplicon separation using "Spreadex" gels

The use of Spreadex gels to define different mini- and micro-satellite alleles of *T. parva* at high resolution has been described previously (Oura et al. 2003, 2004a). Under optimal conditions, these gels provide a resolution of three base pairs (bp). Allele sizes were estimated by direct comparison with the M3 marker (Elchrom Scientific), which contains more than 50 DNA fragments ranging between 75 and 622 bp. The M3 marker has been specifically designed for the accurate sizing of micro- and mini-satellite alleles that may differ in size by as little as three base pairs and contains a range of markers that are three to five base pairs apart.

Data analysis

Two separate datasets were generated for genotypic analyses. The MLG dataset comprised genotypes constructed from only the predominant allele at each locus, while the 'allelic profile' dataset comprised genotypic profiles constructed from all the alleles identified at each locus. Similarity analyses of each dataset was performed using Jaccard's similarity coefficient (Jaccard 1908) employing the web-based application—Clustering Calculator (http://www.biology.ualberta.ca/old_site/jbrzusto/cluster.php). This program was used to cluster the data, produce dendrograms and perform bootstrapping analysis using 1,000 pseudo-replications. Generated trees were visualised using TreeViewX version 0.4 (Page 1996). F_{ST} values (Wright 1921) were calculated using allele frequency data from the full allelic profiles for the 12 micro- and mini-satellite loci.

Results

Haemoparasite carrier prevalence in buffalo, impala, eland and bushbuck from LMNP

In order to investigate the prevalence of haemoparasites in wildlife species in LMNP, an RLB assay was used on blood samples from 12 impala (Fig. 1a), three bushbuck (Fig. 1b), six eland (Fig. 1c) and 19 buffalo (Fig. 1d) grazing inside LMNP, and the results are summarised in terms of the prevalence of tick-borne haemoparasites in Table 1. The majority of buffalo were carriers of many of the *Theileria* species [*T. parva, Theileria mutans, Theileria velifera, Theileria buffeli* and *Theileria* sp. (buffalo) (Allsopp et al.

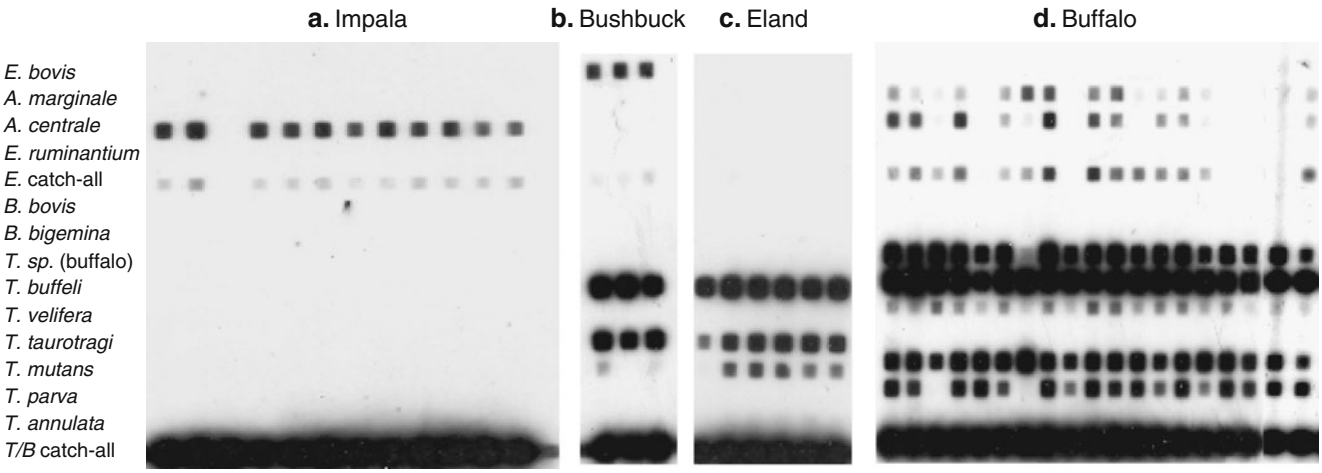

Fig. 1 Reverse line blot of samples from wildlife species grazing in Lake Mburo National Park, **a** impala, **b** bushbuck, **c** eland and **d** buffalo. Species-specific oligonucleotide probes were applied to the horizontal rows of the RLB and are shown to the *left of the blot* (*T/B catch-all—Theileria/Babesia* catch-all, E *catch-all—Ehrlichia* catch-all, *E—Ehrlichia*, *A—Anaplasma*, *B—Babesia*, *T—Theileria*)

Table 1 Prevalence of haemoparasites in wildlife and cattle grazing inside Lake Mburo National Park and in cattle neighbouring the park

