Molecular characterization of *Theileria parva* field strains derived from Cattle and Buffalo sympatric populations of Northern Tanzania

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Abstract

East coast fever is a disease of cattle caused by *Theileria parva* and is transmitted by the tick *Rhipicephalus appendiculatus*. Buffalos are a natural reservoir of the parasite. The objectives of this study were to determine the *T. parva* infection in buffalo and cattle field samples, to identify genotypes of *T. parva* infecting cattle and buffalo and finally to investigate the presence of buffalo-derived *T. parva* genotypes in cattle that graze in close proximity with infected buffalo. Real-Time PCR (RT-PCR) and Fluorescence Resonance Energy Transfer (FRET) was used to diagnose *T.parva*. Genotyping of *T. parva* was conducted by analysing p67, PIM and p104 genes . In addition, amplicons with unique PIM and p104 PCR-RFLP profiles were sequenced to identify shared genotypes. The results have shown the high occurrence of *T. parva* in buffalo and a much higher occurrence of *T. parva* in cattle from the Ngorongoro area. Analysis of the variable region of the p67 loci showed four different *T. parva* alleles in buffalo and cattle. PCR-RFLP analysis of the p104 loci revealed four allelic profiles in buffalo and two in cattle. PCR-RFLP analysis of PIM loci from buffalo gave profiles that were complex and difficult to interpret. On the contrary, cattle showed allelic profiles that resemble Muguga and Marikebuni isolate PIM gene profiles. Multi-locus genotypes (MLGs) and sequence analysis revealed that the majority of buffalo and cattle genotypes were distinct but there is an indication of some sharing of genotypes between buffalo and cattle.

Keywords: *Theileria parva;* p104; p67; Polymorphic immunodominant (PIM); Amplicon; Allele

{**Citation:** Mwega Elisa, Paul Gwakisa**,** Kgomotso Sibeko, Marinda Oosthuizen, Dirk Geysen. Molecular characterization of *Theileria parva* field strains derived from Cattle and Buffalo sympatric populations of Northern Tanzania. American Journal of Research Communication, 2014, 2(7): 10-22} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Theileria parva is the causative agent of East Coast fever (ECF) and Corridor disease. ECF is a cattle disease of economic importance in endemic areas of Eastern, Central and Southern Africa (Morrison *et al*., 1986). Previous studies in many parts of Africa, including Kenya (Nene *et al.,* 1992), Zimbabwe (Bishop *et al.,* 1994), Uganda (Oura *et al.,* 2010) and South Africa (Sibeko *et al.,* 2011), reported a diversity of *T. parva* isolated in buffalo and cattle. Several genes have been investigated in search of discriminatory sequence differences between *T. parva* isolates. Among these are sporozoite antigen genes, p67, p104 and PIM (Nene *et al*., 1996; Iams *et al*., 1990; Toye *et al*., 1999; Bazarusanga *et al*., 2007).

Characterization of the p67 gene sequences of East Africa isolates has revealed the presence of a 129 bp deletion in the central region in cattle-derived *T. parva* isolates, while there is no deletion in buffalo-derived isolates (Nene *et al.,* 1992, 1996). Therefore, the presence or the absences of the 129 bp deletion within the p67 gene has been used to differentiate between cattle, and buffalo-derived *T. parva* isolates (Nene *et al.,* 1996). In order to perform different analyses by using p104 and PIM profiles, novel assays based on PCR amplification and RFLP analysis of single copy genes encoding polymorphic antigens have been developed to ensure a wider coverage of polymorphism within the genome, this method discriminates against field parasites with good resolution (Geysen *et al.,* 1999; Ogden *et al.,* 2003).

This study reports on genotyping of *T. parva* parasites by characterization of genes expressing the sporozoite stage-specific surface antigen protein (p67), the polymorphic immunodominant molecule (PIM), and the microneme–rhoptry protein (p104) in an attempt to identify distinctive features that differentiate *T. parva* isolates from cattle and buffalo in Tanzania.

Materials and methods

DNA Extraction and Screening of *T.parva*

Blood samples were randomly collected from 50 buffalo and 50 cattle in the Ngorongoro area where the two graze within close proximity or on the same pastures. It is also an area where cattle were immunized with the Muguga cocktail vaccine by the infection and treatment method (ITM). Furthermore, a second collection of randomly selected blood samples came from 50 buffalo and 50 cattle in the Serengeti area, where there is no intermingling and cattle were not immunized. Blood was drawn from the jugular vein into 10ml Vacutainer tubes containing EDTA. Genomic DNA was extracted from whole blood using the protocol as described in the pure gene blood core kit (QIAGEN). These were screened for the presence of *T. parva* DNA by using a FRET-based Real-Time PCR approach on the Rotor gene 3000, Corbett Research (Michiel Janssens PHD thesis 2009).

