Theileria lestoquardi in Sudan is highly diverse and genetically distinct from that in Oman

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Abstract

Malignant ovine theileriosis is a severe tick-borne protozoan disease of sheep and other small ruminants which is widespread in sub-Saharan Africa and the Middle East. The disease is of considerable economic importance in Sudan as the export of livestock provides a major contribution to the gross domestic product of this country. Molecular surveys have demonstrated a high prevalence of sub-clinical infections of *Theileria lestoquardi*, the causative agent, among small ruminants. No information is currently available on the extent of genetic diversity and genetic exchange among parasites in different areas of the country. The present study used a panel of *T. lestoquardi* specific micro- and mini-satellite genetic markers to assess diversity of parasites in Sudan (Africa) and compared it to that of the parasite population in Oman (Asia). A moderate level of genetic diversity was observed among parasites in Sudan, similar to that previously documented among parasites in Oman. However, a higher level of mixed-genotype infection was identified in Sudanese animals compared to Omani animals, consistent with a higher rate of tick transmission. In addition, the *T. lestoquardi* genotypes detected in these two countries form genetically distinct groups. The results of this work highlight the need for analysis of *T. lestoquardi* populations in other endemic areas in the region to inform on novel approaches for controlling malignant theileriosis.

Keywords: *Theileria lestoquardi*, population genetics, Sudan, Oman

1. Introduction

Theileriosis is a tick-borne disease caused by protozoan parasites of the genus *Theileria*. These parasites infect a wide range of domestic and wild animals and are transmitted by ixodid ticks of several genera including *Amblyomma*, *Haemaphysalis*, *Hyalomma* and *Rhipicephalus*. The disease is a threat to the agricultural industry of many countries in sub-Saharan Africa and Asia because of the associated animal mortality, reduction in animal productivity and constraints to livestock improvement (Bishop *et al.*, 2004). Globally, the most economically important species affecting cattle are *T. parva* and *T. annulata*, which induce leukocyte transformation and cause East Coast fever and tropical theileriosis, respectively. *Theileria lestoquardi* is the most pathogenic *Theileria* species infecting sheep, and to a lesser degree goats, causing malignant ovine theileriosis (Muleya *et al.*, 2012).

Malignant ovine theileriosis (MOT) was first described in Egypt in an imported animal from Sudan (Littlewood, 1915), and then reported in Iraq (Latif *et al.*, 1977), India (Sisodia, 1981), Sudan (El Ghali & El Hussein, 1995), Turkey (Sayin *et al.*, 1997), Iran (Spitalska *et al.*, 2005), Saudi Arabia (El-Azazy *et al.*, 2001) and the Sultanate of Oman (Tageldin *et al.*, 2005; Shayan *et al.*, 2011). However, it has not been reported in Jordan (Sherkov *et al.*, 1977) or Israel (Pipano, 1991). Although molecular surveys have confirmed the presence of *T. lestoquardi* in Iran (Ghaemi *et al.*, 2012), Sudan (Salih *et al.*, 2003; El Imam & Taha, 2015) and the Sultanate of Oman (Tageldin *et al.*, 2005), there is a paucity of information on the distribution of this pathogen. In Oman, local sheep breeds are highly susceptible to ovine theileriosis caused by both *T. lestoquardi* and *T. ovis* (Al-Fahdi *et al.*, 2017) with a large proportion of sheep hosting mixed infections with these species along with *T. annulata* (Al-Weheibi, 2011). A recent country-wide molecular survey revealed a high prevalence of *T. lestoquardi* among sheep (22 %) and a low prevalence among goats (0.5 %) (Al-Fahdi *et al.*, 2017).