Group	*n*	*T. parva*	*T. mutans*	*T. taurotragi*	*T. velifera*	*T. buffeli*	*T.* sp. (buffalo)	*A. centrale*	*A. marginale*	*E. bovis*
Impala from LMNP	12	0	0	0	0	0	0	11 (92%)	0	0
Eland from LMNP	6	0	5 (83%)	6 (100%)	0	6 (100%)	0	0	0	0
Bushbuck from LMNP	3	0	2 (67%)	3 (100%)	0	3 (100%)	0	0	0	3 (100%)
Buffalo from LMNP	19	17 (85%)	19 (100%)	0	19 (100%)	19 (100%)	19 (100%)	12 (63%)	14 (74%)	0
Calves inside LMNP (1–3 months old)	20	9 (45%)	4 (20%)	5 (25%)	0	6 (30%)	0	0	0	4 (20%)
Calves inside LMNP (3–12 months old)	25	23 (92%)	2 (16%)	19 (76%)	2 (16%)	0	0	0	4 (16%)	6 (38%)
Calves neighbouring LMNP (3–12 months old)	99	68 (69%)	28 (28%)	45 (45%)	15 (15%)	0	0	0	5 (5%)	34 (34%)

The number of positive samples for each species is shown together with percent positivity in parenthesis. *LMNP* Lake Mburo National Park, *T Theileria*, *A Anaplasma*, *E Ehrlichia*

1993)] and were also carriers of *Anaplasma marginale* and *Anaplasma centrale*. All 12 impala were strongly positive in the RLB assay with the *Theileria*/*Babesia* catch-all probe, indicating that they were all carrying *Theileria* and/or *Babesia* haemoparasites; however, none of the individual species represented on the RLB were positive, indicating that these must represent other uncharacterised species. Interestingly, 11 out of 12 of the impala were also positive for *A. centrale* which, until now, has not been identified in cattle in Uganda (Oura et al. 2004b). The three bushbuck and six eland sampled were all carriers of *T. buffeli* and *Theileria taurotragi,* and the three bushbuck were also carriers of *Erhlichia bovis*. None of the four species of wildlife sampled were carriers of *Babesia bigemina, Babesia bovis* or *Ehrlichia ruminantium*.

Haemoparasite carrier prevalence in cattle grazing inside and neighbouring LMNP

In order to investigate the role that wildlife play in the epidemiology of tick-borne haemoparasite infections in cattle in Uganda, an RLB assay was used to compare the haemoparasite carrier prevalence in 45 calves grazing inside and 99 calves grazing outside LMNP. Two groups of cattle were sampled within the park, the first group were 3–12 months old and the second group were between 1 and 3 months old (Fig. 2a, b). Also, 99 3–12-month-old calves from farms neighbouring but separated from LMNP were sampled, and a selection of the data is shown in Fig. 2c. The results are summarised in Table 1. There was no significant difference in the *T. parva* carrier prevalence between calves of 3–12 months grazing inside (92% positive) and neighbouring (69% positive) LMNP; however, the *T. parva* carrier prevalence in the 1–3-month-old calves (45%) was significantly lower than that of the 3–12-month-old calves grazing both inside and neighbouring the park ($p<0.001$, Fisher's exact test). There was no significant difference in *T. mutans* carrier prevalence between 3- and 12-month-old calves sampled inside (16%) and outside (28%) LMNP and also no significant difference was seen in *T. velifera* carrier prevalence between 3- and 12-month-old calves grazing outside (15%) and inside (16%) LMNP. Thirty percent (6/20) of the group of 1–3-month-old calves grazing inside LMNP were carriers of *T. buffeli;* however, none of the 3–12-month-old calves grazing inside or outside the park were positive for this parasite. The *T. taurotragi* carrier prevalence was significantly higher in 3–12-month-old calves grazing inside LMNP (76%) compared to outside LMNP (45%) ($p=0.007$, Fisher's exact test). No significant difference in the carrier prevalence of *E. bovis* was seen in calves neighbouring LMNP (34%) and calves inside LMNP (38%). A low carrier prevalence level (<16%) of *A. marginale* was seen in cattle both inside and outside the park, and none of the cattle sampled were carriers for *Theileria* sp. (buffalo), *B. bigemina*, *B. bovis*, *E. ruminantium* or *A. centrale*.

Variation between cattle and buffalo-derived multi-locus genotypes of *T. parva*

One aim of the study was to compare buffalo and cattle genotypes of *T. parva* in order to address the question of whether the buffalo and cattle gene pools are distinct or whether there is evidence for gene flow. In order to investigate if buffalo-derived genotypes of *T. parva* are capable of infecting cattle in the field, we compared the MLGs of buffalo-associated genotypes with those of the cattle that were co-grazing with buffalo in LMNP. It was hypothesised that these cattle were likely to have hosted ticks that had previously fed on buffalo and were therefore likely to have been exposed to buffalo-derived strains of *T. parva*. DNA from 23 to 68 *T. parva* positive blood samples (Table 1) from 3- to 12-month-old calves that regularly

