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Original Article

Detection of carrier state and genetic diversity of *Theileria parva* in ECF-vaccinated and naturally exposed cattle in Tanzania

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ABSTRACT

Infection and Treatment Method (ITM) has been practiced in Tanzania for over 20 years as a prevention measure against East Coast Fever disease. It is known that ITM, like natural ECF infection, leads to a carrier state, whereby vaccinated cattle become asymptomatic carriers of the parasite. It is expected that ECF vaccination using ITM also leads to generation of combinations of vaccine specific *Theileria parva* and local strains that circulate in the field what contributes to an unknown level of parasite diversity. Moreover, the long term impact of ITM on carrier state and parasite diversity in cattle are largely unknown. To address this question blood was collected from ECF-vaccinated (n = 239) and unvaccinated (n = 97) cattle from Loiborsoit, Emboreet, Esilalei, Manyara ranch and Mswakini villages in the Maasai steppe of northern Tanzania, as well as Mruazi and Leila farms in Tanga in eastern Tanzania. Screening for *T. parva* using nested PCR revealed an overall prevalence of *T. parva* to be 34.5%, with a significant higher prevalence among ECF-vaccinated cattle. Using three VNTR markers (ms2, ms5 and MS7) higher parasite genetic diversity in terms of higher number of alleles and expected heterozygosity was shown in vaccinated than unvaccinated cattle. These parameters were highest in cattle from Manyara ranch. Nevertheless, the principle component analysis (PCoA) showed no distinct clustering patterns as most *T. parva* alleles clustered together throughout the four quadrants implying parasite homogeneity among the sampled populations. However, some of the parasite alleles closely clustered with Muguga vaccine alleles in two of the quadrants, consistent with closer genetic relatedness between the vaccine strains and the *T. parva* populations from the Maasai steppe. Likewise analysis of molecular variance (AMOVA) revealed most of the genetic variation (93%) being contained within populations with only 7% being among populations. This study therefore confirms the role of ECF vaccination in enhancing carrier state and *T. parva* diversity in vaccinated cattle populations. Higher *T. parva* diversity may play an important role in carrier cattle by way of restricting breakthrough infections from field parasite strains.

1. Introduction

Theileria parva is a tick-borne protozoan parasite belonging to the phylum Apicomplexa and causes in cattle a severe disease known as East Coast fever (ECF). The disease is endemic in eleven countries in eastern and Central Africa, where it poses a serious economical problem to the livestock industry (Hayashida et al., 2012). The parasite is transmitted by the tick vector, *Rhipicephalus appendiculatus*, which spreads the infection after feeding on infected/carrier animals (Olds et al., 2018). The African buffalo (*Syncerus caffer*) is the main natural host of *T. parva* but it does not develop clinical signs of infection. The interaction of cattle and the African buffalo may result to the transmission of buffalo derived *T. parva* in cattle which results in Corridor disease (CD) characterized by low levels of parasitized leukocytes in peripheral lymph nodes compared to high parasitosis seen in ECF (Sitt et al., 2015).

Exotic breeds of cattle (*Bos taurus*) and their crosses which are increasingly being used to satisfy demands for milk production, are highly

Abbreviations: T. parva, *Theileria parva*; MC, Muguga cocktail; ECF, East Coast fever; ITM, Infection and treatment method; VNTR, Variable number of tandem repeats; Bp, Base pair; MS, Minisatellite; ms, Microsatellite; nPCR, Nested PCR; gDNA, Genomic DNA; DNA, Deoxyribonucleic Acid; EDTA, Ethylene diaminetetra acetic acid; et al., And others; MHC, Major histocompatibility complex; PCR, Polymerase Chain Reaction; µl, Microlitre; °C, Degrees centigrade; Pmole, picomole; %, Percentage

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susceptible and suffer high rates of mortality. The disease has a particularly devastating impact on poor small-holder farmers, who often do not have adequate resources or access to control measures. While the mortality in exotic cattle may reach 100%, indigenous cattle (*Bos indicus*) breeds develop resistance to the disease following primary natural infection and recovery. Control of the disease by prevention of tick infestation requires essentially continuous application of acaricides and is therefore expensive and difficult to sustain. The use of acaricides also poses a threat to the environment. Treatment of clinically sick cattle is limited by high cost and the need to treat animals during the early stages of disease. Because of the shortcomings of these control measures and the fatal nature of the disease, there is a demand for effective vaccines to provide a sustainable means of controlling the disease (Perry, 2016).

The only available vaccine against ECF in Tanzania is based on an Infection and Treatment Method (ITM) that involves inoculation of live sporozoite-stage parasites and simultaneous treatment with long-acting tetracycline. The live vaccine, also called the Muguga cocktail, consists of the three strains of *T. parva*, namely, Muguga, Serengeti-transformed and Kiambu 5 (Radley et al., 1975). There have been worries that the Muguga cocktail vaccine may introduce parasites with a new genetic background into local parasite populations (Oura et al., 2007). In spite of such worries, the infection and treatment method has found wide adoption in Tanzania, with wider deployment in pastoral areas of northern Tanzania in the last 20 years. According to a GALVmed report (2010), ITM has drastically reduced calf mortality from 80% to < 2%, resulting in cattle herders being able to sell more animals and increase their income. Despite the high cost of up to US$10 per animal (Di Giulio et al., 2009), the ITM remains to be the most cost-effective ECF control option available to farmers in pastoral and agro-pastoral systems in northern Tanzania (Martins et al., 2010).