PCR amplification of the p67, p104 and PIM genes from *T. parva*

A semi-nested PCR was carried out in a total volume of 25 µl. Forward Primer IL613 and reverse primer IL792 (Nene *et al.,* 1996) was used to amplify the variable region of the p67 gene. The amplification conditions applied were as described by Nene *et al.* (1996). The variable regions of the *T. parva* PIM and p104 genes were amplified using the semi-nested PCR as described by De Deken *et al*. (2007). The agarose gel (2%) was stained with ethidium bromide for detection of amplified

product after electrophoresis. The 100 bp DNA molecular weight marker was used to estimate the amplicon sizes.

Restriction analysis of p104 and PIM PCR products

Restriction enzymes Bcl*1* and Alu*1* were used to digest the amplified PCR products generated from the PIM and p104 semi-nested PCR. Digestion was conducted using 1.5µl of the 10X buffer (60mM Tris-HCL (pH 7.5), 500mM NaCl, 60mM $MgCl_2$ and 10mM DDT for Alu*1* and 100mM Tris-HCL (pH 7.9), 500Mm NaCl, 100mM $MgCl_2$ and 10mM DDT for Bcl*1*. In addition, water 9.2µl, 0.3µl restriction enzymes and 4.0 µl of PCR amplified product were added to obtain a total volume of 15µl. Digestions were performed overnight at 50°C and 37°C for the enzymes Bcl*1* and Alu*1*, respectively. An aliquot of the digested sample was subsequently analysed by size separation through a 12% polyacrylamide gel, and thereafter, stained for 40 minutes using 5µl of SYBR green (10x) diluted in 50ml of 1x TBE.

Multi-locus analyses of the *T. parva* positive samples

Multi-locus genotypes were defined for each sample by combining alleles of three loci (p67, p104 and PIM). Alleles were defined by letters, S for p67, F for p104 and M for PIM.

Cloning and sequencing of p104 and PIM PCR products

PIM and p104 Selected PCR products were cloned and sequenced as described by Sibeko *et al.* (2011).

Sequence analysis

Sequences obtained from selected clones prepared from p104 Serengeti buffalo number 11 (accession number: KF267247) and PIM amplicons from Serengeti buffalo number 25, 35 and Ngorongoro buffalo number 41 (accession numbers: KF267248, KF267249, KF267250 respectively) were assembled and edited using GAP4 of the Staden package (version 1.6.0 for Windows) (Staden, 1996; Staden *et al.,* 2000). Multiple alignments of PIM and p104 sequences were performed using ClustalX software. Four p104 nucleotide sequences of allele one, two, three and four obtained from different *T. parva* stocks of cattle and buffalo in East Africa were used as a reference (Skilton *et al.,* 2002). PIM sequences were aligned against previously published *T. parva* PIM sequences (Toye *et al*., 1995 a,b). Muguga (accession number: L06323), Marikebuni (accession number: L41148) and 7014 (accession number: L41833). Muguga, Marikebuni, Uganda and Boleni are cattle-derived *T. parva* stocks. The aligned nucleotide sequences were exported to the Mega 4.1 software (Tamura *et al.,* 2007) and translated into amino acid sequences.

Results

Screening of blood samples

Theileria parva infection in field samples was determined using the FRET assay and results showed that the majority of buffalos were carriers of the *T. parva*. *Theileria parva* DNA was detected in forty-eight out of fifty buffalo samples (96%) from the Ngorongoro area, and forty-nine out of fifty

buffalo samples (98%) from Serengeti. However, different incidence was observed in cattle grazing in the Ngorongoro area, where they intermingle with buffalo. Forty percent of cattle from Ngorongoro were positive for *T. parva* being significantly higher when compared to the 14% of the positives in cattle from the Serengeti area, where cattle do not intermingle with buffalo. Nevertheless, the majority of field samples from buffalo and cattle showed the presence of multiple *Theileria species,*including *T. mutans*, *T. taurotragi*. *T. verifera,* in Cattle and *T. mutans, T. taurotragi* were found in buffalo. Furthermore, *T. parva* was found as dominant species. All positive samples of cattle and buffalo were selected for amplicon size analysis of the p67 gene and profile analysis of the p104 and PIM gene.

