Veterinary Parasitology 224 (2016) 20–26

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Population genetic analysis of *Theileria parva* isolated in cattle and buffaloes in Tanzania using minisatellite and microsatellite markers

Elpidius Rukambile[a,*], Eunice Machuka[b], Moses Njahira[b], Martina Kyalo[b], Robert Skilton[b], Elisa Mwega[c], Andrew Chota[a], Mkama Mathias[a], Raphael Sallu[a], Diaeldin Salih[d]

[a] *Tanzania Veterinary Laboratory Agency (TVLA), P.O. Box 9254, Dar es Salaam, Tanzania*
[b] *Biosciences Eastern and Central Africa—International Livestock Research Institute Hub (BecA-ILRI Hub), P.O. Box 30709-00100, Nairobi, Kenya*
[c] *Faculty of Veterinary Medicine, Genome Science Center, Sokoine University of Agriculture (SUA), P.O. Box 3019, Morogoro, Tanzania*
[d] *Veterinary Research Institute (VRI), P.O. Box 8067, Khartoum, Sudan*

ARTICLE INFO

Article history:
Received 18 December 2015
Received in revised form 22 April 2016
Accepted 24 April 2016

Keywords:
Buffalo
Cattle
Diversity
Population genetic
Tanzania
Theileria parva

ABSTRACT

A population genetic study of *Theileria parva* was conducted on 103 cattle and 30 buffalo isolates from Kibaha, Lushoto, Njombe Districts and selected National parks in Tanzania. Bovine blood samples were collected from these study areas and categorized into 5 populations; Buffalo, Cattle which graze close to buffalo, Kibaha, Lushoto and Njombe. Samples were tested by nested PCR for *T. parva* DNA and positives were compared for genetic diversity to the *T. parva* Muguga vaccine reference strain, using 3 micro and 11 minisatellite markers selected from all 4 chromosomes of the parasite genome. The diversity across populations was determined by the mean number of different alleles, mean number of effective alleles, mean number of private allele and expected heterozygosity. The mean number of allele unique to populations for Cattle close to buffalo, Muguga, Njombe, Kibaha, Lushoto and Buffalo populations were 0.18, 0.24, 0.63, 0.71, 1.63 and 3.37, respectively. The mean number of different alleles ranged from 6.97 (Buffalo) to 0.07 (Muguga). Mean number of effective alleles ranged from 4.49 (Buffalo) to 0.29 (Muguga). The mean expected heterozygosity were 0.07 0.29, 0.45, 0.48, 0.59 and 0.64 for Muguga, cattle close to buffalo, Kibaha, Njombe, Lushoto and Buffalo populations, respectively. The Buffalo and Lushoto isolates possessed a close degree of diversity in terms of mean number of different alleles, effective alleles, private alleles and expected heterozygosity. The study revealed more diversity in buffalo isolates and further studies are recommended to establish if there is sharing of parasites between cattle and buffaloes which may affect the effectiveness of the control methods currently in use.

1. Introduction

Theileriosis in the form of East Coast fever (ECF) is a very devastating tick-borne disease of cattle which is highly endemic in 11 countries in the Eastern, Central and Southern Africa (ECSA) region where it is a major hindrance to cattle production (Mukhebi et al., 1992). East Coast fever is caused by the tick-borne haemoprotozoan parasite *Theileria parva* transmitted by brown ear tick, *Rhipicephalus appendiculatus.* Economic losses resulting from ECF are attributed to high morbidity and mortality in local and exotic cattle and reduced milk production in lactating cows (Mbwambo et al., 2002; Kivaria et al., 2007).

T. parva infection in susceptible cattle results in a rapid proliferation of infected lymphocytes and death within two to three weeks if no treatment is commenced (Irvin and Mwamachi, 1983). During the course of infection, lymphocytes and antigen presenting macrophages of the M phi lineage are transformed, resulting in a loss of phenotypic markers and consequently, a loss of function (Morrison, 2009; Jeong et al., 2009). In Tanzania control of *T. parva* involves application of acaricides once or twice per week to control the vector, use of commercially available chemotherapy and immunization of animals using infection and treatment method (ITM). ITM comprises inoculation of cattle with a homogenate of infected ticks simultaneously with a single dose of a long-acting formulation of oxy-tetracycline to curtail the infection. This results in long term immunity against the homologous parasite strains but variable