Theileria spp. that infect livestock are common in central Sudan. The extraordinary climatic and ecological diversity in Sudan, ranging from the desert zone in the north to wooded savannahs in the south together with a wide range of domestic and wildlife hosts, accommodate the biological requirements of a variety of tick species (Elghali & Hassan, 2012). *Amblyomma exornatum, Rhipicephalus sanguineus* and nine *Hyalomma* spp. are common in northern Sudan (Hoogstraal, 1956), with *H. anatolicum* the most prevalent (A Elghali & Hassan, 2012). *Theileria lestoquardi* and *T. annulata* are known to be widespread across the country (Salih *et al.*, 2003). Serological testing, using the schizont antigen indirect fluorescent antibody test, revealed a high *T. lestoquardi* infection rate of 16.3 % among sheep in the main grazing areas in Sudan. These findings were later confirmed by surveys in multiple sites in central and northern areas of the country using reverse line blot (RLB) analysis (El Imam & Taha, 2015). Recent surveys have demonstrated the spread of *T. lestoquardi* into Western Sudan, in areas where MOT has not been reported previously, with a high infection rate among both goats (41 %) and sheep (69 %) (Osman *et al.*, 2017).

Population genetic analysis of *Theileria* parasites can provide important information about the epidemiology of disease and may facilitate the development of rational control approaches. Genomic sequence analysis has allowed the identification of putatively neutral, polymorphic micro- and mini-satellite markers for economically important *Theileria* spp. which has facilitated population genetic analysis of *T. parva* (Oura et al., 2003) and

T. annulata (Weir *et al.*, 2007; Al-Hamidhi *et al.*, 2015; Gomes *et al.*, 2016) in a number of endemic regions. More recently, a set of genetic markers has been established for *T. lestoquardi* and, at present, only one Omani study has applied these markers to examining diversity in field populations of the parasite (Al-Hamidhi *et al.*, 2016). However, a recent study has examined genetic diversity of *T. lestoquardi* in Sudan using micro- and mini-satellites identified in the *T. annulata* genome (Ali *et al.*, 2017). These two species are phylogenetically closely related and, for this reason, a proportion of *T. annulata* markers can also be used to genotype *T. lestoquardi* isolates. However, *T. annulata* markers may not be appropriate for analysis of *T. lestoquardi* in the field due to the frequent occurrence of mixed species infections in sheep involving *T. lestoquardi* and *T. annulata* (Taha *et al.*, 2013; Al-Fahdi *et al.*, 2017). For this reason, the present study employs a panel of *T. lestoquardi*-specific markers. This approach negates any issues of co-infection with related *Theileria* species and can allow a credible estimate of the level of diversity within and divergence between populations (Al-Hamidhi *et al.*, 2016).

The present study used *T. lestoquardi*-specific markers distributed over the four chromosomes for analysis of natural *T. lestoquardi* populations in Sudan, where MOT is a major problem (El Imam & Taha, 2015). The data from Sudan was then compared to that from Oman in order to assess the level of genetic differentiation between *T. lestoquardi* populations in these exemplar African and Asian countries. This provides the first comparative analysis on population structure of *T. lestoquardi* on sites that are widely geographically separated.

2. Materials and Methods

2.1. Study site and parasite samples

One hundred and ninety-one blood samples were collected from apparently healthy sheep over a period of one year in 2013 (n = 81) and in October 2016 (n = 110) in the River Nile state (Aldamer and Atbra Provinces) in northern Sudan (Figure l). The samples were collected from adult sheep; the majority of samples collected in 2016 were from ten sheep flocks grazing sheep (n = 80) while 30 samples were collected from three farms where the livestock were kept indoors. The River Nile state is characterised by a hot desert climate. The annual average rainfall is 60 mm, which mostly falls between July and August, and the average relative humidity is 29 %. Grazing is limited to seasonal watercourses and low-lying areas following sporadic rain showers. *Hyalomma anatolicum* ticks have been detected in the River Nile state, where 74 % of the ticks feeding on sheep were found to be *H. anatolicum* (Salih *et al.*, 2004; Ahmed *et al.*, 2005).