Analysis of the p67 gene

Theileria parva-positive samples were selected for amplicon size analysis of the p67 gene. The analysis showed four amplicons of different sizes present in buffalo and cattle examined during this study. The 0.9 and 1.1 kb bands were obtained from buffalo-derived samples while 0. 8 and 1.0 kb were identified from cattle-derived *T. parva* isolates (Table 1). A hundred percent of cattle-derived *T. parva* isolates from Serengeti and 57% from Ngorongoro produced one bands of 1.0 kb. However, 29% of *T. parva* isolates from Ngorongoro cattle produced a single band amplicon of 0.8 kb in size and 14% showed alleles with double band amplicons at 0.8 and 1.0kb (Table 1) . The 0.8 kb allele has been designated allele 3 and was identified from buffalo samples (Sibeko *et al.,* 2009). The majority of amplicon sizes obtained from buffalo samples from Serengeti and Ngorongoro buffalo consisted of the 0.9 and 1.1kb bands designated alleles 1 and 2, respectively (Sibeko *et al.,* 2009). However approximately 11% of the buffalo samples had a single band of 0.9 kb in size, the typical cattle-type allele (Nene *et al.,* 1996; Sibeko *et al.,* 2009).

Table 1 Amplicons obtained from amplification of the *T. parva* single copy gene, p67

Amplicon size(s)	Serengeti		Ngorongoro	
(Kb)	Buffalo (%)	Cattle (%)	Buffalo (%)	Cattle (%)
0.8	0	0	0	29
0.9	11	0	12	0
1.0	0	100	0	57
1.1	0	0	0	0
0.8&1.0	0	0	0	14
0.9&1.1	89	0	88	0

PCR-RFLP analysis of the p104 loci by PAGE electrophoresis

PCR-RFLP profile patterns were obtained from four distinct p104 amplicons derived from buffalo samples (Figure 1a). The profiles had a varying band composition ranging from seven to ten bands per individual profile. Profile one was the predominant profile (77%) buffalo sample from Ngorongoro while profiles 2, 3 and 4 were present at 13%, 5% and 5% respectively (Table 2). Frequencies of profiles 1, 2 and 3 in Serengeti buffalo samples were 77%, 20% and 3% respectively (Table 2).

PCR-RFLP analysis of p104 loci in the cattle gave allelic profiles identical to those previously obtained from Muguga and Marikebuni *T. parva* isolates (Geysen *et al.* 1999, Skilton *et al.,* 2002). It can be seen that the allelic profile 5a (Figure 1a) being the predominant one in all Serengeti cattle is identical with the Marikebuni profile. Likewise, allelic profile 6a (Figure 1a) which was predominant in 33% of the Ngorongoro cattle is identical to that of the Muguga isolate (Table 2). Multiple bands as seen in lane 8 (Figure 1a) suggest a co-presence of Muguga and Marikebuni isolate-specific profiles in a single sample. This mixed profile was observed in 33% of Ngorongoro cattle-derived *T. parva* isolates (Table 2).

PCR-RFLP analyses of the PIM loci by PAGE electrophoresis

PCR-RFLP analysis of PIM amplicons obtained from *T. parva* isolates from buffalo produced six different profile patterns with varying band composition ranging from 2 to 12 bands (Figure 1b). This study has revealed that the allelic profile 6b was most dominant in both Ngorongoro (35%) and Serengeti (42%) buffalo (Table 2). However, the frequency of other allelic profiles varied between the two populations occurring at different and lower frequencies (Table 2).

RFLP analysis of PIM loci in cattle gave different allelic profiles. Forty percent of Ngorongoro cattle produced allelic profile 7 (Figure 1b) which is identical to the Kiambu *T. parva* isolate, while another 20% produced allelic profile 8, which is similar to that of the Muguga *T. parva* isolate (Figure 1b). In addition, 20% of Ngorongoro cattle produced allelic profiles 9 and 11. Multiple allelic profiles were produced in 67% and 33% (Table 2) of Serengeti cattle as observed in lanes 9 and 10 in Figure 1b respectively). Profiles 7, 8 and 11 were unique to Ngorongoro cattle samples while profile 10 was unique to *T. parva* isolates from Serengeti cattle.