A previous study in northern Tanzania (Kazungu et al., 2015a) in ECF-vaccinated herds showed that continuous natural tick challenge provides an incremental effect on acquired immunity, resulting into enhanced carrier state and seroprevalence (Kazungu et al., 2015b), low disease incidence with low case fatality and eventual endemic stability. Nevertheless the implication of the potential exchange of genetic information is largely unknown as regards ECF vaccination outcomes. In order to better understand the long term impact of ITM, we investigated the carrier state and diversity *T. parva* in relation to ECF vaccination status. The results generated from this study are relevant to better define ECF vaccination outcomes and hence improve the delivery ECF vaccination in pastoral systems, where this is most demanded.

2. Materials and methods

2.1. Study sites

The study was carried out in Maasai steppe of northern Tanzania and Tanga region of eastern Tanzania. The Maasai steppe is made of Simanjiro plains, Tarangire National Park and Lake Manyara National Park. It lies between 3°52′ and 4°24' South and 36°05 and 36°39 East. This area consists of a wildlife corridor which is bordered by Monduli district and Manyara ranch located in the north and Tarangire National park on the south-western side. Due to its proximity to protected areas the area has high interaction of wildlife reservoirs such as African buffalo and domestic animals which increase circulation of *T. parva* through tick bites hence making ECF endemic in the area. The Maasai steppe represents one of the areas in Tanzania, where ITM is intensively deployed to prevent ECF. Tanga region is situated at the north-east corner of Tanzania between 4° and 6° degrees below the Equator and 37° -39°10′ degrees east of the Greenwich Meridian. Mruazi and Leila farms in Tanga were selected for this study purposively due to the lack of interactions with wildlife.

2.2. Blood samples and DNA extraction

A total of 336 clinically healthy cattle were conveniently sampled from each of the following villages as follows: Loiborsoit ($n = 35$), Emboreet ($n = 41$), Esilalei ($n = 69$), Manyara ranch ($n = 72$), Mswakini ($n = 63$), Leila farm ($n = 36$) and Mruazi Farm ($n = 20$). The five villages were selected from the Maasai steppe in northern Tanzania while the other two herds (Leila farm and Mruazi farm) were selected from Tanga, north-eastern Tanzania. All herds in northern Tanzania were purposively selected based on their location with reference to Tarangire and Manyara National parks (proximity to African buffaloes) and individual cattle were selected based on their ECF vaccination status. Vaccinated cattle were easily identified by ear-tag numbers, which indicated the year of vaccination which was also confirmed by cattle owners. Consent from local village authorities and individual farmers was sought prior to blood collection done from March to May 2018.

Whole blood samples were collected through jugular vein puncture using 10-ml EDTA vacutainer tubes (Becton Dickinson, Vacutainer Systems, England). Tubes were labeled and stored in a cool box with ice packs while in the field and later stored in the fridge (4 °C) at district veterinary offices before transportation to the laboratory, where they were kept frozen at −20 °C until DNA extraction.

DNA was extracted using the Quick-g DNA™ Blood miniprep (D 3073, Zymo Research, USA) following the manufacturer's protocol. The eluted DNA was checked for quality on agarose gels prior to amplification.

2.3. Nested PCR for screening of T. parva positive samples using p104 gene

All DNA samples were screened for *T. parva* carrier state 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 GACTAT 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-TGGGTG TGT TTC CTC GTC ATC TGC-3). Primers designed based on p104 antigenic gene were previously described by Odongo et al. (2010). The primary PCR was composed of 6.25 µl quick-load ® Taq 2× Master mix, 3.25 µl nuclease free water, 0.25 µl of each of the primers (For and Rev) and 2.5 µl of g DNA to have a final volume of 12.5 µl. The reaction was briefly vortexed and then centrifuged for 1 min at 14,000 rpm to bring all the droplets down. The nuclease free water was used as negative control and the in-house *T. parva* positive DNA sample (Muguga vaccine DNA) was used as positive control. The amplification conditions for primary PCR were 95 °C for 1 min followed by 30 cycles of 95 °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. The reactions were conducted in a thermocycler (ProFlex PCR system, Applied Biosystems, Foster City, CA, USA). The secondary round PCR products were analyzed by electrophoresis run at 80 V for 40 min in 1.5% agarose gels. Positive nPCR products were identified as 277 bp DNA fragments.

2.4. PCR amplification and fragment analysis of mini and microsatellite loci

Three VNTR markers (MS7, ms5 and ms2) chosen from the chromosome number one of the Muguga isolate of *T. parva* were used to study genetic diversity of *T. parva* within and among cattle populations. The nested PCR deploying the outer and inner primers designed by Oura et al., (2003) and Salih et al. (2018), respectively (Table 1) was

Table 1
Panel of mini and microsatellite markers used to genotype *T. parva* positive samples.