2.2. Identification of *Theileria* species

DNA was extracted using a Qiagen QIAamp DNA mini Kit (Qiagen, Germany) following the manufacturer's instructions and stored at -20 °C. *Theileria* genus-positive samples were identified by polymerase chain reaction (PCR) using pan-*Theileria* primers, targeting the 18S rRNA locus: F 5'-GGCGTTTATTAGACCTAAAACCAAAC-3' and R 5'-TTTGAGCACTCTAATTTTCTCAAAGT-3' (Al-Hamidhi *et al.*, 2016). A total reaction volume of 25 µl included 2 µl of template DNA, 25mM $MgCl_2$, 10 pmol of each primer, 1 U DNA *Taq* polymerase and 10 mM dNTPs. The PCR temperature profile was as follows: denaturation at 95 °C for 5 minutes, 35 cycles at 95 °C for 30 seconds, 58 °C for 30 seconds

and 65 °C for 30 seconds, followed by a final extension step at 65 °C for 5 minutes. The amplified products were then separated on a 2 % ethidium bromide pre-stained agarose gel. To differentiate between the common *Theileria* species in Oman (*Theileria annulata*, *T. lestoquardi* and *T. ovis*), PCR products were subjected to restriction fragment length polymorphism (RFLP) analysis with *HpaII* restriction enzyme (Biolab, UK), as described elsewhere (Al-Hamidhi *et al.*, 2015).

2.3. Micro- and mini-satellite genotyping

A panel of nine polymorphic micro- and mini-satellite markers designed for the genetic analysis of *T. lestoquardi* (Al-Hamidhi *et al.*, 2016) was used to genotype each sample. The forward PCR primer in each marker set was labeled with a fluorescent dye (HEX) at the 5' end. PCR was carried out in a total volume of 25 µl containing 2 µl of template DNA, 1 µl of each primer (10 pmol), 1 U of *Taq* DNA polymerase (Biolab, UK) and 10 mM dNTPs. PCR amplification was carried out at 95 °C for 5 minutes, then 32 cycles of 95 °C for 30 seconds, 42-62 °C for 30 seconds and 65 °C for 30 seconds followed by a final extension step at 65 °C for 5 minutes. PCR products were then denatured at 95 °C for 5 minutes and subjected to capillary electrophoresis on an ABI3130 xl Genetic Analyser (Applied Biosystems, UK). DNA fragment sizes were analysed relative to the ROX-labeled GS500 set of size-standards (Applied Biosystems) using Genemapper software (Applied Biosystems).

Multiple products from a single PCR reaction indicated the presence of a mixture of genotypes. To determine the relative concentration of each allele/amplicon, the area under each peak was measured. In this way, the predominant allele at each locus was identified for each sample and this data was combined to generate a multi-locus genotype (MLG) that represents an estimate of the most abundant genotype in each sample. The MLG data was then used to determine a number of indices including genetic diversity, linkage disequilibrium and population differentiation.

2.4. Genetic analysis

The MLG dataset was compared to a previously generated dataset from *T. lestoquardi* isolates in Oman (Al-Hamidhi *et al.*, 2016) in order to determine the extent of diversity and genetic relatedness among the two populations. Tandem software (Matschiner & Salzburger, 2009) was utilised to facilitate consistent allele-calling and, since most loci were located in exons, a 3 bp repeat unit was used to differentiate alleles. Genetic diversity parameters were calculated for the entire dataset using GenAlex v6.5 (Peakall & Smouse, 2012). This included determining the number of alleles per locus and expected heterozygosity (H_e). These two parameters were used to assess the level of polymorphism at each locus and determine diversity overall and within the sub-populations. Expected heterozygosity was calculated using the formula for 'unbiased heterozygosity' also termed haploid genetic diversity, $H_e = [n/(n-1)][1-\sum p^2]$, where n is the number of isolates and p is the frequency of each different allele at a locus (Anon, 1996).

To determine whether *T. lestoquardi* parasites in Aldamer and Atbra Provinces comprised a single panmictic population with a high degree of genetic exchange, multi-locus linkage disequilibrium (LD) of the alleles at pairs of loci was measured using the standard index of

association (I^S_A). Both I^S_A and variance of data were calculated using the program LIAN version 3.5 (Haubold & Hudson, 2000).