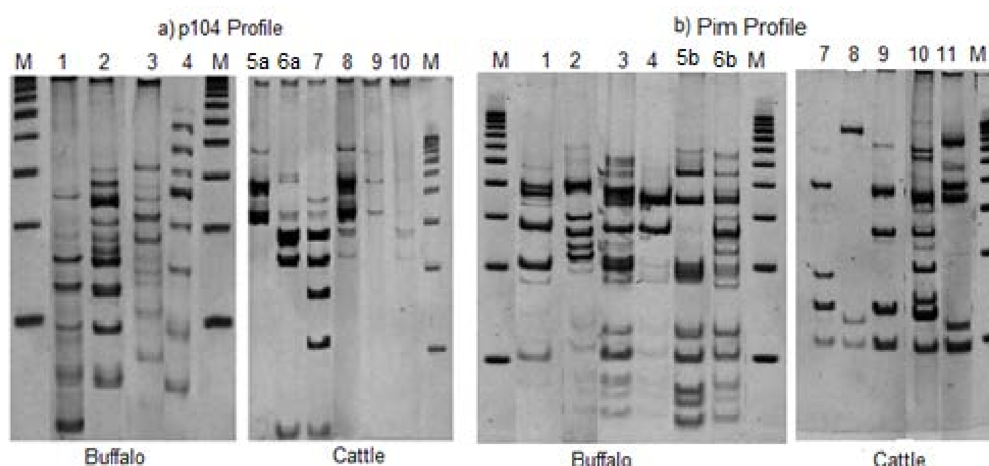

Figure 1 a) PCR-RFLP analyses of p104 loci. Lane 1= profile F1, Lane 2= profile F2, Lane 3= profile F3, Lane 4= profile F4, Lane 5a = profile F5, Lane 6a = profile F6, Lane 7= profile F7, Lane 8 = profile F8, Lane 9 = Katete, Lane 10 = Muguga. **b) PCR-RFLP analyses of PIM loci.** M = 1kb Marker. Lane 1 = M1, Lane 2 = M2, Lane 3 = M3, Lane 4 = M4, Lane 5b = M5, Lane 6b = M6, Lane 7 = M7, Lane 8 = M8, Lane 9 = M9, Lane 10 = M10, Lane 11 = M11.

Table 2 Frequencies of p104 and PIM profiles appeared among *T. parva* isolates from Ngorongoro and Serengeti buffalo and cattle

Host of sample origin	Profile	Occurrence Frequency (%)			
		Ngorongoro		Serengeti	
		p104	PIM	p104	PIM
Buffalo	1	77	18	77	8
	2	13	12	20	0
	3	5	9	3	17
	4	5	14	0	4
	5a	-	12	-	29
	6a	-	35	-	42
Cattle	5b	0		100	-
	6b	33		0	-
	7	33	40	0	0
	8	33	20	0	0
	9	-	20	-	67
	10	-	0	-	33
	11	-	20	-	0

Multi-locus analyses of the *T. parva* positive samples

Multi-locus genotypes were defined for each sample by combining alleles of three loci p67, p104 and PIM (Table 3). The multilocus profiles which appeared more than once were selected for further analysis. A total of twenty three multilocus profiles from Serengeti buffalo-derived *T. parva* were analyzed. Results revealed four dominant genotypes (S1F1M6, S1F1M5, S1F1M3, S2F1M5) whereby the most occurring genotype (S1F1M6) accounted for 39% of the population while the remaining genotypes were significantly lower and accounted for 17%, 13% and 9% (S1F1M5, S1F1M3, S2F1M5, respectively) of the parasite population. The other five genotypes identified from the remaining 12% of the Serengeti buffalo-derived *T. parva* isolates appeared only once in the population. Two dominant genotypes (S1F1M6, S1F1M1) were observed in Ngorongoro buffalo-derived *T. parva* positives. Whereby, the most frequent genotype (S1F1M6) accounted for 17% of the population while the S1F1M1 genotype accounted for 9%. The remaining eight genotypes were observed in 74% of the samples and appeared only once. In addition, shared genotypes have been identified in Ngorongoro and Serengeti buffalo-derived *T. parva*. Six distinct genotypes were found to be shared, and the dominant genotype (S1F1M6) among these accounted for 30% of the parasite population. Analysis of cattle-derived *T. parva* multilocus profiles revealed two distinct genotypes present in Serengeti cattle (S4F5M9, S4F5M10) and five distinct genotypes presented in Ngorongoro cattle (S3F5M7, S4F8M7, S4F6M8, S4F5M9 and S5F7M9). Only 5% of the parasite population shared genotypes (S4F5M9) between Ngorongoro and Serengeti cattle. The dominant genotype was (S4F5M9) and appeared in Ngorongoro and Serengeti cattle.