* Corresponding author.
E-mail addresses: rukambile@yahoo.com (E. Rukambile), enidm32@gmail.com (E. Machuka), wanjahira@gmail.com (M. Njahira), M.Kyalo@cgiar.org (M. Kyalo), rskilton@icipe.org (R. Skilton), elisa_mwega@yahoo.co.uk (E. Mwega), chotaandrew@gmail.com (A. Chota), matsonics2000@yahoo.co.uk (M. Mathias), raphaelsallu@yahoo.com (R. Sallu), diaeldin2000@hotmail.com (D. Salih).

http://dx.doi.org/10.1016/j.vetpar.2016.04.038
0304-4017/

protection against challenge with heterologous parasite strains (Irvin et al., 1983). Population genetic studies of *T. parva* are important to determine the genetic exchange among the parasite isolates. The levels of recombination among parasites determine the extent of general population diversity which have great implication in the success and/or failure of ITM (Katzer et al., 2006; McKeever, 2007; Kuo and Kissinger, 2008; Katzer et al., 2010). Recombination under selection can support rapid dissemination of an allele within a population. In *Theileria* spp., recombination can play an important role in generating diversity at loci that are relevant for manipulating host immunity during evasion (Katzer et al., 2011).

Mini and microsatellites have high rates of mutation due to replication slippage and repair. The high rates of mutation result in high levels of polymorphism at the variable number tandem repeat (VNTR) loci making them ideal markers for pathogen diversity study (Oura et al., 2003). Mini and microsatellites have been successfully used to determine population diversity of *T. parva* in different studies. Mini and microsatellite markers were used to characterize the three strains which are present as components of the Muguga *T. parva* vaccine cocktail, to determine the ability of each component to induce carrier state (Oura et al., 2004) and the possibility of being transmitted from vaccinated animals to other susceptible animals (Oura et al., 2007). Four mini and microsatellite markers were used to study diversity of the three stocks which are the components of the Muguga cocktail vaccine whereby 14 different genotypes were revealed (Patel et al., 2011). On the other hand, 9 minisatellites were used to study population genetics and sub-structure of *T. parva* population in the samples collected from two districts in Zambia with success (Muleya et al., 2012). The current study focused on the population structure of *T. parva* isolated from cattle in two districts in the Eastern zone and one district in the Southern highland zone and buffaloes from selected National Parks of Tanzania using 11 mini and 3 microsatellite markers. Determination of parasite diversity in buffalo is equally important because the buffalo-derived parasites can be maintained between cattle and tick passage and yet the parasite population in buffalo is often more diverse than in cattle (Bishop et al., 1994; Potgieter et al., 1988; Collins and Allsopp, 1998; Sibeko et al., 2010).

2. Materials and methods

2.1. Study sites

The study was carried out in 3 districts namely, Kibaha and Lushoto in the Eastern and Njombe in the Southern Highland agro-ecological zones of Tanzania. Kibaha and Njombe are 675 km apart, Kibaha and Lushoto are 419 km apart, Lushoto and Njombe are 847 km apart. East Coast fever has been more frequently reported in Kibaha district and immunization has been practiced. Previously there were fewer reports of the disease in Njombe and Lushoto highlands but in recent times, ECF cases have been reported and the vectors are frequently seen (Personal communications). No vaccination had been reported in the sites where sampling was conducted in Njombe and Lushoto and the cattle sampled at Kibaha, Lushoto and Njombe had no history of co-grazing with buffaloes. Ruaha and Mikumi National parks are along the road towards Njombe and they are about 247 km apart, the closest National park to Njombe being Ruaha which is 161 km from Njombe. Mkomazi National Park is located in the North Eastern Tanzania (Fig. 1)

2.2. Blood sampling

A total of 150 blood samples were randomly collected from cattle which have no history of co-grazing with buffalo in Njombe, Kibaha and Lushoto districts, 50 blood samples being collected

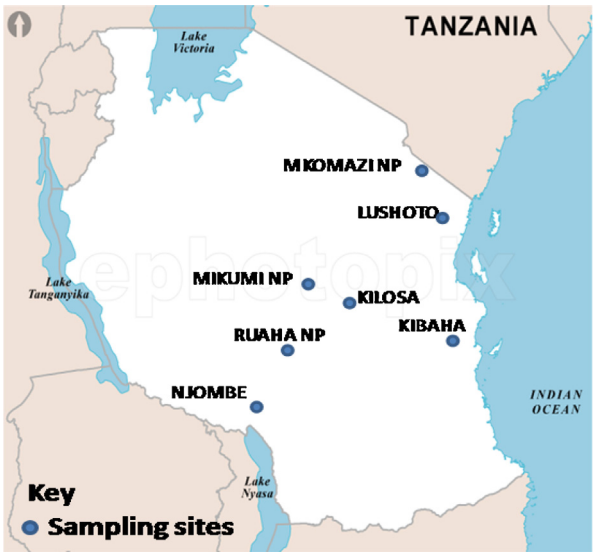


Fig. 1. A Map of Tanzania showing sampling areas.