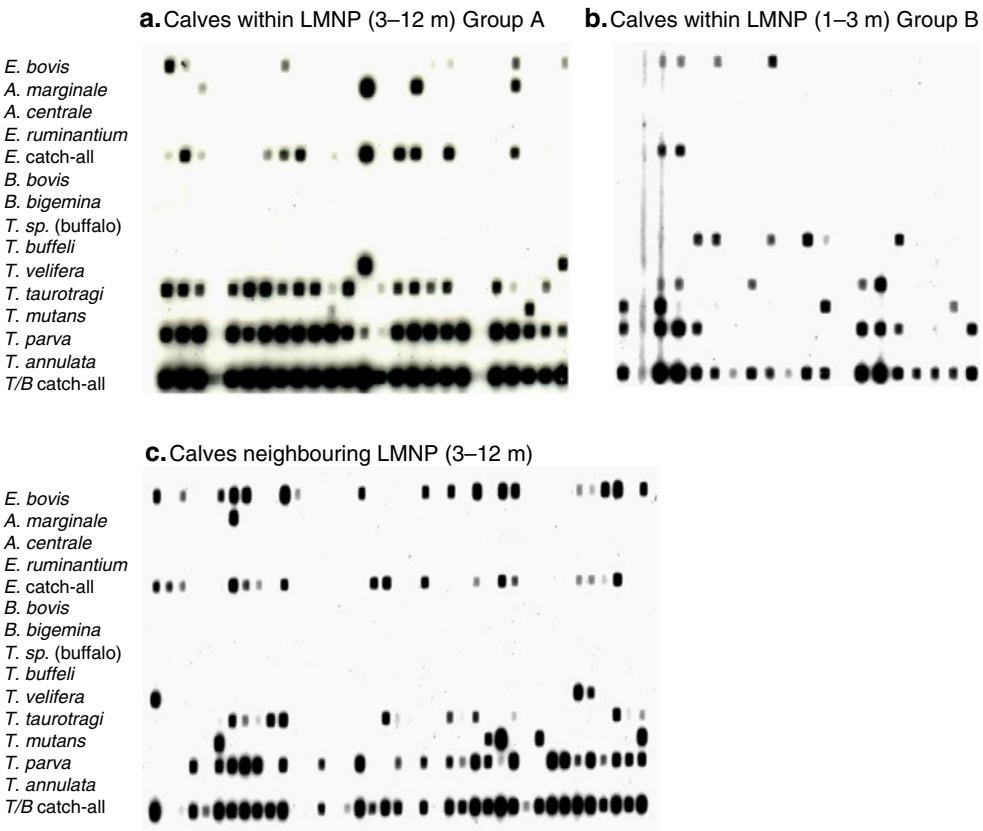

Fig. 2 Reverse line blot of samples from cattle grazing inside and neighbouring Lake Mburo National park, **a** 3–12-month-old calves grazing within LMNP, **b** 1–3-month-old calves grazing within LMNP, **c** 3–12-month-old calves grazing outside and separated from LMNP. Species-specific oligonucleotide probes were applied to the horizontal rows of the RLB and are shown to the *left of the blot* (*T/B catch-all—Theileria/Babesia* catch-all, *E catch-all—Ehrlichia* catch-all, *E—Ehrlichia*, *A—Anaplasma, B—Babesia*, *T—Theileria*)

grazed inside and outside LMNP was purified and PCR amplified using the panel of ten mini-satellite and two micro-satellite markers (Oura et al. 2003, 2004a, 2005). The resultant products were separated on high-resolution Spreadex gels. An example of the data for four of the mini- and micro-satellite markers (MS 27, MS33, ms 5 and ms 7) is shown in Fig. 3, using samples from cattle (left panel) and buffalo (right panel) from within LMNP. The alleles were sized by direct comparison to the M3 marker run to the left and right of each gel, and identically sized alleles were assigned a letter. Of the 68 *T. parva* positive samples from calves grazing outside LMNP, 40 amplified a single or one predominant allele with the majority of the panel of mini- and micro-satellite markers (data not shown), while 14 of the 23 *T. parva* positive samples from calves grazing inside LMNP amplified a single or predominant allele with the majority of the panel of markers (selected data shown in Fig. 3, left panel). From the 19 buffalo samples (all the samples amplified with the micro-satellite markers) from LMNP, three showed a single or predominant allele with at least eight of the panel of 12 satellite markers, but the remaining samples showed many alleles. In order to quantify the relationships between the different samples, a multi-locus genotype (MLG) was defined for each sample by combining the predominant micro-satellite allele at all twelve loci. Jaccard's coefficient of similarity was calculated for all pair-wise comparisons between each of the MLGs from the 14 samples from calves grazing inside LMNP (Mu samples), the 40 samples from calves grazing outside LMNP (Mb samples) and the three samples from buffalo (Mb-Buff samples) as well as one buffalo sample from Kenya (Ke-Buff) and one buffalo sample from Zimbabwe (Zim-Buff). These coefficients were used to construct a similarity dendrogram between the MLGs (Fig. 4). The buffalo-derived MLGs were very different from the cattle-derived MLGs and considerable variation was found among the small number of buffalo-derived MLGs. The buffalo-derived MLGs consistently branched from nodes at the root of the dendrogram, while the cattle-derived MLGs always formed a single, discrete cluster. Although the cattle MLGs appeared to form two distinct groups, bootstrap support for the node defining these groups was low (8%). Notably, the MLGs derived from the cattle grazing inside LMNP (labeled Mu) and the MLGs derived from cattle grazing in pastures neighbouring LMNP (labeled Mb) did not form separate clusters.