Table 3 Shared allelic profiles and multi-locus genotypes results from buffalo (B) and cattle (C) derived *T. parva* from Ngorongoro (N) and Serengeti (S) areas

	p67					P104								PIM											Mult-
	S1	S2	S3	S4	S5	F1	F2	F3	F4	F5	F6	F7	F8	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	Locus
NB10	S1					F1								M1											S1F1M1
SB30	S1					F1								M1											S1F1M1
NB31	S1					F1										M3									S1F1M3
SB14	S1					F1										M3									S1F1M3
NB16	S1					F1												M5							S1F1M5
SB2	S1					F1												M5							S1F1M5
NB3	S1					F1													M6						S1F1M6
SB13	S1					F1													M6						S1F1M6
NB18	S1							F3											M6						S1F3M6
SB16	S1							F3											M6						S1F3M6
NC45				S4						F5												M9			S4F5M9
SC17				S4						F5												M9			S4F5M9

Alleles are defined by letters, S for p67, F for p104 and M for PIM.

p104 gene sequences analysis

Four p104 clones representing different PCR-RFLP profiles were selected for sequencing (figure 2a). The p104 amino acid sequence alignment revealed sequence similarities ranging from 92-98% with reference sequences from the GenBank (Figure 2b). Amino acid sequences from the p104 clones 10, 11 and 12 showed 95% identity to the p104 allele 2 (originally obtained from *T. parva* Marikebuni, a cattle-derived stock) sequence while clone 9 showed 95% identical to allele 4 (originally obtained from *T. parva* 7014, a buffalo-derived stock) sequence.

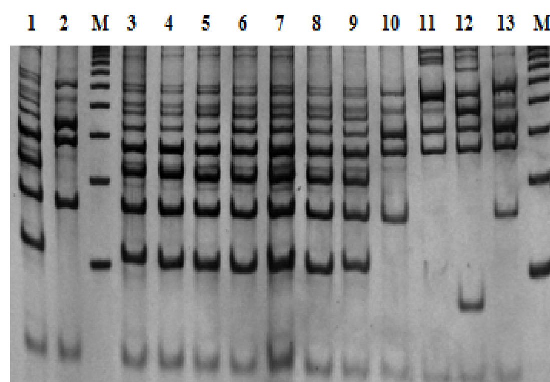

Figure 2a PCR-RFLP profiles obtained from cloned p104 PCR product from Serengeti buffalo (SB 11). PCR-products were digested with restriction enzymes *Alu* 1. Clones 9, 10, 11 and 12 represent four different profiles identified and these were selected for sequencing. M is 1Kb Marker.

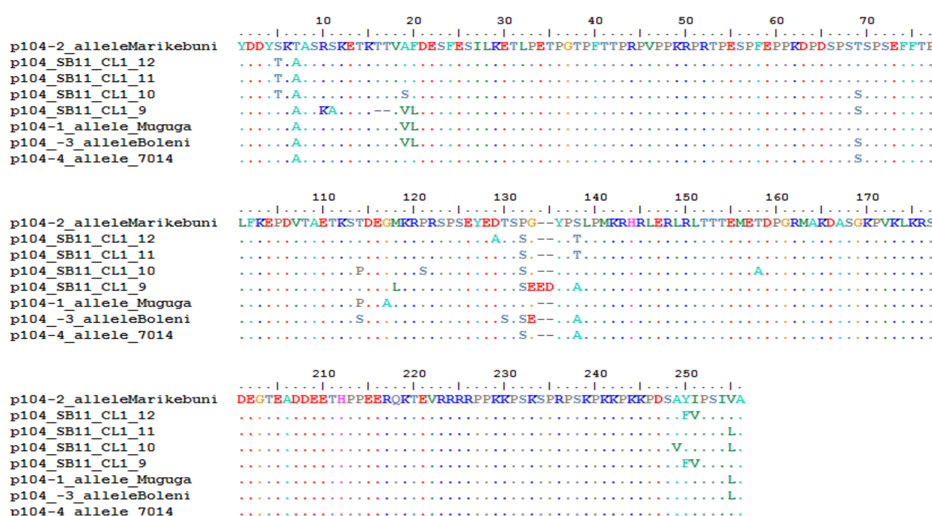

Figure 2b Multiple sequence alignment of the p104 amino acid sequences obtained from Serengeti buffalo (SB 11). Serengeti buffalo clones 9, 10, 11 and 12 sequences were aligned with previously published *T. parva* p104 amino acid sequences. Colored dots-line shows similarity of the amino acid sequences, broken-line shows "deletions" of the amino acids sequences and the sequence differences is marked by specific amino acids.

PIM gene sequences analysis

Clones of amplicons representing distinct PIM PCR-RFLP profiles from three buffalo samples from Ngorongoro were selected for sequencing. Five different PIM profiles were identified in this study showing varying band compositions ranging from three to ten bands (figure 3a). The results of this PIM amino acid sequence alignment revealed a high diversity in buffalo. The specific patterns of diversity were observed in the central region being highly polymorphic containing several large deletions in the amino acid region between 40bp and 355bp (Figure 3b).