Table 1
Locations and hosts where the samples were collect from.

Location	Number of sample	Host
Kibaha	50	Cattle
Njombe	50	Cattle
Lushoto	50	Cattle
Mikumi National park	7	Cattle
	25	Buffalo
Ruaha National park	10	Cattle
	16	Buffalo
Mkomazi National park	8	Cattle
	20	Buffalo

from each district. Ten millimeter of blood sample from each animal was collected in EDTA Vacutainer tubes (Becton Dickinson, Oxford, UK) through jugular vein puncture, kept in cool box and later were frozen at −20 °C at Central Veterinary Laboratory, Dar Es Salaam, Tanzania. The blood samples from buffalo and the cattle sharing pasture with buffalo were obtained from the sample archive at Centre for Infectious disease and Biotechnology, Dar Es Salaam, Tanzania (Table 1).

2.3. Screening for T. parva positive samples

DNA was extracted from thawed blood using a commercial extraction kit according to the manufacturer's instructions (PureLink® Genomic DNA Mini Kit, Invitrogen, USA). All samples were screened for *T. parva* using a nested polymerase chain reaction (PCR) assay targeting the 104 kDa antigen (p104) gene (Skilton et al., 2002). The assay was run in two PCR rounds; the primary and secondary PCR; using outer primers for primary PCR (For1 5′-ATT TAA GGA ACC TGA CGT GAC TGC-3′ and Rev1 5′-TAA GAT GCC GAC TAT TAA TGA CAC C-3′), and inner primers for secondary (nested) PCR (For2 5-GGC CAA GGT CTC CTT CAG AAT ACG-3 and Rev2 5′-TGG GTG TGT TTC CTC GTC ATC TGC-3′). Primers were designed based on p 104 antigenic gene (Genebank M29954). In both, primary and secondary PCR, Bioneer AccuPower® PCR Pre-mixes (Seoul, South Korea) was used. The primary PCR composed of 17 μl nuclease free water, 10 pmole of each of the primers (For and Rev) and 2 μl of gDNA at 20 ng/μl to have a final volume of 20 μl. The reaction was briefly vortexed and then centrifuged for 2 min at 3000 rpm to bring all the droplets down. The nuclease free water was used as negative control and the DNA sample known to be *T. parva* positive from BecA-ILRI Hub sample repository was used as positive

Table 2
Panel of mini and microsatellite markers used to genotype *T. parva* samples used in this study with their respective chromosomes, outer nested primers (Oura et al., 2003) and inner nested primers (Salih et al. unpublished data).

	Outer nested primers		Inner nested primers	
Chromosome	Name	Sequence	Name	Sequence
1	ms 2 For O	aagttagtatcaccaccaggctgg	ms 2 For N	gcccaatgtaccgagaatcctcac
	ms 2 Rev O	ggctcatctaccactccaactcc	ms 2 Rev N	attctccgcattctccaccacctc
1	ms 5 For O	aacacagtaactaacccaggcc	ms 5 For N	aatcttccaatcccaaccacatac
	ms 5 Rev O	aactccagcggaatcccgaaata	ms 5 Rev N	cccgaaataaaaccaaattccacc
2	ms 7 For O	tgggtagagattggaatacgcgag	ms 7 For N	ttaacttatctccctctccctccc
	ms 7 Rev O	aataccgacgcgttccgaggaatc	ms 7 Rev N	acactctcaacaactcactcttcc
1	MS 3 For O	cccgatctcactcacatacaacc	MS 3 For N	ccaccgtaaccctctataccat
	MS 3 Rev O	cagcaaatccaactcgtcgtcctg	MS 3 Rev N	gacatctccctcaaatcagactc
1	MS 7 For O	ctcctcagcatcctgctgctcattg	MS 7 For N	gttcagtcctatggcaattcag
	MS 7 Rev O	gcgcatgactgcttttacattaaccc	MS 7 Rev N	caaacctcttcaaattcactctagg
1	MS 8 For O	ggcgtgacggtaatacaccttcc	MS 8 For N	gcctcctcaagcaattcagta
	MS 8 Rev O	cctcctagacactcccgaagatg	MS 8 Rev N	ctggttcaaacacatcaaggtaca
2	MS 16 For O	catggcattcctaggcatcacatc	MS 16 For N	cctcctccatactactaacctacc
	MS 16 Rev O	ccaagggaattaatactgttggag	MS 16 Rev N	cagcgctcagattcacttgtact
2	MS 19 For O	ccgcgtacagactaaactcgccg	MS 19 For N	ccagacacctcaaatcccaagta
	MS 19 Rev O	cctccaactgatccaccctcgcag	MS 19 Rev N	ccacactgccacctaatacaaa
3	MS 21 For O	gatgagcacaaggagttcctggtg	MS 21 For N	ttctacccaacgccactctatgcg
	MS 21 Rev O	gacggcgtctgagatggcgatgac	MS 21 Rev N	tgactcccgtcttatccaaattcg
3	MS 25 For O	ccagagatctcggacacaactcc	MS 25 For N	acacacccatcaacgtagtaac
	MS 25 Rev O	taaggtgccaaacggcggacac	MS 25 Rev N	caccatcacactcttaaccat
3	MS 27 For O	ccgccacctcagtctcgaga	MS 27 For N	cctgcgatacatttctaatcc
	MS 27 Rev O	cacaactcacaccggaatctcac	MS 27 Rev N	gtaataccattcccacctctac
4	MS 33 For O	ctcattaccactctacccatcgc	MS 33 For N	cttctcaaggtaccgtaaacc
	MS 33 Rev O	catggtccatcttcatctggtcc	MS 33 Rev N	cctcactactccaatagttcttc
4	MS 34 For O	gtgcctagagagggaacggatatg	MS 34 For N	gattacgccactgtcataaccacc
	MS 34 Rev O	cctccggctgagattagtggcagg	MS 34 Rev N	aacactccacgctccacattcacc
4	MS 40 For O	cggtggaggctcggatgtgtgc	MS 40 For N	catatcacctcatggtacacac
	MS 40 Rev O	gcaggatcaacatcgccaaccac	MS 40 Rev N	ccagccctaatacacaaatc