Population differentiation was assessed by estimating Wright's F_{ST} index using the Fstat computer package Version 2.9.3.2. Two estimators of F_{ST} (G'_{ST} and θ) (Cockerham & Weir, 1984; Nei, 1987) were used to estimate genetic differentiation between sub-populations. Principal Co-ordinate analysis (PCoA) was performed using GeneAlex6 in order to visualise the relationships between MLGs. In addition, the partitioning of genetic diversity among and between populations was investigated using analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992).

The software Structure V. 2.3.4 was used to deduce the underlying structure of parasite population and determine how many sub-populations it comprised. This method detects clusters without prior information on the origin of samples. Ten iterations for K = 2 to K = 5 (K being the number of clusters) were run, each with a burn-in period of 10,000 steps and then 20,000 Markov Chain Monte Carlo (MCMC) iterations. In addition, the optimal number of clusters was assessed using the program Structurama (Huelsenbeck *et al.*, 2011).

2.5. Multiplicity of infection

Multiplicity of infection (MOI), defined as the presence of multiple genotypes in an individual, was determined by the presence of more than one allele at a locus in a sample. To avoid over-estimation of low abundance alleles, only minor alleles having a peak height >33 % of the corresponding predominant allele were accepted. This eliminated spurious peaks in the vicinity of major peaks, which if unaccounted for could overestimate the presence of rare alleles thus artificially inflating the calculated MOI. The mean number of alleles across the nine loci in each sample was calculated and this index value was used to represent the MOI within each sample. The overall mean for the index values for each sample was then calculated.

3. Results

3.1. Diversity of micro- and mini-satellites

A total of 191 apparently healthy sheep in Aldamer and Atbra Provinces, Sudan, were screened for the presence of *T. lestoquardi* by PCR-RFLP of the *18S rRNA* locus. *Theileria lestoquardi* was detected in a total of 98 animals (51 %). For samples collected in 2016, a total of 85 of 110 (77.2 %) were found to be positive for *T. lestoquardi*. This comprised 12 positives among 30 animals kept indoors (40 %) and 73 of 80 grazing animals (91.2 %). In 2013, 13 of 81 animals (16 %) were found to be positive for *T. lestoquardi*. Of the 98 *T. lestoquardi* isolates, 90 (13 from 2013 and 77 from 2016) were successfully genotyped using nine species-specific micro- and mini-satellites (supplementary Table 1).

A high level of polymorphism was observed among the ninety *T. lestoquardi* isolates. The total number of alleles in the population sample ranged from 2 for TL_MS25 to 17 for TL_MS13 (Table 1). The number of alleles per locus within a single sample ranged from one to seven (Table 1). Seven alleles were detected in a single sample using the marker TL_MS13, which was the most polymorphic of the loci analysed, possessing 17 defined

alleles across the entire dataset. A high-level of polymorphism was observed among six other loci (TL_MS281, TL_MS280, TL_MS04, TL_MS16, TL_MS07 and TL_MS18) (Figure 2) and a lower level of allelic polymorphism was observed for TL_MS 19 and TL_MS25.

The average heterozygosity among *T. lestoquardi* parasites in the Aldamer and Atbra provinces in Sudan was found to be moderate (H_e = 0.572), ranging from 0.355 to 0.816. This is comparable to that reported for the parasite population in Oman (H_e = 0.582), examined using the same panel of markers (Al-Hamidhi *et al.*, 2016).

3.2. Multiplicity of infection

Eighty-three (92 %) of the 90 Sudanese *T. lestoquardi* isolates analysed carried multiple parasite genotypes. The minimum number of alleles per locus per isolate ranged between 1 and 7 alleles, with average of 3 (SD ±1.3). In contrast, among Omani isolates, the number of alleles per locus ranged between 1 and 6, with an average of 2.6 (SD ±0.9). The overall prevalence of isolates showing a MOI was higher in Sudan (92 %) than in Oman (44 %) (P = 0.0001).