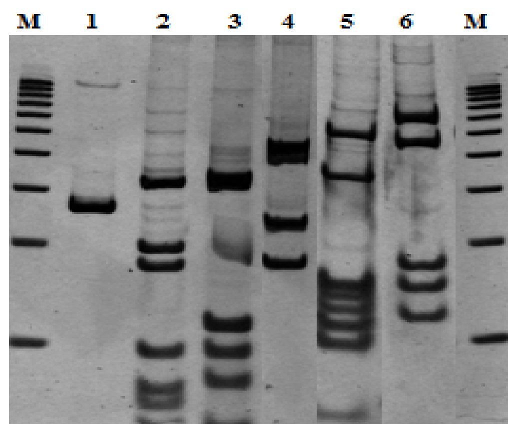

Figure 3a PCR-RFLP profile obtained from cloned PIM PCR product from Ngorongoro buffalo (NB). The PCR product were digested with restriction enzymes Bcl *1*. Clones 2 and 3 are from NB 25, clone 4 from NB 35 and clones 5 and 6 are from NB 41, M is 1kb DNA molecular weight marker. All clones were selected for sequencing.

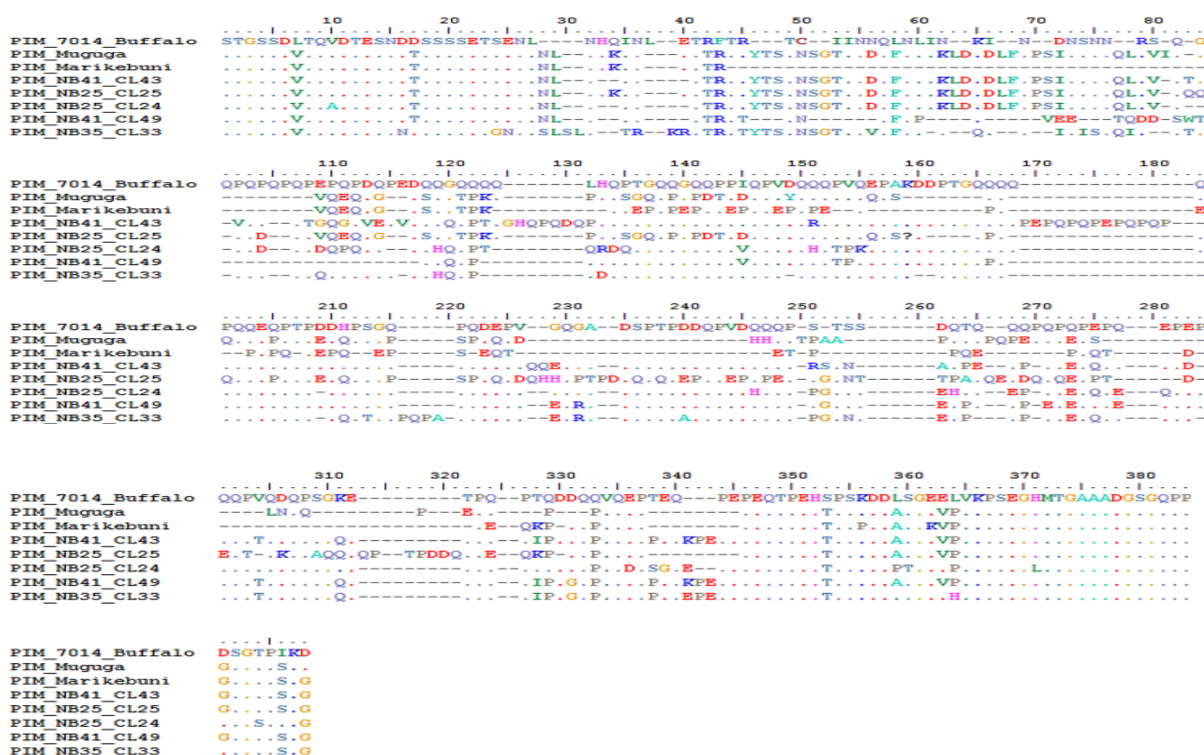

Figure 3b Multiple sequence alignment of the PIM amino acid sequences obtained from buffalo samples. Three samples from Ngorongoro buffalo PIM unique profiles (NB 35 clone 3.3, NB 25- clones 2.4, 2.5 and NB 41- clones 4.3, 4.9) were aligned with previously published *T. parva* PIM amino acid sequences. Colored dots show similarity of the amino acid sequences while the broken-line shows "deletions" of the amino acids sequences and the sequence differences is marked by specific amino acids.