Markers		Outer nested primers sequence	Inner nested primers sequence
MS7[a]	For[d]	CTCCTCAGCATCCTGCTGCTCATTG	GTTCAGTCCTATGGCAATTCAG
	Rev[e]	GCGCATGACTGCTTTTACATTAACCC	CAAACCTCTTCAAATTCACTCTAGG
ms5[b]	For	AACACAGTAACTAACCCAGGCC	AATCTTCCAATCCCAACCACATAC
	Rev	AACTCCAGCGGAATCCCGAAATA	CCCGAAATAAAACCAAATTCCACC
ms2[c]	For	AAGTTAGTATCACCACCAGGCTGG	GCCCAATGTACCGAGAATCCTCAC
	Rev	GGCTCATCTACCACTCCAACTCC	ATTCTCCGCATTCTCCACCACCTC

[a] Minisatellite 7.
[b] Microsatellite 5.
[c] Microsatellite 2.
[d] Forward nested primer.
[e] Reverse nested primer.

Table 2
Proportions of cattle categories based on *T. parva* infection status.

Category	Sub-category	*T. parva* infection status		Total	*P* value
		Positive, n (%)	Negative, n (%)		
ECF vaccination status	Vaccinated	103 (43.1)	136 (56.9)	239	P < .000
	Unvaccinated	13 (13.4)	84 (86.6)	97	
Proximity to wildlife interface	Yes[a]	107 (38.2)	173 (61.8)	280	P < .001
	No[b]	9 (16.1)	47 (83.9)	56	
Overall		116 (34.5)	220 (65.5)	336	

[a] Cattle sampled from Loiborsoit, Emboret, Manyara ranch, Mswakini and Esilalei.
[b] Cattle sampled from TangaLeila and Mruazi farms.

performed to amplify each of the VNTR markers for each DNA sample that was *T. parva* positive as identified by the p104 nested PCR. The primary PCR amplification was done in 10 µl comprising of 2 µl of 20 ng/µl genomic DNA, 5 µl of quick-load ® Taq 2× Master mix, 0.4 µl of each outer primers at 10 pmole and 2.2 µl of nuclease free water. The nuclease free water was used as negative control and an in-house *T. parva* positive DNA sample isolated from the Muguga vaccine 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 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 30s, annealing at 58 °C for 1 min, extension at 72 °C for 1 min plus a final extension at 72 °C for 20 min. Five microliters of the amplicons were analyzed on 1.5% agarose gels to check for amplification success. DNA fragment sizes (Table 3) were scored from gel pictures using the Vision capt software version 15.0 (Vilber Lourmat).

2.5. Data analysis

Data were entered and cleaned using Microsoft Excel (2013). GenALEX software version 5 (Peakall and Smouse, 2012) was used to calculate genetic diversity parameters for the entire data set. This included determining the mean number of alleles, number of effective alleles and expected heterozygosity (He). These parameters were used to determine diversity overall and within the populations. Analysis of molecular variance (AMOVA) was used to study *T. parva* diversity by estimating the percentage variations within and among the cattle populations. Principal Component Analysis (PCoA) was used to investigate the genetic relationships between the *T. parva* from different sampling locations. Descriptive statistics were computed at 95% Confidence Interval (CI). Chi-square test was used to determine association between outcome variables (*T. parva* positivity) and categorical variables such as vaccination status and cattle proximity to wildlife interface areas. Statistical significance was determined at $p < .05$.

3. Results

3.1. Prevalence of T. parva carrier state in ECF-vaccinated and unvaccinated cattle using the p104 gene

Three hundred and thirty six (336) clinically healthy cattle were screened for *T. parva* using p104 nested PCR. A 277 bp PCR product was observed in 116 (34.5%) out of a total of 336 cattle. The proportion of p104 PCR positive cattle was significantly higher among previously ECF-vaccinated cattle (43%; 103/239) than unvaccinated cattle (13.4%; 13/97) ($p < .000$). Likewise, a higher frequency of *T. parva* positivity was seen among cattle in herds grazing closer to wildlife interface areas (107/280; 38%) than those sampled farther from wildlife (9/56, 16%) ($P < .001$) (Table 2).

Prevalence of *T. parva* also varied between different cattle populations. Hence highest prevalence was found in cattle sampled from Manyara ranch (82%) while no infections were detected in Mruazi farm (0%). Thirteen ECF unvaccinated cattle were also *T. parva* positive and these were from Leila farm ($n = 4$) in Tanga, and Loiborsoit ($n = 2$) and Esilalei ($n = 7$) villages in the Maasai steppe in northern Tanzania.