Allelic diversity of cattle and buffalo genotypes of *T. parva*

A typical sample of the data from 23 co-grazed calf samples (age 3–12 months, Fig. 3, left panel) and the 19 buffalo samples from LMNP (Fig. 3, right panel) analysed with two mini-satellite and two micro-satellite markers is shown in Fig. 3. This clearly illustrates that multiple alleles are

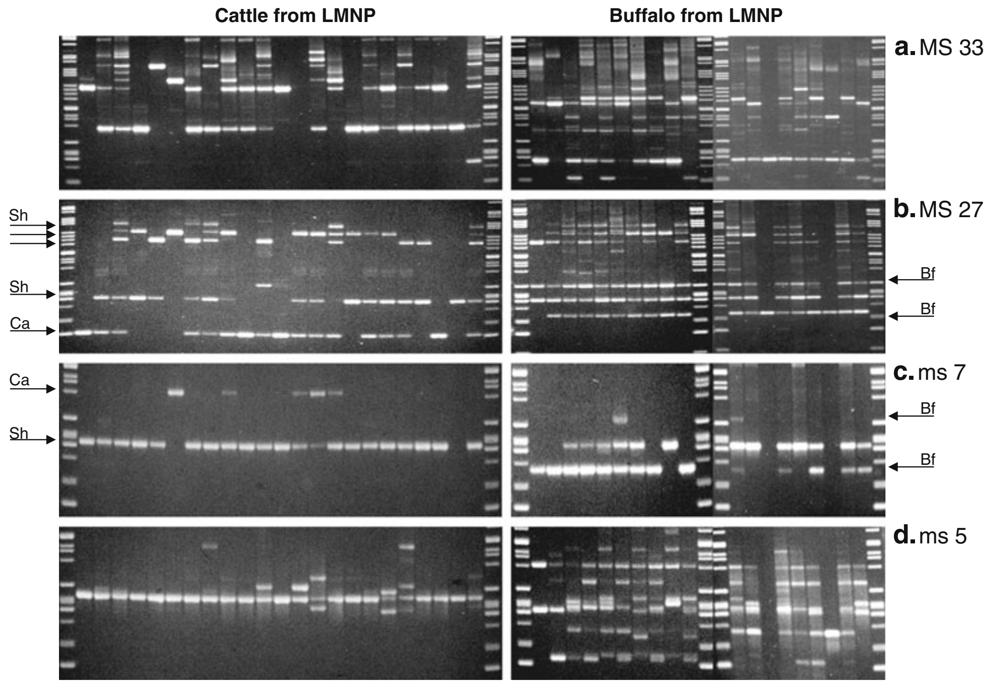

Fig. 3 Spreadex gels showing PCR products generated using mini-satellite primers **a** MS 33, **b** MS 27 **c** ms 7 and **d** ms 5 to amplify DNA from a selection of buffalo samples from LMNP (*right panel*) and cattle grazing within LMNP (*left panel*). Alleles were sized by direct comparison with the M3 marker shown in the *left* and *right* of each gel. A number of identically sized alleles shared between cattle and buffalo are arrowed *Sh*, alleles specific to buffalo are arrowed *Bf* and alleles specific to cattle are arrowed *Ca*