Discussion

This study was carried out to investigate the genetic diversity of *T. parva* isolates existing in cattle and African buffalo in the Serengeti and Ngorongoro in Tanzania. Furthermore, to investigate if *T. parva* genotypes are shared between buffalo and cattle that are grazing in same pasture or in close proximity. Throughout this study, the diagnosis of *T. parva* was conducted using FRET-RT-PCR. All positive samples were characterized for the p67, PIM and p104 genes.

The results obtained by the FRET assay revealed the presence of *T. parva* parasites in cattle and African buffalo in Tanzania. In addition, a high number of samples had mixed infection with other *Theileria* species, including *T. mutans*, *T. taurotragi*. *T. verifera* in Cattle and *T. mutans, T. taurotragi* were found in buffalo. The different species could be easily discriminated using the melting curve analysis (FRET RT-PCR). *Theileria parva* was found to be the dominant infection. Moreover, mixed infection of *T. parva* in the field samples was previously reported in Rwanda (Bazarusanga *et al.,* 2007) South Africa (Chaisi *et al.,* 2010) and Uganda (Oura *et al.,* 2010), whereby cattle and buffalos were found to have different theileria parasites, including *T. parva, T. mutans, T. velifera, T. buffeli and Theileria sp. (buffalo).* High incidence of *T. parva* was observed

in buffalo from Ngorongoro and Serengeti area. This is not surprising since buffalos are thought to be the major natural host of *T. parva* (Grootenhuis *et al.,* 1987).

The results showed a higher incidence of *T. parva* in Ngorongoro cattle as compared to Serengeti cattle, it seems likely that buffalo-derived *T. parva* have been transmitted to cattle in Ngorongoro. This could be possible since traditional livestock owners practice co-grazing that brings their cattle in close contact with buffalo. Therefore, ticks would feed on both buffalo and cattle thus transmitting parasites. In addition, detection of genotypes having a similar profile to those of vaccine strains suggests that these strains could have contributed to the high *T. parva* incidence in Ngorongoro area. The low incidence of *T. parva* in cattle from the Serengeti area would have resulted from the absence of a vaccination program and nonexistence of contact between cattle and buffalo in this area.

Four different p67 alleles were found in buffalo and cattle in Serengeti and Ngorongoro areas. The majority of buffalo-derived *T. parva* in Ngorongoro and Serengeti national park had identical p67 profiles with double bands. Few buffalos from Serengeti national park showed allelic profiles consisting of a single band. These alleles were previously identified as allele 1 and allele 2 (Nene *et al.,* 1996). One can see that there is an identical buffalo-derived p67 genotype shared between the buffalo populations of the two parks. This situation has been previously reported by Sibeko *et al.,* (2009) in buffalo populations from two national parks in South Africa. The p67 profiles consisted of a two and a three-band, with both allelic profiles shared between the two different populations.

Analysis of cattle-derived *T. parva* allelic profiles revealed the presence of two p67 alleles, alleles 3 and 4 as previously described by Sibeko *et al.,* 2009. All Serengeti cattle samples had allele 4, which was also observed in some Ngorongoro cattle samples. It is possible that this allele originated in the Serengeti or was introduced by bringing to the area infected cattle from Ngorongoro. Extensive diversity of cattle-derived *T. parva* has been observed within the Ngorongoro area, where mixed p67 allelic profiles were found. Because there is no limitation of cattle movement between Serengeti and Ngorongoro, this may increase the genotype flow in these two areas.

The analysis of *T. parva* p104 amplicons obtained from cattle revealed allelic profiles identical to those of *T. parva* Muguga and Marikebuni. All Serengeti cattle-derived *T. parva* produced p104 allelic profiles identical to the *T. parva* Marikebuni isolates. As no vaccination has been conducted in this area, it is possible that these alleles have been introduced by infected cattle from Ngorongoro or from Kenya. Moreover, Ngorongoro cattle-derived *T. parva* showed allelic profiles identical with that of the *T. parva* Muguga isolates. Muguga and Marikebuni *T. parva* isolates originating from Kenya. *T. parva* Muguga is one of the parasite strains that constitute the sporozoites-based live cocktail vaccine, also containing the Kenyan Kiambu isolate and the Tanzanian Serengeti isolate. According to Bishop *et al.* (2000), the *T. parva* Kiambu p104 genotype is identical to the Marikebuni profile and Serengeti p104 profile is identical to the *T. parva* Muguga p104 profile. Considering that the Muguga cocktail has been used to immunize cattle into the Ngorongoro area, it is possible that these alleles have been introduced from vaccinated cattle. Hence a mixture of Marikebuni and Muguga p104 genotypes were found in some isolates examined.