3.2. Determination of T. parva diversity in carrier cattle using mini and microsatellite markers

T. parva allelic patterns at the three satellite loci, MS7, ms5 and ms2 in the cattle populations are shown in Fig. 1, a, b and c, respectively. The number of MS7 alleles ranged from 2 (Tanga Leila farm, Esilalei and Mswakini cattle populations) to 5 (Manyara ranch cattle population). Similarly, number of effective alleles (3.269) was highest in Manyara ranch population and least in Esilalei population (1.6). Likewise, diversity within the ms5 and ms2 markers indicated that the Manyara ranch population scored highest mean number of different

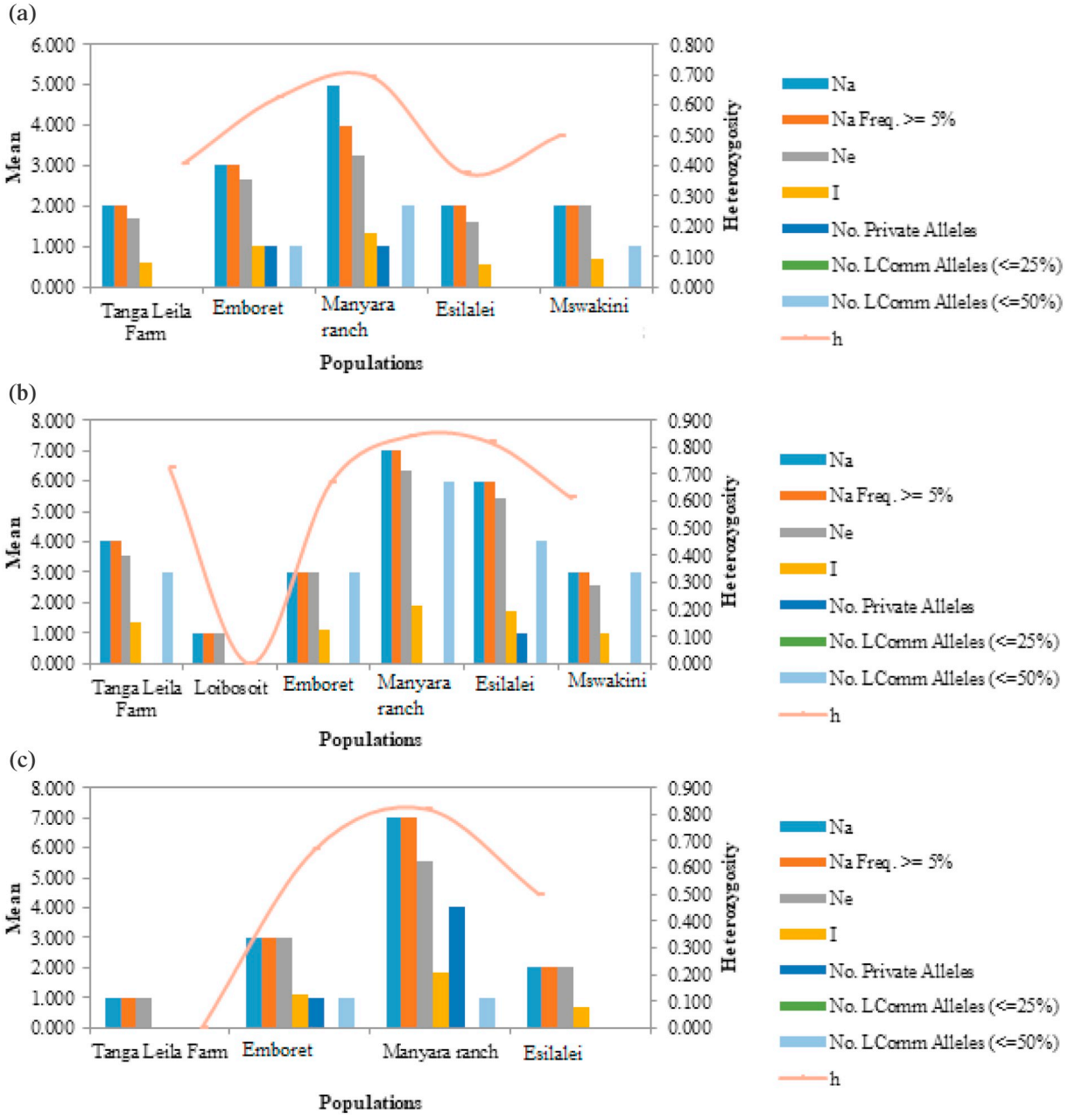

Fig. 1. a. The allelic patterns for MS7 *T. parva* marker across cattle populations used in this study. 1b: The allelic patterns for ms5 *T. parva* marker across cattle populations used in this study. 1c: The allelic patterns for ms2 *T. parva* marker across cattle populations used in this study.

Table 3
Number of alleles detected in the three VNTR markers.

Marker	Number of alleles		Size range (bp)
	Total in populations	Total in Muguga vaccine	
MS7	6	2	125–450
ms5	3	2	150–450
ms2	4	3	150–500

alleles, mean number of effective alleles and expected heterozygosity. These parameters were lower in the other cattle populations. The average expected heterozygosity for MS7 marker was highest in Manyara ranch population (0.694) and lowest in Esilalei population (0.375). The number of alleles amplified on the Muguga vaccine DNA using the MS7, ms2 and ms5 markers was 2, 3 and 2, respectively (Table 3). Allelic patterns amplified on the Muguga vaccine DNA were also amplified in majority of the vaccinated and some of the unvaccinated cattle. Some of the parasite alleles detected in the Muguga vaccine were not detectable in some vaccinated cattle, although there were also several non-Muguga vaccine alleles that were detected in the cattle. Generally, ECF-vaccinated cattle possessed more alleles when compared to unvaccinated cattle (Fig. 2).