3.3. Linkage disequilibrium

LD was estimated using the standard index of association (I^S_A) to investigate whether the high diversity observed in *T. lestoquardi* in Sudan could be explained by a high rate of recombination, a consequence of sexual reproduction. Linkage analysis was first performed by combining data from samples collected in 2013 and 2016. In addition, linkage analysis was performed for the combined population in Sudan and Oman. When all samples from Sudan were treated as a single population, an I^S_A value of 0.116 and a V_D value (3.812) greater than L (2.171) was obtained, indicating LD (Table 2). Similar results were obtained when each year in Sudan was examined separately (Table 2). The combined Sudanese and Omani dataset showed a slightly higher degree of association between loci than for the Sudan dataset alone (I^S_A = 0.124).

3.4. Population sub-structuring

To measure the level of genetic differentiation within *T. lestoquardi* in Sudan and between Sudanese and Omani populations, F_{ST} values were estimated. Two estimates of F_{ST} (G'_{ST} and θ) (Cockerham & Weir, 1984; Nei, 1987) were calculated. No differentiation was observed between the *T. lestoquardi* population samples collected in Sudan in 2013 and 2016 (G'_{ST} = 0.029 and θ = 0.027). Additionally, no genetic differentiation was detected between *T. lestoquardi* populations in grazing sheep and those kept indoors (F_{ST} = 0.011). However, a high level of differentiation (G'_{ST} = 0.295 and θ = 0.295) was evident between parasite populations in Oman and Sudan.

The above finding was supported by subsequent PCoA analysis. There was no evidence of differential clustering of parasite samples collected in 2013 and 2016 (Figure 3A). However, discrete clustering of parasites from Sudan and Oman was evident, indicating geographical isolation may be associated with genetic isolation (Figure 3B). The amount of molecular variation obtained represented by the first and second axes was 45.03 % and 12.95 %, respectively (Figure 3B), indicating that this diagram is able to illustrate much of the underlying genetic variation in the dataset. Analysis of molecular variance (AMOVA),

suggested that most of the genetic variation, 72 %, was contained within sub-populations with only 28 % explained by differences between sub-populations.

Structure analysis clearly indicated the presence of two discrete clusters representing underlying sub-populations (K = 2). Sudanese MLGs formed one cluster while Omani MLGs formed another (Figure 4).

4. Discussion.

The present study examined the diversity of *T. lestoquardi* using species-specific mini- and micro-satellites and assessed the population structure of the parasites in Sudan, comparing it to that in Oman. This is the first comparative genetic analysis of *T. lestoquardi* parasites in geographically separated sites. The parasites in Sudan and Oman were shown to form genetically distinct groups. However, the extent of diversity among *T. lestoquardi* in Sudan (H_e = 0.572) was similar to that documented among parasites in Oman (H_e = 0.582). Nonetheless, a higher prevalence of MOI was inferred among isolates from Sudan (92 %) compared to those from Oman (44 %).