ms—Microsatellite.
MS—Minisatellite.
For O—Forward outer nested primer.
Rev O—Reverse outer nested primer.
For N—Forward inner nested primer.
Rev N—Reverse inner nested primer.

control. The amplification conditions for primary PCR were 94 °C for 1 min followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min with additional 10 min at 72 °C as final extension. The components of the secondary PCR remained the same as for the primary PCR except the template which was primary PCR products diluted at 1:10. The amplification conditions were the same except the annealing temperature and the number of cycles was reduced to 55 °C and 30 cycles, respectively. All amplifications were done using a programmable thermal cycler (MJ Research, Watertown, MA, USA). The secondary round PCR products were analyzed by electrophoresis run at 100 V for 40 min in 2% agarose gel stained with GelRed (Biotium Inc. USA).

2.4. PCR amplification and analysis of mini and microsatellite loci

The nested PCR deploying the outer and inner primers designed by Oura et al. (2003) and Salih et al. (unpublished data) respectively (Table 2) was performed to amplify each of 11 mini and 3 microsatellites used in the study for each sample that was positive by p104 nested PCR amplification. The inner forward primer of each marker was fluorescently labeled at the 5′ end with one of the following four standard dyes; 6-FAM (Blue), NED (Yellow), PET (Red) and VIC (Green) (Bioneer, Korea), to enable detection on an ABI genetic analyser. The primary PCR amplification was done in 10 μl comprising of 2 μl of 20 ng/μl genomic DNA, 0.08 μl of 0.5 U/μl DreamTaq polymerase (Thermo Scientific, Lithuania, EU), 0.2 μl of 10 mM dNTPs, 0.4 μl of each outer primers at 10 pmole and 1 μl of 10X DreamTaq buffer with 20 mM $MgCl_2$ (Thermo Scientific, Lithuania, EU). The nuclease free water was used as negative control and the DNA sample known to be *T. parva* positive from BecA-ILRI Hub sample repository was used as positive control. The cycling conditions for the primary PCR were as follows; Initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, extension at 72 °C for 1 min plus a final extension at 72 °C for 10 min. For the secondary PCR, all other reagents remained the same except that 0.5 μl of the primary PCR was used as the template and the volume of water was increased accordingly to give a total of 10 μl reaction volume. The cycling conditions for the secondary PCR were as follows; Initial denaturation at 95 °C for 5 min followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 1 min, extension at 72 °C for 1 min plus a final extension at 72 °C for 20 min. Three microliter of the amplicons were analyzed on a 1.5% agarose gel stained with GelRed (Biotium Inc. USA) to check for amplification success. One and half to two μl of 2 – 4 PCR products of the same sample amplified by forward primers labeled with different dyes were co-loaded on the same well of 96 wells plate with 8 μl mixture of HiDi and LIZ500 mixed at 1 ml LIZ500 and 15 μl HiDi. The samples on the plate were denature at 95 °C by thermocycler for 3 min, fast cooled on ice for 10 min then the plates were loaded in an ABI 3730xl genetic analyser (Applied biosystem) for capillary electrophoresis (Mwega et al., 2014; Muleya et al., 2012). Capillary electrophoresis was carried out at Bioscience eastern and central Africa (BecA) sequencing and genotyping unit. DNA fragment sizes were analyzed by LIZ500 standard size marker (Applied Biosystems) using GeneMapper software (Applied Biosystems).