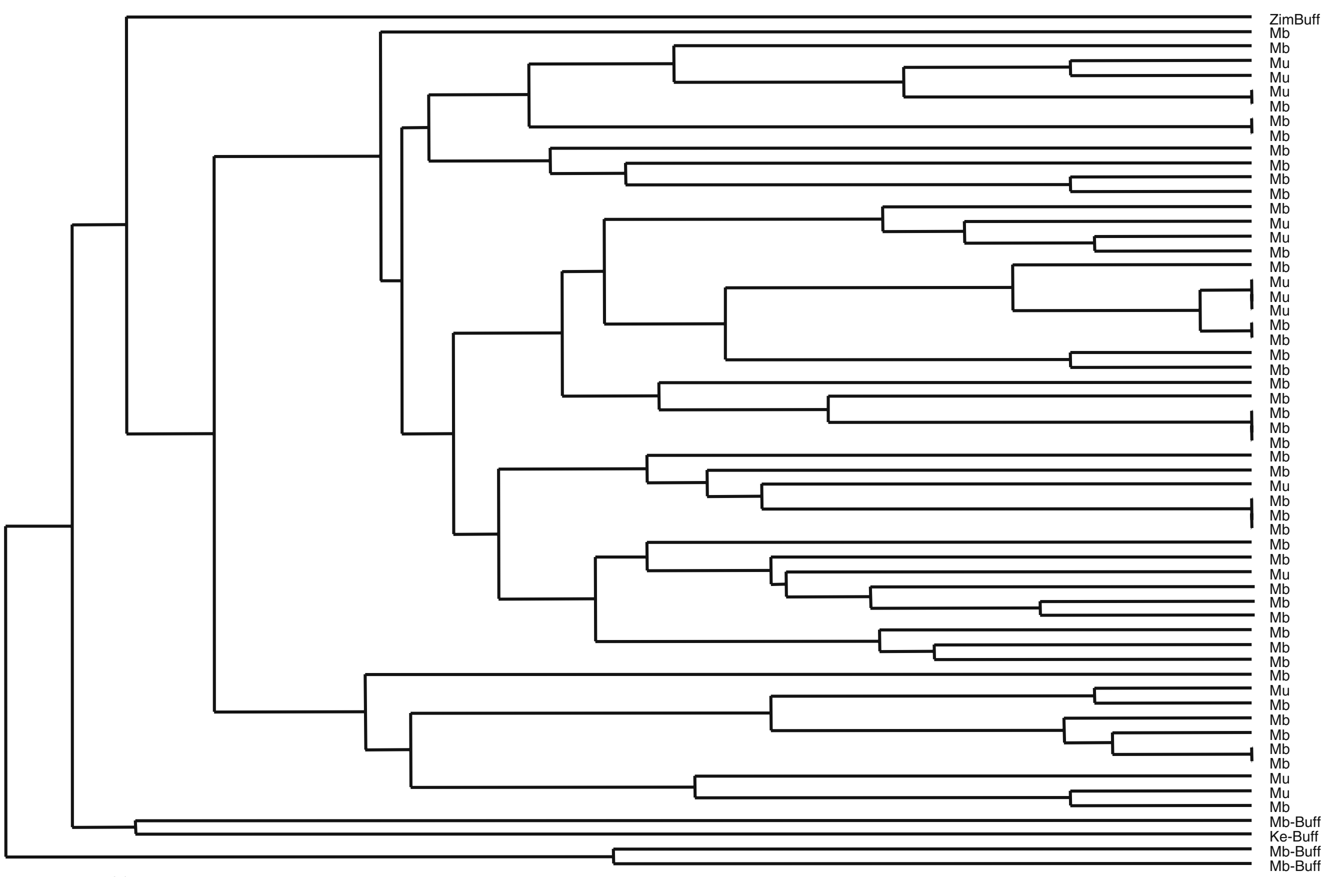

Fig. 4 Dendrogram illustrating the relationship between the multi-locus genotypes of isolates from calves grazing inside LMNP and calves grazing on pastures neighbouring LMNP. MLGs from three buffalo from LMNP (Mu-Buff) as well as two buffalo-derived *T. parva* isolates, 7014 (Ke-Buff) and Zimbabwe (T.p/.BAL/25) (Zim-Buff) are shown. Samples labeled *Mb* (Mbarara) are from calves grazing in pastures neighbouring LMNP and those labeled *Mu* (Mburo) are from calves grazing within the park

detected in a large proportion of both the buffalo and cattle samples. Almost all the buffalo-derived samples contain a mixture of genotypes, and overall, a greater number of alleles are seen in the buffalo compared to the cattle samples, and this is particularly evident with micro-satellite ms 5 (Fig. 3, bottom panel). Many alleles are unique to buffalo (marked Bf on Fig. 3), many are unique to cattle (marked Ca on Fig. 3), and some alleles are shared between cattle and buffalo (marked Sh on Fig. 3). Since a large proportion of the buffalo and cattle samples contained a number of alleles at one or more loci, it was impossible to construct MLGs for the majority of isolates. In order to perform similarity analyses, full allelic profiles of each sample were compared. Alleles were sized by direct comparison to the M3 marker run to the left and right of each gel, and each allele in the population was identified. For each sample, an allelic profile or fingerprint was generated, and this incorporated every allele present at each locus in that sample. Jaccard's coefficient of similarity was calculated between the complete allele profiles representing each sample, and these coefficients were used to construct a dendrogram (Fig. 5). The dendrogram clearly illustrates that the combinations of alleles present in the buffalo-derived samples are distinct from those found in cattle (100% bootstrapping support). To an extent, the Mbarara cattle-derived samples (Mb samples) and the Mburo cattle-derived samples (Mu samples) form separate clusters, although two discrete groups cannot be identified as the tree is relatively unstable. However, the clear distinction between cattle and buffalo genotypes agrees with the results of MLG dataset, which was based solely upon predominant alleles (Fig. 4).

