Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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

Genetic diversity and population structure of *Theileria parva* in South Sudan

Diaeldin A. Salih[a,f,*,3], Joram M. Mwacharo[b,1], Roger Pelle[a], Moses N. Njahira[a,2], David O. Odongo[c,d], Mary N. Mbole-Kariuki[c], Wani L. Marcellino[e], Agol K. Malak[e], Henary Kiara[c], Abdel Rahim M. El Hussein[f], Richard P. Bishop[c], Robert A. Skilton[a,2]

[a] *Biosciences Eastern and Central Africa-International Livestock Research Institute Hub (BecA-ILRI Hub), P.O. Box 30709-00100, Nairobi, Kenya*
[b] *School of Life Sciences, Centre for Genetics and Genomics, University of Nottingham, Nottingham, NG7 2RD, UK*
[c] *International Livestock Research Institute (ILRI), P.O. Box 30709-00100, Nairobi, Kenya, Kenya*
[d] *School of Biological Sciences, The University of Nairobi, P.O. Box 30197, Nairobi, 00100, Kenya*
[e] *Ministry of Animal Resources and Fisheries, P.O. Box 126, Juba, South Sudan*
[f] *Central Veterinary Research Laboratory, P.O. Box 8067, Khartoum, Sudan*

ARTICLE INFO

Keywords:
Theileria parva
ECF
South Sudan
Genotyping

ABSTRACT

Theileria parva is a parasitic protozoan that causes East Coast fever (ECF), an economically important disease of cattle in eastern, central and southern Africa. In South Sudan, ECF is considered a major constraint for livestock development in regions where the disease is endemic. To obtain insights into the dynamics of *T. parva* in South Sudan, population genetic analysis was performed. Out of the 751 samples included in this study, 178 blood samples were positive for *T. parva* by species-specific PCR, were collected from cattle from four regions in South Sudan (Bor = 62; Juba = 45; Kajo keji = 41 and Yei = 30) were genotyped using 14 microsatellite markers spanning the four chromosomes. The *T. parva* Muguga strain was included in the study as a reference. Linkage disequilibrium was evident when populations from the four regions were treated as a single entity, but, when populations were analyzed separately, linkage disequilibrium was observed in Bor, Juba and Kajo keji. Juba region had a higher multiplicity of infection than the other three regions. Principal components analysis revealed a degree of sub-structure between isolates from each region, suggesting that populations are partially distinct, with genetic exchange and gene flow being limited between parasites in the four geographically separated populations studied. Panmixia was observed within individual populations. Overall *T. parva* population genetic analyses of four populations in South Sudan exhibited a low level of genetic exchange between the populations, but a high level of genetic diversity within each population.

1. Introduction

Theileria parva is a tick-transmitted protozoan parasite that causes East Coast fever (ECF), an economically devastating disease of cattle in eastern, central and southern Africa (Mukhebi et al., 1992). After entering the host during tick feeding, the parasite rapidly invades and transforms host leukocytes. This stage, the schizont, is responsible for most of the disease pathology. After a period of division in synchrony with the host leukocytes, merozoites are produced and released from the infected leukocyte. Free merozoites invade erythrocytes and develop into piroplasms, which are infective for the tick vector, *Rhipicephalus appendiculatus* (Dobbelaere and Heussler, 1999). The *Theileria* parasite undergoes haploid asexual replication in the vertebrate host following a diploid sexual phase in the tick vector (Gauer et al., 1995).