2.5. Categories of the population used in study

The samples were divided into 5 groups. Samples collected from Kibaha, Njombe and Lushoto were from cattle which are semi-intensively grazed and have no history of sharing the grazing areas

Table 3
Major allele frequency, gene diversity, number of alleles and polymorphic information content (PIC) of the mini and microsatellite markers used to characterizing 5 populations and Muguga isolate used in this study.

Marker	Major allele frequency	Number of allele	Gene diversity	PIC
ms2	0.40	26.00	0.79	0.78
ms5	0.43	39.00	0.79	0.78
ms7	0.52	22.00	0.69	0.66
MS3	0.51	46.00	0.73	0.72
MS7	0.31	37.00	0.84	0.83
MS8	0.53	53.00	0.71	0.71
MS16	0.36	44.00	0.85	0.84
MS19	0.25	91.00	0.93	0.93
MS21	0.65	38.00	0.58	0.57
MS25	0.30	82.00	0.90	0.90
MS27	0.36	37.00	0.85	0.84
MS33	0.56	45.00	0.68	0.67
MS34	0.27	68.00	0.91	0.91
MS40	0.33	50.00	0.88	0.87
Mean	0.41	48.43	0.79	0.79

with buffalo. Samples from these areas were treated as individual populations named as Kibaha, Njombe and Lushoto populations. Samples from buffaloes were categorized as buffalo population regardless of their origin and the samples from cattle which graze close or within the areas where buffaloes are grazing were categorized as cattle close to buffalo population. Muguga vaccine strain was also included in the study and was referred as Muguga isolate.

2.6. Data analysis

The powerMarker software (Liu and Muse, 2005) was used to calculate the diversity parameters of the markers used in this study whereby the major allele frequency, gene diversity, number of alleles and polymorphic information component (PIC) of each marker were generated. Allelic richness as the mean across all loci for each population and Analysis of Molecular variance (AMOVA) was estimated using GenAlEX software version 6.5 (Peakall and Smouse 2012). The same software was used to calculate Principle component analysis (PCoA). DARwin Version 5 software (Perrier and Jacquemoud-Collet, 2006) was used to construct the dendrogram to determine the relationship among 5 populations used in the study and the Muguga isolate.

3. Results

3.1. Screening for T. parva and PCR amplification of mini and microsatellite markers

A total of 105 (70%) cattle (Lushoto 41, Njombe 32, Kibaha 23 and cattle close to buffalo 9) and 30 (49%) buffalo samples were positive by p104 amplification. Samples positive for p104 PCR amplification were amplified for mini and microsatellite markers. Out of 135 samples subjected to mini and microsatellite markers amplification, 133 samples were successively amplified, 2 samples were poorly amplified by most of the markers used and were excluded from the study.

3.2. Markers and allelic variation

The major allele frequency, gene diversity, number of allele and polymorphic information component across all 14 makers ranged from 0.24 (MS 19) to 0.64 (MS 21) with mean of 0.41, from 0.57 (MS 21) to 0.92 (MS 19) with a mean of 0.79, from 22 (ms 7) to 91 (MS 19) with mean of 48.4 and from 0.57 (MS 21) to 0.93 (MS 19) with mean of 0.79, respectively (Table 3). MS 19 maker was more diverse and informative and MS 21 was less diverse and informative than other markers used in this study.

3.3. T. parva population diversity

The diversity across populations was determined by the mean number of different alleles, mean number of effective alleles, mean number of private allele and expected heterozygosity. The mean number of allele unique to populations for Cattle close to buffalo, Muguga, Njombe, Kibaha, Lushoto and Buffalo populations were 0.18, 0.24, 0.63, 0.71, 1.63 and 3.37, respectively. The mean number of different alleles ranged from 6.97 (Buffalo) to 0.07 (Muguga). Mean number of effective alleles ranged from 4.49 (Buffalo) to 0.29 (Muguga). The mean expected heterozygosity were 0.07, 0.29, 0.45, 0.48, 0.59 and 0.64 for Muguga, cattle close to buffalo, Kibaha, Njombe, Lushoto and Buffalo populations, respectively (Fig. 2). The population which was close to Buffalo in mean number of different alleles, effective alleles, private alleles and expected heterozygosity was Lushoto and the values were 6.71, 4.17, 1.63 and 0.59 respectively.