In order to investigate if there was evidence for mixing between the buffalo and the cattle gene pools, the level of differentiation was measured between the two cattle and the buffalo populations. Pair-wise F_{ST} values were calculated using the full allelic profile dataset for the three populations, namely the buffalo inside LMNP (Mburo buffalo), the cattle that co-grazed with buffalo inside the park (Mburo cattle) and the cattle that were located outside the park which did not co-graze with buffalo (Mbarara cattle). The results of this analysis are presented in Fig. 6, revealing a moderate amount of differentiation both between the Mburo cattle and the buffalo (F_{ST}=0.134) and the Mbarara cattle and the buffalo (F_{ST}=0.126). In contrast, little differentiation was identified between the two cattle populations (F_{ST}=0.018). These results agree with the similarity analysis and demonstrate that genotypes from the two cattle-derived populations show some similarity to each other but are clearly distinct from the buffalo population. It can be concluded, therefore, that there is no evidence indicating the transmission of buffalo genotypes into the co-grazed cattle parasite population.

Discussion

This study set out to investigate the role that buffalo and other wildlife may play in the epidemiology of tick-borne haemoparasite infections in cattle in Uganda, as well as whether there is evidence for the transmission of buffalo-derived genotypes of *T. parva* to cattle that are in close contact with buffalo. Using an RLB assay, we measured the haemoparasite carrier prevalence in four common species of wildlife (buffalo, impala, bushbuck and eland) present inside LMNP in Uganda. We then proceeded to compare the haemoparasite prevalence in wildlife to that in cattle co-grazing with wildlife inside the park and cattle with no direct contact with wildlife from farms neighbouring the park.

Buffalo were found to be carriers of many tick-borne haemoparasites including *T. parva, T. mutans, T. velifera, T. buffeli, Theileria* sp. (buffalo), *A. marginale* and *A. central,* indicating that they have the potential to spread these parasites to cattle. In contrast, impala were found to carry only *A. centrale*. In a previous study (Oura et al. 2004b) as well as in this study, *A. centrale* was not identified in cattle in Uganda, so it is possible that the *A. centrale*, found in both buffalo and impala, is unable to transmit to cattle. Interestingly, all the impala were strongly positive in the RLB assay with the *Theileria*/*Babesia* catch-all probe, indicating that they were carrying *Theileria* and/or *Babesia* haemoparasites; however, the species was not identified. These results indicate that these impala in Uganda are carriers of an as-yet unidentified *Theileria* or *Babesia* parasite that may be wildlife- or impala-specific. Further work is necessary to confirm the identity of this parasite. The bushbuck and eland sampled were all carriers of *T. buffeli* and *T. taurotragi*, indicating that these two species of wildlife are likely to be reservoirs of these parasites. *E. bovis* was not present in the buffalo, eland or impala that were sampled, although the three bushbuck sampled were carriers of *E. bovis*, indicating that bushbuck may be a reservoir of this parasite, which was also present in cattle grazing both inside and neighbouring LMNP. None of the four species of wildlife sampled or the cattle grazing in and around LMNP were carriers of *B. bigemina, B. bovis* or *E. ruminantium*, indicating that these parasites are not circulating in this region of Uganda.

The majority of buffalo sampled in LMNP were carriers of *T. parva*; however, *T. parva* was not identified in the impala, bushbuck or eland that were sampled. This indicates that *T. parva* may be incapable of being transmitted to eland, bushbuck and impala. Despite an apparent difference in prevalence of *T. parva*, no statistically significant difference was identified between calves grazing inside LMNP that had direct contact with wildlife (92%) and calves grazing outside the park with no wildlife