Some of Ngorongoro cattle showed allelic profiles with multiple bands, most likely caused by a mixed infection of uncharacterized field strains suggesting that there is extensive genetic diversity in *T. parva* parasites circulating in cattle in this area. Moreover, some of Ngorongoro *T. parva* isolates obtained from cattle showed typical buffalo-derived p104 profiles, which could be evidence of contact between buffalo and cattle resulting in transmission of buffalo-associated parasites into cattle. A p104 profile observed during this study was similar to one previously reported for *T. parva* isolates obtained from a Kenyan buffalo and a Zambian buffalo-derived bovine isolate (Zam5) (Geysen *et al.*, 1999). PCR-RFLP analysis of p104 locus from buffalo-derived *T. parva* samples

showed four distinct and complex allelic profiles. The profiles showed complexity and were difficult to interpret as they might likely represent different *T. parva* genotypes in a single sample**.**

PCR-RFLP analysis of the PIM locus from buffalo gave profiles that are complex and difficult to interpret; this is because PIM is highly polymorphic as previously reported (Geysen *et al.,* 2004). However, six distinctive PIM PCR-RFLP profiles were observed in buffalo-derived *T. parva* isolates and five different profiles in cattle-derived isolate. Extensive allelic diversity was observed in samples from Ngorogoro cattle and buffalo compared with the Serengeti samples, particularly of cattle. This limited diversity within the parasite population of Serengeti cattle could be explained by the lack of buffalo contact being known to harbor a more diverse parasite population. Similar findings were reported by Oura *et al.,* 2003 using the micro and mini satellite genotyping system to analyse populations of *T. parva*-derived from buffalo and buffalo associated cattle against cattle with no contact with buffalo. Analysis of allelic profiles of buffalo-derived parasites suggested a higher multiplicity of infection than that obtained in cattle-derived isolates, which showed PIM allelic profiles that resembled those of Muguga and Kiambu isolates. Muguga cocktail has been used to immunize cattle in the Ngorongoro area, and it is possible that these alleles have been introduced from vaccinated cattle giving to even rise to a mixture of Kiambu and Muguga PIM genotypes in some samples. These results correlate well with the findings obtained from analysis of p104 PCR – RFLP.

Multi-locus genotypes of buffalo and cattle-derived *T. parva* was compared in order to look for shared genotypes. The results showed diversity between cattle and buffalo genotypes. Clearly, there are genotypes that were exclusively specific for Serengeti buffalo and other genotypes that were limited to Ngorongoro buffalo. Interestingly, we found also genotypes shared between Serengeti and Ngorongoro buffalo, suggesting that *T. parva* populations in buffalo from the two areas have been exchanged and is possible because buffalos are moved from one park to another, and this unlimited or uncontrolled buffalo migration could promote transmission of parasites from one buffalo population to the other. Analysis of multi-locus genotypes also revealed a high diversity in Ngorongoro and Serengeti cattle-derived *T. parva.* However, one shared multi-locus genotype was found indicating that genotypes are circulating between cattle in Ngorongoro and Serengeti. Due to the uncontrolled movement of cattle between these areas, there is also the possibility of selective transmission of specific *T. parva* genotypes that adapt and circulate in cattle in these areas.

Amino acid sequence comparison of the cattle-derived p104 clones differed from the known three cattle-type p104 sequences suggesting the existence of the *T. parva* diversity. In addition, PIM gene sequences obtained from selected *T. parva* clones revealed highly polymorphic buffalo-derived *T. parva* alleles. These results are in agreement with the high genetic diversity reported previously for PIM (Geysen *et al.,* 2004).

Conclusion

Generally, results revealed that there is a difference between buffalo and cattle alleles for the three loci examined. There is an indication of some sharing between buffalo and cattle alleles as revealed by PCR-RFLP analyses of p104 loci. Moreover, it is difficult to confirm these putative shared genotypes as this study generated limited sequence data. A higher number of buffalo and cattle samples need to be cloned and sequenced for the p104 and PIM genes in order to confirm shared genotypes of buffalo and cattle-derived *T. parva.* The preliminary findings obtained from this study offer an early indication of *T. parva* diversity in buffalo and cattle in Tanzania. In order to have a

more comprehensive view of the *T. parva* parasite interactions along the buffalo-cattle interface, a larger set of isolates need to be characterized and sequenced.

Conflict of interest

The authors have no known conflict of interest with other people or any organization.

Acknowledgements

We are grateful to Belgian Directorate General for Development Co-operation Framework agreement ITM/DGCD and the Institute of Tropical Medicine (ITM) for financial support. Our sincere gratitude to Lieve Vermeiren and Niels Krol (ITM-Antwerp-Belgium) for their tireless technical support.

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