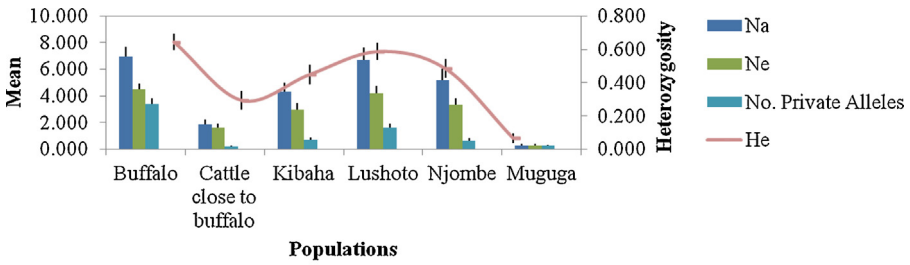
The results from constructed principle component analysis (PCA) showed three patterns of clustering, the buffalo genotypes which clustered on the top right quadrant comprising the buffalo individuals and few genotypes from Njombe, Kibaha and Lushoto; the bottom right quadrant comprising most of Lushoto, Kibaha and Njombe and few buffalo genotypes. The left top and down quadrant was the admix group comprising the genotype from all populations including Muguga isolate (Fig. 3). It is in this quadrant only where the cattle close to buffalo population genotypes were found. Analysis of molecular variance (AMOVA) showed high within population variation (90%) compare to among population variation (10%) where as among individual variation was 0% (Table 4).

The dendrogram generated showed three main clusters, the cluster where most of the buffalo genotypes were found and formed its own sub-cluster with one genotype from Njombe; the cluster comprising high percentage of the individuals from each population except Muguga isolate. In this cluster there is a sub-cluster comprising only the genotypes from all 3 populations of cattle which have no history of co-grazing with buffaloes. The last cluster was small cluster comprising one genotype from buffalo and small number of genotypes from all other populations. It is in this cluster where the Muguga isolate was found (Fig. 4).

4. Discussion

The population genetic analysis study deploying 11 mini and 3 microsatellites was conducted to determine *T. parva* population diversity. In this study a total of 103 and 30 blood samples collected from clinically health cattle and buffaloes, respectively were used. The study revealed the prevalence of 70% and 49% of *T. parva* in cattle and buffaloes respectively. The problem was bigger than it was previously thought before possibly because of the lower sensitivity of the methods which are routinely used compared to the p104 nested PCR assay which is able to detect even sub-clinical and carrier cases (Skilton et al., 2002). There is high possibility that most of the animals sampled were in carrier state, a phenomenon which is common following natural infection in both hosts or post immunization in cattle (Oura et al., 2007; Oura et al., 2011).

The type and number of mini and microsatellite markers used in this study were previously used by Mwega et al. (2014) and differ from the set of 9 minisatellites used by Muleya et al. (2012) to study population genetic diversity of *T. parva*. The mean polymorphic information component (PIC) of the mini and microsatellite markers used ranged between 0.57 and 0.93 with a mean of 0.79. Loci are considered to be highly informative when the PIC is greater



Na = Number of Different Alleles
Ne = Number of Effective Alleles
No. Private Alleles = No. of Alleles Unique to a Single Population
He = Expected Heterozygosity

Fig. 2. Allelic patterns presenting mean number of different alleles, effective alleles, private alleles and expected heterozygosity across 5 populations and Muguga isolates used in this study.