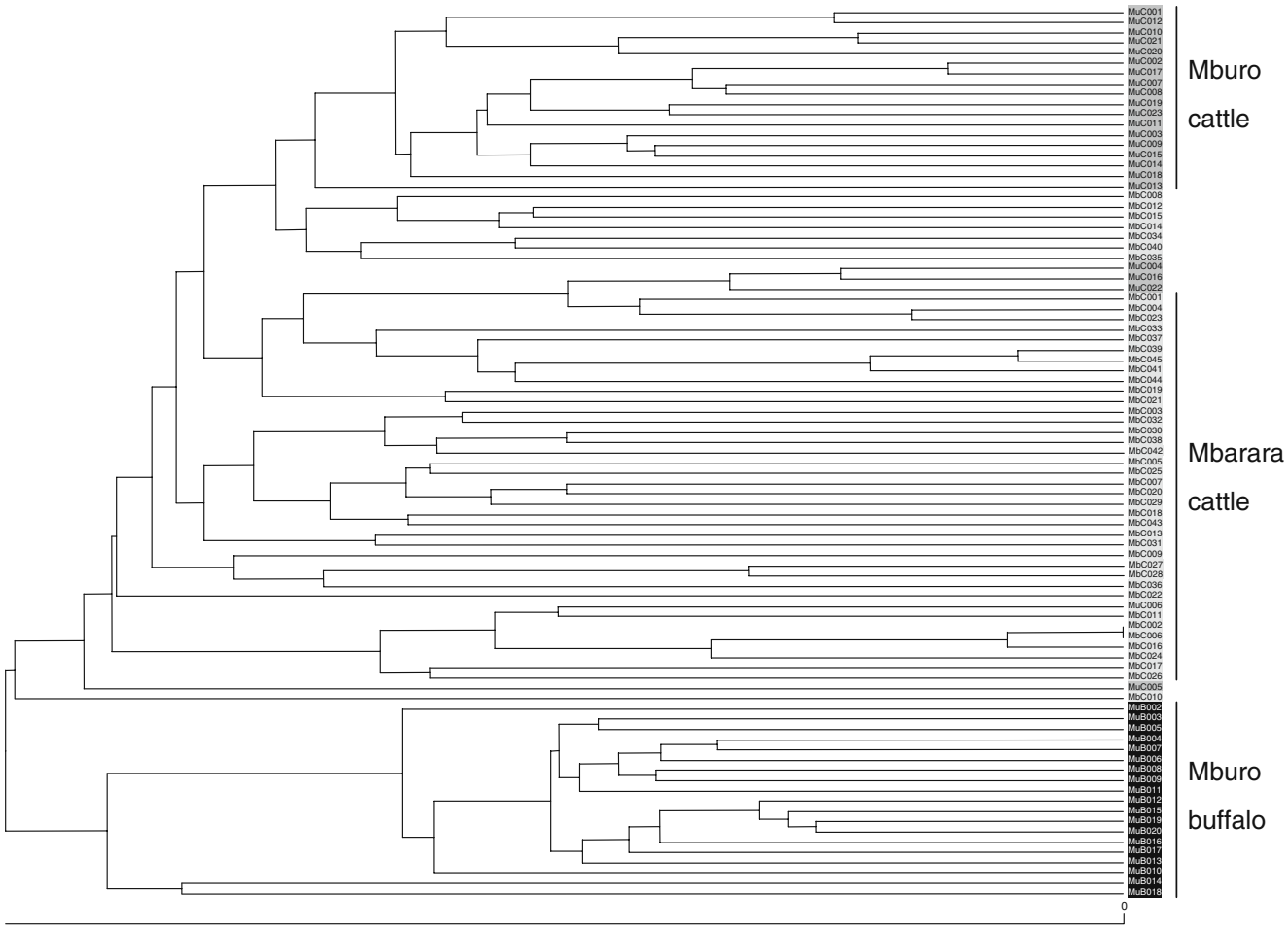

Fig. 5 Dendrogram illustrating the relationship between complete allelic profiles representing buffalo grazing within LMNP (*Mburo buffalo*), cattle grazing within LMNP (*Mburo cattle*) and cattle grazing outside LMNP (*Mbarra cattle*)

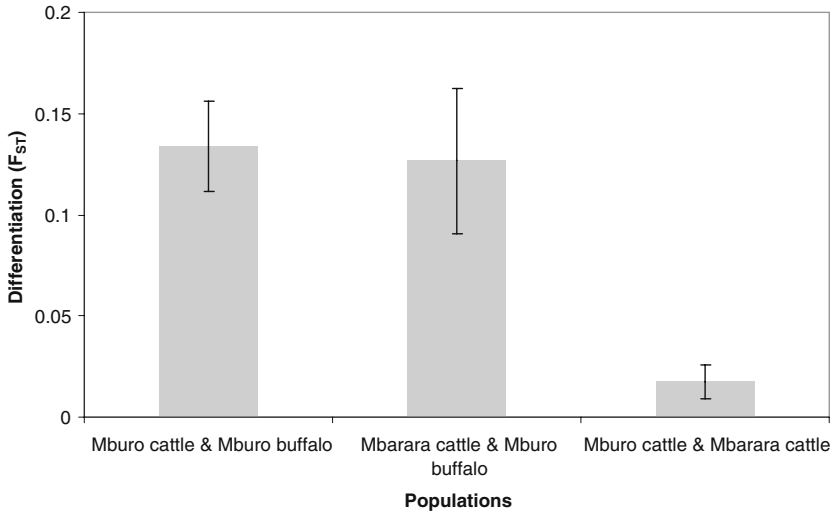

Fig. 6 Pair-wise comparisons of differentiation between the cattle and buffalo populations. F_{ST} values were calculated using allele frequency data from the full allelic profiles at 12 micro- and mini-satellite loci. The error bars represent the standard error

contact (69%). Although this suggests that proximity to wildlife does not affect the prevalence of *T. parva* in cattle, a larger sample size may confirm that the presence of wild animals correlates with increased prevalence in co-grazed cattle.

T. taurotragi is known to be a parasite of eland (Grootenhuis et al. 1980), and there are large populations of eland present in LMNP. Therefore, it is unsurprising that calves grazing inside the park had a higher prevalence of *T. taurotragi* than calves grazing outside the park. This suggests that this parasite can be passed from eland to cattle; however, the fact that *T. taurotragi* is present in cattle outside the park that had no direct contact with eland, indicates that it is likely that *T. taurotragi* can also be maintained in a cattle–tick–cattle cycle.