The presence of a high level of genotypic diversity and a high prevalence of MOI among infected sheep in Aldamer and Atbra provinces, northern Sudan, is indicative of intense transmission. Previous surveys demonstrated high rates of tick infestation in this area, as well as other sites in the country (Osman *et al.*, 2017). A higher rate of tick infestation and transmission may be linked to a significantly higher MOI in Sudan compared to that in Oman. MOI and mean number of genotypes within individual hosts reflect the transmission intensity, abundance of vector (ticks) and the level of host (sheep) infestation (Weir *et al.*, 2011). *Hyalomma anatolicum* is the most abundant vector of *Theileria* spp. both in Sudan (El Hussein *et al.*, 2012) and Oman (Al-Fahdi *et al.*, 2017). A previous study in Sudan reported a high rate of *Theileria* infection in *H. anatolicum,* ranging from 8.6 % to 49.6 % (Salih *et al.*, 2004; Osman *et al.*, 2017), however similar information is not available for Oman. Such a high vector infection rate can result in a high level of transmission and super-infection that can lead to the acquisition of multiple genotypes among vertebrate hosts (Conway *et al.*, 1992). This may explain why MOI was two-fold higher in Sudan than that in Oman, with almost every infected animal harbouring more than one parasite genotype. The high rate of asymptomatic carriage of *T. lestoquardi* among local sheep coupled with the elevated rate of transmission presumably, allow continuous inoculation of new genotypes to local animals and high prevalence of disease. However, it is of some interest that each animal contained only a limited number of genotypes, at least on the days of sampling, with an average of 3 genotypes/infection in Sudan compared to 2.6 genotypes/infection in Oman. This may be attributed to the fact that some host factors could limit acquisition of multiple clones in the vertebrate hosts of apicomplexan parasites, such as age, due to mounting immunity resulting from increased exposure to infection (Weir *et al.*, 2011; Pacheco *et al.*, 2016) and breed/ethnic group (Pacheco *et al.*, 2016). Acquired immunity and prevalence of MOI are age structured in malaria endemic areas, suggesting that acquired immunity may influence the risk of subsequent infection (Smith *et al.*, 1999). Additional factors that can limit MOI in infected sheep may include within-host competitive interaction between multiple genotypes (Huijben *et al.*, 2011) or presence of some genotypes below the PCR detection threshold. Similar observations of limited MOI in a single infection have been reported for the malaria parasite

P. falciparum in countries with high malaria transmission (Ntoumi *et al.*, 1995; Smith *et al.*, 1999). However, MOI has been found to be a reliable indicator for success of control efforts, and a significant reduction can reflect a reduction in the rate of transmission and superinfection locally (Nkhoma *et al.*, 2013). Therefore, control efforts that cut transmission can reduce rates of MOI in *Theileria* endemic areas and the likelihood of emergence of novel recombinant genotypes that can spread the disease to less immune competent animals.

The moderate diversity among *T. lestoquardi* reported in the present study in Sudan, and that previously reported in Oman (Al-Hamidhi *et al.*, 2016), was lower than that observed in *T. annulata* in different endemic sites, such as Portugal (Gomes *et al.*, 2016), Turkey and Tunisia (Weir *et al.*, 2011). However, it is important to note that the set of markers used to define *T. annulata* genotypes is different from those used in *T. lestoquardi* and so the results at each locus are not directly comparable. Nevertheless, given that each set of markers was selected from their respective genome using very similar criteria, they may be taken as representative sub-sets of highly polymorphic markers from each species. Compared to *T. annulata* in Tunisia and Turkey, lower genetic diversity was found in *T. lestoquardi* in both Sudan ($P < 0.01$) and Oman ($P < 0.001$) (Student's T test). Even in the same region where the two species co-exist, *T. annulata* showed higher diversity than *T. lestoquardi* (Al-Hamidhi *et al.*, 2016). For example, in two areas of Oman where *T. lestoquardi* isolates were collected from small ruminants and *T. annulata* was collected from cattle living in the same farms, lower diversity was noted in *T. lestoquardi* ($P < 0.01$ (Batinah) and $P < 0.05$ (Sharqia), Student's T test) (Al-Hamidhi *et al.*, 2016). The generally lower diversity of *T. lestoquardi* compared to that seen in *T. annulata*, supports the hypothesis that *T. lestoquardi* has evolved from an ancestral *T. annulata*, and that parasite speciation occurred as the parasite adapted to the small ruminant host (Schnittger *et al.*, 2000). This hypothesis is consistent with the high identity at the 18S rRNA locus between *T. lestoquardi* and *T. annulata* (Schnittger *et al.*, 2003). The feature that stands out in the present study in both *T. lestoquardi* populations in Africa and Asia is the lower genetic diversity compared to *T. annulata* (Al-Hamidhi *et al.*, 2016). Comparative genomic analysis, using genome sequence data of *T. lestoquardi*, will shed more light on this hypothesis (Professor Arnab Pain, King Abdullah University of Science & Technology, Thuwal, Kingdom of Saudi Arabia; Personal communication;).