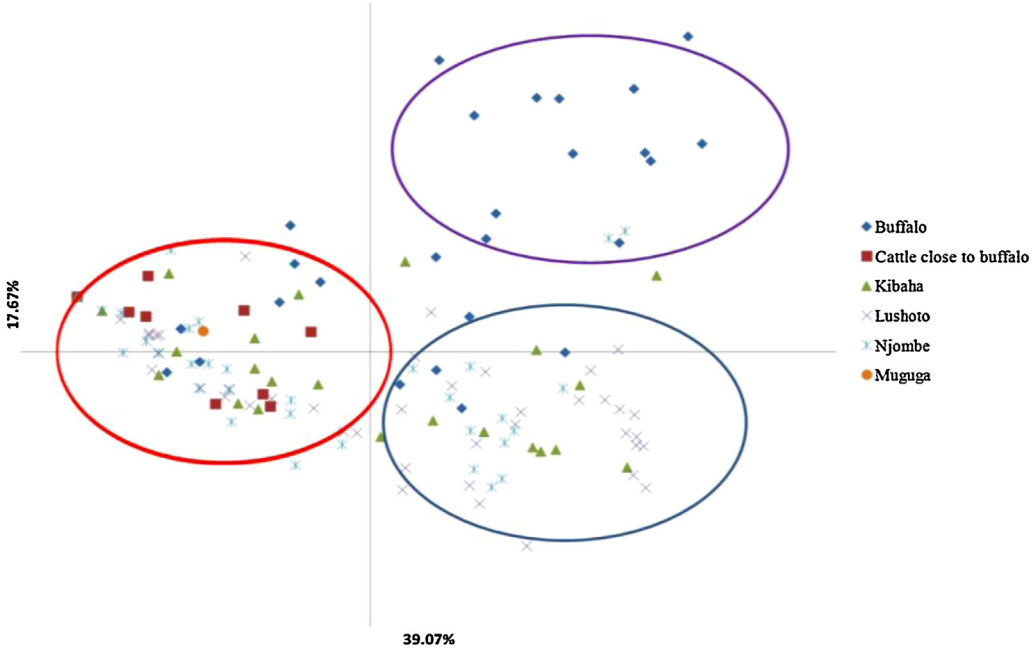


Fig. 3. Principal component analysis (PCA) of *T. parva* as generated from 5 populations and Muguga isolate. The proportion of the variation in the data set is indicated in each axis. The analysis presents three main clusters; right top quadrant (purple oval bordered) comprising most of the buffalo individuals, left top and bottom quadrants (red oval bordered) presenting admixed group and the right bottom quadrant (blue oval bordered) cluster comprising most of Njombe, Lushoto and Kibaha population genotypes.

Table 4
Analysis of molecular variance (AMOVA) calculated from 5 populations and Muguga isolate.

Source of variation	Degree of freedom	Sum of square	Mean Square	Estimated Variation	Percentage variation
Among Populations	5	128.67	25.73	0.82	10
Within Populations	135	1006.11	7.45	7.45	90
Total	140	1134.79		8.27	100

than 0.5 (Ngono Ema et al., 2014; Ya-Bo et al., 2006). Consequently, the results obtained in this study are informative and should be considered reliable as an assessment for *T. parva* population genetic diversity in the areas assessed. Furthermore the high polymorphism of the mini and microsatellite markers used is revealed by high mean gene diversity (0.79) and relatively higher mean major alleles frequency (0.41).

The Buffalo population showed a high polymorphism compared to other populations studied. This is supported by larger mean number of different alleles, effective alleles, private alleles and expected heterozygosity than other populations, followed by Lushoto. Buffaloes are believed to carry a heterogeneous population of parasites (Sibeko et al., 2011), therefore; these results were not surprising. The presence of unique alleles in all populations indicate that there is a unique set of isolates in the populations used in this study as each allele is a representative of a different isolate. This may compromise ITM, the only available control method of this parasite through immunization since guarantee for cross protection among different isolates is very limited (Pellé et al., 2011; Patel et al., 2011). The variation of the individuals within population was further revealed by AMOVA which showed 90% within individual variations.

T. parva parasites which originate from buffalo are genotypically and antigenically closely related to *T. parva* maintained in cattle