Most communities in South Sudan comprise small-scale mixed livestock-crop farmers and/or pastoralists, and mainly depend on livestock production for their livelihood. Due to civil war in the country, there has been a drastic change in animal husbandry practices and consequently an increase in the occurrence of various animal diseases including ECF. Following the signing of the Comprehensive Peace Agreement (CPA) in January 2005, there has been extensive movement of people and their livestock, with concomitant reports of ECF spreading to additional areas further north of the country, that were previously ECF free (Marcellino et al., 2017; Nyoap et al., 2015). ECF is ranked high in terms of its impact on the livelihood of resource poor farming community in South Sudan (Marcellino et al., 2011). It is

* Corresponding author at: Biosciences Eastern and Central Africa-International Livestock Research Institute Hub (BecA-ILRI Hub), P.O. Box 30709-00100, Nairobi, Kenya.
E-mail address: diaeldin2000@hotmail.com (D.A. Salih).
[1] Current address: International Centre for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 5689 Addis Ababa, Ethiopia.
[2] Current address: International Centre of Insect Physiology and Ecology (icipe), P.O. Box 30772-00100, Nairobi, Kenya.
[3] Current address: Central Veterinary Research Laboratory, Khartoum, Sudan.

https://doi.org/10.1016/j.ttbdis.2018.03.002
Received 1 June 2017; Received in revised form 28 February 2018; Accepted 1 March 2018
1877-959X/ © 2018 Elsevier GmbH. All rights reserved.

considered to be a major constraint for the development of livestock in regions where the disease is endemic. Management of ECF is primarily through control of the tick vector using acaricides, although this is unsustainable in the medium term, due to increasing acaricide resistance and food safety concerns (George et al., 2004). A highly effective 'infection and treatment' live vaccine based on the injection of sporozoites together with a dose of long acting tetracycline has long been available for control of ECF, but this has not yet been deployed in South Sudan (Di Giulio et al., 2009). There are concerns that the introduction of foreign parasites through vaccination to a region might result in parasites with novel genotypes and potentially more virulent phenotypes (De Deken et al., 2007). Therefore, prior to the deployment of a live vaccine in South Sudan, there is an urgent need to characterize the *T. parva* genotypes circulating in the region.

Previous studies on the molecular diversity in *T. parva* were based on application of a panel of monoclonal antibodies, directed against a single immunodominant polymorphic antigen (Minami et al., 1983). Later, this method was combined with detection of restriction fragment length polymorphisms in genomic DNA using probes derived from multi-copy gene families (Bishop et al., 1993). A panel of micro- and minisatellites markers derived by systematic screening of the *T. parva* genome sequence was developed more recently (Oura et al., 2003), and this has greatly enhanced the power and utility of molecular analyses. The use of these markers confirmed earlier observations indicating that the molecular diversity of *T. parva* in East Africa is high. Analysis with 12 micro- and minisatellites markers revealed 84 multilocus genotypes (MLGs) in blood samples from three geographical localities in Uganda (Oura et al., 2005), and a total of 183 alleles were observed at 30 micro- and minisatellites loci from 20 Kenyan tissue culture isolates (Odongo et al., 2006). Infection of a single animal with multiple *T. parva* genotypes appears to be a common occurrence (Oura et al., 2007). Recently, however, the analysis of 14 micro- and minisatellites markers revealed a low level of genetic diversity in *T. parva* from cattle populations in Tanzania (Mwega et al., 2014; Rukambile et al., 2016).

The objective of the present study was twofold: first, to provide a comprehensive understanding of *T. parva* genetic diversity in cattle using microsatellite markers, which can inform rational implementation of future control strategies; and second, to provide baseline data on the population genetic structure that will provide insights into the origin of *T. parva* present in South Sudan and enable monitoring of live vaccine deployment and possible future spread to adjacent regions.

2. Materials and methods

2.1. Ethics statement

The study reported here was carried out in strict accordance with the recommendations in the

standard operating procedures of the ILRI IACUC (The ILRI's Institutional Animal Care and Use Committee). We confirm that the studies that the samples were initially collected for received specific approval from ILRI IACUC.

2.2. Bovine blood samples

A total of 751 blood samples spotted on FTA™ cards (Whatman Biosciences, UK) were collected from cattle from four regions in South Sudan, Yei (n = 89), Kajo Keji (n = 78) and Juba (n = 167), in Central Equatoria State (CES), and Bor (n = 417), in Jonglei State (JS) (Fig. 1). Detailed information on the sampling locations, date of collection and references are provided in Table S1.