3.3. PCoA and AMOVA analysis

The principal component analysis (PCoA) revealed existing relationships between *T. parva* in the six cattle populations (Fig. 3). The patterns of clustering suggested that most of the parasite alleles (genotypes) were clustered throughout the four quadrants. Most of the parasite alleles found in Manyara ranch population clustered at the top left and bottom left quadrants together with alleles from Esilalei,

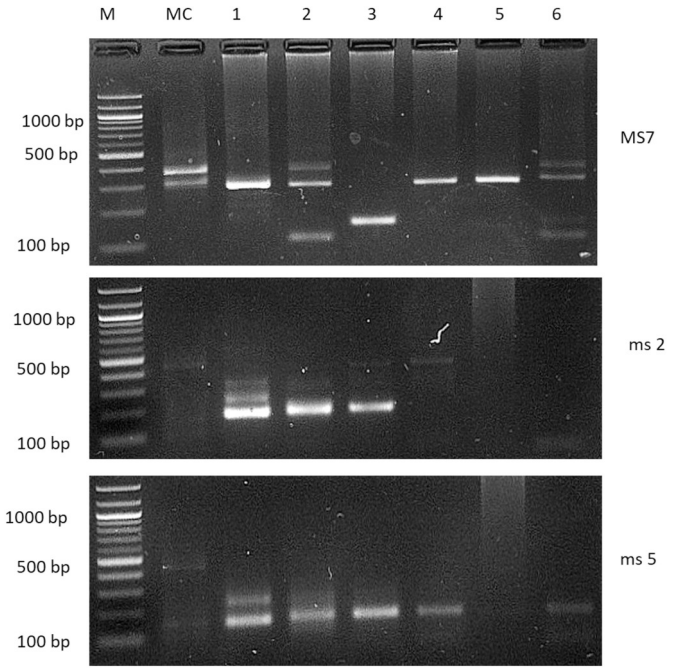

Fig. 2. Agarose gel showing example allelic profiles of MS7 (top gel), ms2 (middle gel) and ms5 (bottom gel) amplified in Muguga cocktail DNA (MC); Calves vaccinated 21 days ago (1,2) in Emboreet village, northern Tanzania; unvaccinated adult cows from Tanga Leila farm (3,4); cattle vaccinated 4 years ago in Emboreet village, northern Tanzania (5,6). The Muguga cocktail was used to vaccinate against ECF in all cases.

Emboreet and Tanga Leila farm populations. The bottom right quadrant contained alleles from Esilalei, Emboreet and Manyara Ranch populations as well as from Tanga Leila farm, which also clustered with one Muguga vaccine allele. Interestingly, three out of the four Muguga vaccine alleles appeared in the top right quadrant, clustering with majority of ECF-vaccinated populations from Manyara ranch, Msakwini and Esilalei. Generally, parasite alleles from the populations tended to spread across the four quadrants, although high representation of alleles was found in the top left, bottom left and bottom right quadrants. The analysis of molecular variance, when used to assess the structure of *T. parva* in the cattle populations indicated that most of the genetic variation (93%) was contained within populations with only 7% variation found between populations (Table 4).

Table 4
The summary of AMOVA for the cattle groups.

Source	Df[a]	SS[b]	MS[c]	Est. Var.[d]	%[e]
Among populations	5	6.790	1.358	0.050	7%
Within populations	102	65.293	0.640	0.640	93%
Total	107	72.083		0.690	100%

[a] Degrees of freedom.
[b] Sum of squares.
[c] Mean squares.
[d] Estimated variance.
[e] Percentage variation.

4. Discussion

This study investigated the carrier state and genetic diversity of *T. parva* in pastoral cattle previously vaccinated against ECF by the infection and treatment method. We found an overall prevalence of *T. parva* to be 34.5% in 336 cattle sampled from six distant cattle populations. Prevalence of *T. parva* was by threefold higher in ECF-vaccinated than unvaccinated cattle. Previous studies conducted in the same areas reported a similar pattern of results, whereby prevalence was higher in ECF-vaccinated than unvaccinated cattle (Kazungu et al., 2015; Kimaro et al., 2017). These results justify the beneficial deployment of the ITM vaccine in pastoral areas of Tanzania, where majority of the cattle become carriers, an asymptomatic tick-transmissible state of infection, allowing cattle to remain immune to ECF (Bishop et al., 1992).