T. buffeli and *Theileria* sp. (buffalo) are thought to be parasites exclusive to buffalo and are believed to be incapable of infecting cattle (Oura et al. 2004b). In this study, all the buffalo, eland and bushbuck sampled in LMNP were carriers of *T. buffeli,* indicating that this parasite is capable of infecting wildlife species other than buffalo. Interestingly, 30% of the younger calves grazing inside the park were also carriers of *T. buffeli*, whereas none of the older cattle grazing inside the park or the cattle outside the park were carriers. This suggests that *T. buffeli* can be transmitted via ticks from buffalo to cattle and younger cattle may be more susceptible to the parasite. However, it is likely that *T. buffeli* is unable to then be passed via ticks from cattle to cattle as only younger calves in close proximity to buffalo inside the park were infected. With *Theileria* sp. (buffalo), all the buffalo sampled in LMNP were carriers of this parasite but it was absent in the cattle population. We can conclude that *Theileria* sp. (buffalo) is only present in buffalo, and it is unlikely that it can be passed from buffalo to cattle.

A high percentage of the buffalo sampled (74%) were carriers of *A. marginale*; however, lower carrier prevalence levels (<10%) were seen in cattle grazing both inside and outside LMNP. This suggests that, as with *A. centrale*, buffalo may be infected with a buffalo-specific strain of *A. marginale*. Further work is necessary in order to characterise these *Anaplasma* species present in wildlife (buffalo and impala) and cattle.

The micro- and mini-satellite genotyping system (Oura et al. 2003) was used to analyse populations of *T. parva* derived from buffalo, buffalo-associated cattle and cattle which did not come into contact with buffalo. Analysis of allelic profiles of buffalo-derived parasite isolates suggested a higher multiplicity of infection than that encountered in cattle-derived isolates, and this agrees with a recent study (Oura et al. 2010, paper under review). The results from the present study suggest there is relatively little genetic differentiation between *T. parva* isolates derived from cattle within and outside the park and that considerable gene flow occurs between these two populations. This conclusion is strongly supported by the F_{ST} results (Fig. 6) and the cluster analysis of predominant MLGs (Fig. 4). Interestingly, the cluster analysis using full allelic profiles (Fig. 5), which represents a more extensive dataset, provides evidence that allelic frequencies are subtly different between the two cattle populations, suggesting that these two populations may be sub-structured. The data suggests that *T. parva* genotypes are transmitted between cattle in the park and areas bordering the park at an appreciable level; however, it is difficult to quantify the dynamics of this effect, particularly with respect to time. As cattle are moved in and out of the park, this could account for transmission between the two populations. This study clearly demonstrates that the buffalo-derived parasite population is separate from the cattle populations (Figs. 4, 5 and 6). Both cattle parasite populations show a moderate degree of differentiation from the buffalo population, and our results provide strong evidence that the buffalo and buffalo-associated cattle harbour distinct genotypes of *T. parva*. A reasonable interpretation of this data is that the *T. parva* genotypes identified in cattle correspond to the ECF-causing parasite population, and these are maintained in a cattle–tick–cattle cycle. Examination of the genotyping dataset (Fig. 3) suggests that several alleles occur that are specific to and frequent within the cattle or buffalo populations, respectively. Assuming a significant level of within-population recombination, as shown by previous population genetic analysis (Oura et al. 2005), the relative abundance of private alleles in both populations would argue against any significant amount of gene flow between the buffalo-derived and cattle-derived populations. With Corridor disease believed to arise from the transmission of buffalo-derived genotypes to cattle, our results concur with previous studies which have concluded that infection is self-limiting in the cattle population. This has been attributed to low numbers of schizont-infected leukocytes giving rise to low piroplasm parasitaemia or the death of the host before parasitaemia develops (Norval et al. 1991) resulting in limited or no transmission. However, it has been demonstrated that faced with a low infective dose, cattle may be capable of surviving, generating piroplasms (Young and Purnell 1973) and that recovered animals can be carriers of disease (Potgieter et al. 1988). Given the high level of mixing of cattle and buffalo in LMNP, the opportunity for recombination between the cattle and buffalo populations would be predicted to arise. There are however very few, if any, reports of Corridor disease occurring in cattle in and around LMNP, thus indicating that buffalo-derived strains of *T. parva* are not transferring to cattle in the area. The results of our study agree with this and suggest that true genetic isolation may already be a feature of *T. parva* and that perhaps the original view of sub-speciation between cattle-

maintained *T. parva parva* and Corridor disease-inducing *T. parva lawrencei* should be re-examined. Consequently, further genotyping studies are required in particular to characterise Corridor disease-inducing strains to determine their relationship with the existing cattle and buffalo-associated parasite populations.

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