2.3. DNA extraction and nested p104 PCR

DNA was extracted from blood spotted on FTA cards using the PureLink™ Genomic DNA Mini extraction kit (Invitrogen, Germany) following the manufacturer's protocol. A nested p104 PCR assay (Odongo et al., 2010) was used to confirm that blood samples were infected with *T. parva*.

2.4. Micro- and mini-satellite PCR assay

The inner and outer nested primers used in this study were as described by Oura et al. (2003, 2005), except the external primers for ms5 and MS8, which were designed in this study. Details of the primers are provided in Table S2. The selected 11 minisatellite (MS) and three microsatellite (ms) markers are highly polymorphic and distributed over the four chromosomes of *T. parva* genome. The primers were synthesized by Bioneer, South Korea.

The primary PCR amplification was carried out in a 10 µl reaction volume consisting of 0.04 U/µl of Dream Taq polymerase (Promega, USA), 1 × PCR buffer (10 mM Tris–HCl [pH 8.4], 50 mM KCl, 2 mM $MgCl_2$), 0.2 mM of each dNTP, 0.2 µM of each forward and reverse primers, and 20 ng genomic DNA. The nuclease free water was used as negative control. Genomic DNA extracted from a schizont-infected lymphocyte culture derived from *T. parva* Muguga (Radley et al., 1975), which is a component of the live vaccine, was used as a positive control.

The PCR was performed in a Gene Amp PCR System 9700 (Applied Biosystems-USA). 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 30s, primer annealing at 60 °C for 1 min, and primer extension at 72 °C for 1 min, and then a final extension step at 72 °C for 10 min. For the secondary PCR, all reagents and the reaction volume was the same except that 0.5 µl of the primary PCR product was used as the template. The cycling profile for the secondary PCR was as follows: initial denaturation at 95 °C for 5 min, followed by 25 cycles of denaturation at 94 °C for 30s, primer annealing at 58 °C for 1 min, primer extension at 72 °C for 1 min, and then a final extension step at 72 °C for 20 min.

2.5. Genotyping and capillary electrophoresis

The PCR amplicons were analysed on a ABI 3730 Genetic Analyzer (Applied Biosystems-USA) at the SegoliP sequencing unit of the BecA-ILRI Hub, Nairobi, Kenya. The Gene Scan 500 LIZ internal lane size standard (Applied Biosystems-USA) was used for size fractionation. Scoring was carried out using the Gene Mapper programme (Applied Biosystems-USA) which allowed for the resolution of 1 base pair (bp) differences with multiple products from a single PCR reaction. Amplicons with maximum peak height were scored, and the predominant allele was defined as that with the largest area under the curve. All data generated from Gene Mapper were re-sized by the Allelobin software (Idury and Cardon, 1997) based on consensus sequence repeats of each marker (Table S2). Two types of files were generated: the first named multi locus genotype (MLG) comprised genotypes constructed from only the predominant allele at each locus. The second file, the allelic profile dataset, comprised genotypic profiles constructed from all alleles identified at each locus (when minor peaks were > 33% the height of the predominant allele present). The MLG file was used to assess population genetic diversity and structure, while the allelic profile file was used to assess the multiplicity of infection (MOI), as well as to test the null hypothesis of linkage equilibrium.

2.6. Population genetic analysis

The MLG dataset was used to derive population genetic indices specifically expected heterozygosity and parameters relating to population differentiation. Since *Theileria* is haploid and heterozygosity cannot be observed directly, the expected heterozygosity was calculated using Arlequin v. 3.5 http://cmpg.unibe.ch/software/arlequin35/ (Excoffier and Lischer 2010).

Based on the genetic distance (GD) calculated in GenAlEx6.5

Download English Version:

https://daneshyari.com/en/article/8507149

Download Persian Version:

https://daneshyari.com/article/8507149

Daneshyari.com