Previously, there has been an extensive debate concerning the risk of vaccination with the Muguga cocktail vaccine, as the live vaccine may introduce parasites with an exotic genetic background into the local parasite population (Geysen et al., 1999; Oura et al., 2004, 2007; McKeever, 2007). This was proven to be a real risk when Oura et al. (2004) demonstrated the transmission of a strain of vaccine constituent to unvaccinated cattle under field conditions in Uganda. Given that *Theileria* parasites could recombine between divergent strains during the sexual stage in ticks, vaccine-derived 'exotic' and 'local' strains could exchange genetic information, resulting in parasites with genetic mosaics and diversity. Thus, it is important to monitor ECF-vaccinated cattle, especially pastoral cattle populations as those in the Maasai steppe, which are naturally exposed to field tick challenge continuously. This study was purposively conducted in the Maasai steppe because ITM has been practiced in these areas, specifically on pastoralist cattle herds, for close to 3 decades with great success (Di Giulio et al., 2009) and hence this ecosystem offers a niche to explore long term impacts of ITM deployment. Monitoring of vaccinated populations

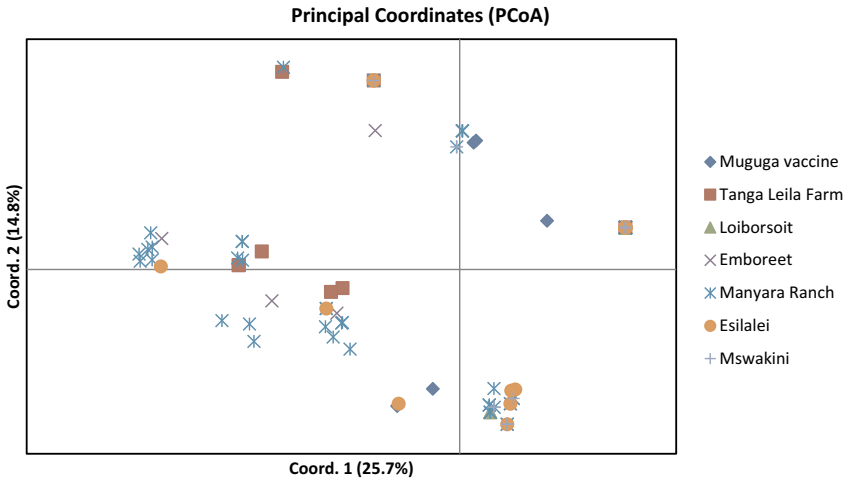

Fig. 3. Principal component analysis (PCoA) of *T. parva* as generated from 6 populations.

would not only reveal longevity of protection but also indicate extent of admixture of vaccine with local field parasite strains over time post ITM. This is important in pastoral and agro-pastoral farming systems of Northern Tanzania, where herders continuously move their cattle in search of areas with pasture and water during dry months of the year. Such livestock movements often expose cattle to pastures used by wildlife, including African buffalo, reservoirs of *T. parva*. This situation explains our finding of higher prevalence of *T. parva* in cattle populations sampled closer to buffalo-grazed interface areas.

Genetic diversity between different *T. parva* strains has been studied using various approaches, including polymerase chain reaction (PCR) or PCR-restriction fragment length polymorphism (RFLP) of polymorphic antigen-encoding genes (Geysen et al., 1999; Bishop et al., 2001). A panel of micro- and mini-satellite markers has also been developed (Oura et al., 2003, 2005) that is widely used in the genetic analysis of field populations (Oura et al., 2004, 2007) and has also been used to characterize vaccine stabilates (Patel et al., 2011) and genetic recombination analysis (Katzer et al., 2006, 2010, 2011). In this study, we compared parasite diversity in six different cattle populations using three satellite marker loci. The analysis enabled us to determine the allelic diversity patterns among cattle populations in relation to the Muguga vaccine. Some of the Muguga vaccine-specific alleles were detected in both, vaccinated and unvaccinated cattle, implying that either such alleles are part of the pool of local *T. parva* alleles that circulate in the cattle populations or that some vaccine-specific alleles are potentially transmitted by ticks from vaccinated to unvaccinated cattle. Interestingly, some of the Muguga vaccine component alleles were not detected in all vaccinated cattle, and there were also several non-Muguga vaccine alleles that were detected in vaccinated as well unvaccinated cattle. ECF-vaccinated cattle possessed higher allelic diversity when compared to unvaccinated cattle although majority of the vaccinated cattle carried only some of the alleles from either MS7, ms2 or ms5 marker, and only a few vaccinated cattle (12/103) simultaneously carrying all the three markers. It is not entirely clear as to why some of the vaccine alleles were not detected in all the vaccinated cattle. An appealing reason to this observation may be the apparent tick pick-up of some parasites that can happen on cattle, which are continuously exposed to tick infestation in the field. The apparent non-detection or disappearance of some *T. parva* genotypes in vaccinated cattle may also be the result of active clearance of parasites from blood circulation by the cattle acquired immune response (Asiimwe et al., 2013). Persistence or disappearance of the Muguga vaccine alleles have also been described to differ between individual strains as previously shown by Oura et al., (2004, 2007) and Skilton et al., (2002), whereby one of the vaccine components, Kiambu 5, induces a long term carrier state and the other (Muguga-Serengeti) induces a short term carrier status.