Despite the high rate of infection and MOI observed among *T. lestoquardi* isolates in Sudan, a significant association between genetically unlinked loci was observed, implying LD. This LD is not explained by sub-structuring between parasites isolated in 2013 and 2016, as both PCoA and F_{ST} analysis showed no evidence of between-year population variation. It is possible that factors such as influx of new genotypes (Wahlund effect) or an epidemic population structure may cause the observed LD. A lower level of LD has been observed within *T. lestoquardi* in some regions in Oman, even at the level of a single farm (Al-Hamidhi *et al.*, 2016). Similar findings have been observed among *T. annulata* in Tunisia, Turkey (Weir *et al.*, 2011) and Portugal (Gomes *et al.*, 2016). Recombination, as measured by genetic linkage, in the Portuguese *T. annulata* population is undoubtedly frequent and is at a broadly similar level to that occurring in Tunisia. Although animals are kept exclusively on farms and are not free-roaming, it is known that there are movements of cattle from farm to farm particularly in neighboring regions (Baptista & Nunes, 2007). Our data suggests that sheep movement is insufficient to homogenise the present parasite population and that geographical

sub-structuring is maintained at the country level. The parasite population is essentially panmictic with only a minor disturbance showed by the low levels of I^S_A and only a limited level of LD.

Very low levels of genetic differentiation were detected between *T. lestoquardi* parasites collected in 2013 and 2016 in Sudan, with a pair-wise F_{ST} value of 0.029. However, a high level of genetic differentiation was measured between *T. lestoquardi* parasites in Sudan and Oman, with a F_{ST} value of 0.295. This is consistent with similar findings of genetic differentiation in populations of other *Theileria* species such as *T. annulata* (Gomes *et al.*, 2016) and *T. parva* (Muwanika *et al.*, 2016), separated by geographical barriers. A higher level of differentiation has been documented between *T. annulata* in Oman and Turkey (F_{ST} = 0.102) than Oman and Tunisia (F_{ST} = 0.063) (Al-Hamidhi *et al.*, 2015). One hypothesis to explain this finding would be more frequent demographic exchange between livestock populations in Oman and Tunisia than Oman and Turkey (Al-Hamidhi *et al.*, 2015). The differentiation between populations in widely separated countries is consistent with geographical and trade barriers hindering gene flow. Nonetheless, there was some overlap between parasites in Sudan and Oman, and complete separation between the parasite populations in the two countries was not observed (Figure 3). Similarly, incomplete separation has been observed in *T. annulata* from widely separated countries (Weir *et al.*, 2007; Al-Hamidhi *et al.*, 2015). This suggests that despite ecological and epidemiological barriers between the parasite populations, there are likely to have been some gene flow between them, and this may be related to movement of animals since tick migration can be ruled out in absence of host movement. Alternatively, the two populations might have not been separated for enough evolutionary time for them to drift apart.

In summary, a moderate level of genetic diversity was observed in the *T. lestoquardi* population in sheep in the Aldamer and Atbra provinces in Sudan. There was a high level of MOI in infected animals and this indicates a large parasite reservoir and gene pool. The lower extent of MOI observed among *T. lestoquardi* in Oman compared to Sudan can be attributed to differences in epidemiology in each country which may be related to the abundance and dynamics of the tick vector.

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Figure 1. *Theileria lestoquardi* sampling sites in River Nile state, Sudan

Figure 2 (A & B). The frequency of alleles, as a percentage (%), is shown for two microsatellites, TL_MS07 and TL_MS13, in Sudanese and Omani parasite populations. The size of each allele (in bp) is given on the x- axis.

Figure 3 (A & B): Principal Co-ordinate analysis (PCoA) of *T. lestoquardi* genotypes from Sudan and Oman. The amount of variation in the dataset represented by each axis is shown as a percentage.

Figure 4: 'Structure' analysis of *T. lestoquardi* genotypes from Sudan and Oman. The presence of two sub-populations was inferred (K = 2).

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