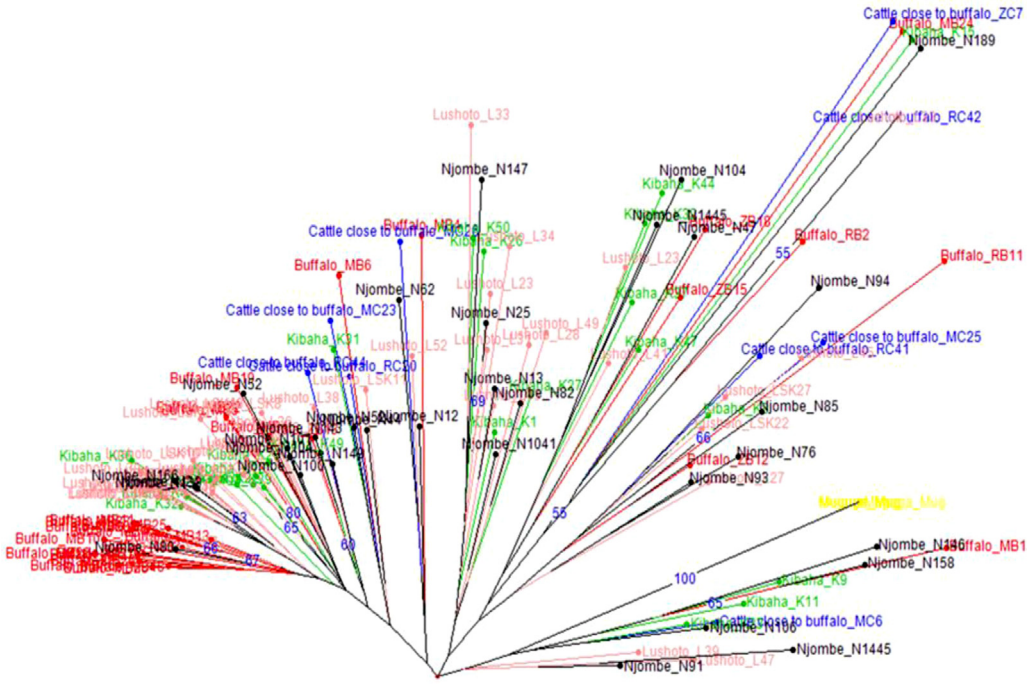


Fig. 4. The neighbor-joining dendrogram presenting the relationship among 5 populations used in this study and the Muguga isolate. Majority of the buffalo individuals formed one separate sub-cluster of individuals while other buffalo individuals being randomly distributed in other cattle individuals. Muguga formed the cluster with few genotypes from other 5 populations.

and only a limited proportion of the buffalo-derived population can be adapted and transmitted between cattle. This study revealed a buffalo-derived *T. parva* sub-cluster different from cattle isolates, this may affect the effectiveness of ITM control methods if parasite sharing occurs between these two hosts. Parasite sharing is more likely to occur since pasture sharing is common under the nomadic system which is prevailing in the country.

Previously there were few cases of ECF reported in Lushoto highlands and Njombe but this study shows that there is a high prevalence of the parasite in these areas. Occurrence of the parasite in these areas may be caused by extensive movement of cattle to these areas for stock establishment, replacement and searching for pastures from the low land of Lushoto and other parts of the country where the vector and parasite is dominant. There is no clear explanation to why the Lushoto population is more diverse than the rest of cattle populations but extensive influx of the cattle may be the reason. Kibaha has been practicing immunization by ITM for many years hence; it was expected to be more diverse than other cattle isolates as immunization increase diversity of the parasite (Weir et al., 2011). Genotype similarity analysis showed Muguga vaccine isolate clustering with genotypes from all populations used in the study as an admix group. This was unexpected since there is no immunization which has been conducted in these areas except at Kibaha. Genotype sharing among these populations may be caused by unrestricted movement of cattle among these areas. These results are concurrent with those of Mwega et al. (2014) which revealed genotype sharing among Muguga vaccine isolate, Eastern and Southern zone isolates although there was no vaccination exercise which had been conducted in Southern zone.

This study revealed the genotypic population structure of *T. parva* in areas where the isolates were collected from. In this study buffalo-derived isolates were found to be more diverse than the cattle-derived isolates. This situation poses more risk on the sustainability of ITM in the feature due to uncontrolled movement of cattle which may lead sub-populations of *T. parva* from buffaloes to adapt to cattle since parasite sharing between these two hosts is possible. On the other hand there was no strong evidence shown by this study on parasite sharing between cattle and buffaloes grazing in the same area. It is still possible that sharing of isolates may be occurring but was not evident given the relatively small population examined in this study. Therefore, it is suggested that future studies be conducted including more samples from cattle that are co-grazing with buffaloes. This will help to establish the potential risks of cross-infection between cattle and buffaloes grazing in the same area.

Financial support

We want to acknowledge the financial support provided to the Biosciences Eastern and Central Africa Hub at International Livestock Research Institute (BecA-ILRI Hub) by the Australian Agency for International Development (AusAID) through a partnership between Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO) and the BecA-ILRI Hub; and by the Syngenta Foundation for Sustainable Agriculture (SFSA); the Bill & Melinda Gates Foundation (BMGF); and the Swedish Ministry of Foreign Affairs through the Swedish International Development Agency (Sida). The financial support granted by Eastern Africa Agricultural Productivity Programme (EAAPP) on field samples collection is highly appreciated.

Conflict of interest

None of the authors (Elpidius Rukambile, Eunice Machuka, Moses Njahira, Martina Kyalo, Robert Skilton, Elisa Mwega, Andrew Chota, Mkama Mathias, Raphael Sallu) has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the paper entitled "Population genetic analysis of *Theileria parva* isolated in cattle and buffaloes in Tanzania using minisatellite and microsatellite markers".

Acknowledgement

We send our sincere gratitude to Joyce Njuguna and James Wainaina for their valuable support on data analysis.

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