Mean number of alleles, mean number of effective alleles and expected heterozygosity were used to compare *T. parva* genetic diversity among cattle populations. We found higher parasite diversity among vaccinated than unvaccinated cattle. We ascribe our findings to the fact that the Muguga cocktail vaccine contains only a limited portion of the diversity found in field populations (Table 3), however, due to continuous natural tick challenge this diversity may be enhanced through influence of sexual recombination what may eventually lead to wider diversity and hence broader immune protection. This finding only confirms the positive role of ECF vaccination in increasing the diversity of the *T. parva* parasites (Sibeko et al., 2011; Patel et al., 2011). The live Muguga vaccine is a cocktail comprising three heterologous strains each of which potentially contributes to the genetic diversity of the *T. parva* in vaccinated cattle, and hence confers protection broader than expected in natural infections in the field. The genetic relationships established between *T. parva* parasites circulating in different cattle populations were consistent with a high level of variation existing within individual populations than between populations. The principle component analysis further revealed closer clustering patterns of the parasite alleles with Muguga vaccine alleles in two of the quadrants, consistent with closer genetic relatedness between the vaccine strains and the *T. parva* populations from the Maasai steppe.

Geographically separated populations, such as those sampled from Tanga (eastern Tanzania) were expected to carry distinct satellite alleles, separate from those found in cattle from the Maasai steppe (northern Tanzania), however, this was not the case in this study. One possible explanation to this finding is the high level of intermingling of cattle due to free livestock movement practiced in the pastoral management system. Livestock movements inevitably facilitates gene flow of the parasites, and hence low variation between populations.

5. Conclusion

This study has brought forth clear findings that vaccination against ECF has a discernible impact on the diversity of *T. parva* parasites, whereby greater number of alleles was shown in vaccinated compared to unvaccinated cattle. It is expected that under conditions of continuous natural tick exposure in the field the live vaccine strains may recombine with naturally tick-transmitted local strains to generate more genotypes, hence bringing about higher diversity among vaccinated cattle.

The study took into consideration separate ecological and geographical locations. Interestingly, analysis of *T. parva* populations revealed that geographical separation did not necessarily imply differences in the genetic structure of *T. parva* populations. Majority of the cattle investigated in this study were sampled from wildlife interface areas. Such areas support constant interaction between cattle, wildlife reservoirs, tick vectors and the parasites. Ecological pressure in such an interface presumably drive the establishment of a carrier state in cattle differently as it would happen in cattle populations grazed far from wildlife. Therefore, the role of the wildlife interface on the diversity of *T. parva* may not be negated, as highest parasite diversity shown in this study was among ECF vaccinated cattle found in close proximity to wildlife interface.

In summary, the deployment of ITM in Tanzania for over 20 years plays an important role in the protection of cattle from ECF, and the protection is mediated by a carrier state, characterized with wide *T. parva* diversity in the vaccinated cattle.

Conflict of interests

None of the authors (Emelesiana Magulu, Fatuma Kindoro, Elisa Mwega, Sharadhuli Kimera, Gabriel Shirima and Paul Gwakisa) has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the paper entitled “Detection of carrier state and genetic diversity of *Theileria parva* in ECF-vaccinated and naturally exposed cattle in Tanzania”.

Ethical statement

Ethical clearance for the study was obtained from the Research, Publications & Ethics Committee of the College of Veterinary Medicine and Biomedical Sciences of Sokoine University of Agriculture, Tanzania and verbal consent was obtained from livestock owners during data collection.

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Declarations of interest

None.

References

Asiimwe, B.B., Weir, W., Tait, A., Lubega, G.W., Oura, C.A.L., 2013. Haemoparasite infection kinetics and the population structure of *Theileria parva* on a single farm in Uganda. Vet. Parasitol. 193 (1–3), 8–14.

Bishop, R., Geysen, D., Spooner, P., et al., 2001. Molecular and immunological characterisation of *Theileria parva* stocks which are components of the 'Muguga cocktail' used for vaccination against East Coast fever in cattle. Vet. Parasitol. 94, 227–237.

Bishop, R., Sohanpal, B., Kariuki, D.P., Young, A.S., Nene, V., Baylis, H., Allsopp, B.A., Spooner, P.R., Dolan, T.T., Morzaria, S.P., 1992. Detection of a carrier state in Theileria parva-infected cattle by the polymerase chain reaction. Parasitology 104 (Pt 2), 215–232.

Di Giulio, G., Lynen, G., Morzaria, S., Oura, C., Bishop, R., 2009. Live immunization against East Coast fever–current status. Trends Parasitol. 25 (2), 85–92.

Geysen, D., Bishop, R., Skilton, R., Dolan, T.T., Morzaria, S., 1999. Molecular epidemiology of *Theileria parva* in the field. Tropical Med. Int. Health 4, A21–A27.

Hayashida, K., Hara, Y., Abe, T., Yamasaki, C., Toyoda, A., Kosuge, T., others, …., 2012. Comparative genome analysis of three eukaryotic parasites with differing abilities to transform leukocytes reveals key mediators of *Theileria*-induced leukocyte transformation. MBio 3 (5).

Katzer, F., Ngugi, D., Oura, C., et al., 2006. Extensive genotypic diversity in a recombining population of the apicomplexan parasite *Theileria parva*. Infect. Immun. 74, 5456–5464.

Katzer, F., Ngugi, D., Walker, A.R., McKeever, D.J., 2010. Genotypic diversity, a survival strategy for the apicomplexan parasite *Theileria parva*. Vet. Parasitol. 167, 236–243.

Katzer, F., Lizundia, R., Ngugi, D., Blake, D., McKeever, D., 2011. Construction of a genetic map for *Theileria parva*: identification of hotspots of recombination. Int. J. Parasitol. 41, 669–675.

Kazungu, Y.E.M., Mwega, E., Kimera, S.I., Gwakisa, P., 2015a. Seroprevalence and carrier state of *Theileria parva* in cattle under two tick control regimes in small-holder farming systems of Tanzania. Livest. Res. Rural. Dev. 27 (6), 2015.

Kazungu, Y.E.M., Mwega, E., Neselle, M.O., Sallu, R., Kimera, S.I., Gwakisa, P., 2015b. Incremental effect of natural tick challenge on the infection and treatment method-induced immunity against *T. parva* in cattle under agro-pastoral systems in Northern Tanzania. Ticks and Tick-Borne Diseases 6 (5), 587–591.

Kimaro, E.G., Mor, S.M., Gwakisa, P., Toribio, J.-A., 2017. Seasonal occurrence of *Theileria parva* infection and management practices amongst Maasai pastoralist communities in Monduli District, northern Tanzania. Vet. Parasitol. 246, 43–52.

McKeever, D.J., 2007. Live immunisation against Theileria parva: containing or spreading the disease? Trends Parasitol. 23, 565–568.

Odongo, D.O., Sunter, J.D., Kiara, H.K., Skilton, R.A., Bishop, R.P., 2010. A nested PCR assay exhibits enhanced sensitivity for detection of *Theileria parva* infections in bovine blood samples from carrier animals. Parasitol. Res. 106, 357–365.

Olds, C.L., Mason, K.L., Scoles, G.A., 2018. *Rhipicephalus appendiculatus* ticks transmit Theileria parva from persistently infected cattle in the absence of detectable parasitemia: implications for East Coast fever epidemiology. Parasit. Vectors 11 (1), 126.

Oura, C.A.L., Odongo, D.O., Lubega, G.W., Spooner, P.R., Tait, A., Bishop, R.P., 2003. A panel of microsatellite and minisatellite markers for the characterisation of field isolates of *Theileria parva*. Int. J. Parasitol. 33 (14), 1641–1653.

Oura, C.A., Bishop, R., Wampande, E.M., Lubega, G.W., Tait, A., 2004. The persistence of component *Theileria parva* stocks in cattle immunized with the 'Muguga cocktail' live vaccine against East Coast fever in Uganda. Parasitology. 129, 27–42.

Oura, C.A., Asiimwe, B.B., Weir, W., Lubega, G.W., Tait, A., 2005. Population genetic analysis and sub-structuring of *Theileria parva* in Uganda. Mol. Biochem. Parasitol. 140, 229–239.

Oura, C.A.L., Bishop, R., Asiimwe, B.B., Spooner, P., Lubega, G.W., Tait, A., 2007. *Theileria parva* live vaccination: parasite transmission, persistence and heterologous challenge in the field. Parasitology 134 (9), 1205–1213.

Patel, E.H., Lubembe, D.M., Gachanja, J., Mwaura, S., Spooner, P., Toye, P., 2011. Molecular characterization of live *Theileria parva* sporozoite vaccine stabilates reveals extensive genotypic diversity. Vet. Parasitol. 179 (1–3), 62–68.

Peakall, R., Smouse, P.E., 2012. GenAlEx 6.5: genetic analysis in excel. Population genetic software for teaching and researchdan update. Bioinformatics 28, 2537–2539.

Perry, B.D., 2016. The control of East Coast fever of cattle by live parasite vaccination: a science-to-impact narrative. One Health 2, 103–114.

Radley, D.E., Brown, C.G.D., Cunningham, M.P., Kimber, C.D., Musisi, F.L., Payne, R.C., 1975. East coast fever: 3. Chemoprophylactic immunization of cattle using oxytetracycline and a combination of theilerial strains. Vet. Parasitol. 1, 51–60.

Salih, D.A., Mwacharo, J.M., Pelle, R., Njahira, M.N., Odongo, D.O., Mbole-Kariuki, M.N., et al., 2018. Genetic diversity and population structure of *Theileria parva* in South Sudan. Ticks and Tick-Borne Diseases 9 (4), 806–813.

Sibeko, K.P., Collins, N.E., Oosthuizen, M.C., Troskie, M., Potgieter, F.T., Coetzer, J.A.W., Geysen, D., 2011. Analyses of genes encoding *Theileria parva* p104 and polymorphic immunodominant molecule (PIM) reveal evidence of the presence of cattle-type alleles in the south African *T. parva* population. Vet. Parasitol. 181 (2–4), 120–130.

Skilton, R.A., Bishop, R.P., Katende, J.M., Mwaura, S., Morzaria, S.P., 2002. The persistence of *Theileria parva* infection in cattle immunized using two stocks which differ in their ability to induce a carrier state: analysis using a novel blood spot PCR assay. Parasitology 124 (3), 265–276.