Ticks and Tick-borne Diseases xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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

Investigating the diversity of the 18S SSU rRNA hyper-variable region of *Theileria* in cattle and Cape buffalo (*Syncerus caffer*) from southern Africa using a next generation sequencing approach

Ben J. Mans[a,b,c,*,1], Ronel Pienaar[a,1], John Ratabane[a], Boitumelo Pule[a], Abdalla A. Latif[a,d]

[a] *Parasites, Vectors and Vector-Borne Diseases, Agricultural Research Council-Onderstepoort Veterinary Institute, Onderstepoort 0110, South Africa*
[b] *The Department of Veterinary Tropical Diseases, University of Pretoria, Pretoria, South Africa*
[c] *Department of Life and Consumer Sciences, University of South Africa, South Africa*
[d] *School of Life Sciences, University of Kwa Zulu-Natal, South Africa*

ARTICLE INFO

Article history:
Received 7 January 2016
Received in revised form 8 April 2016
Accepted 8 April 2016
Available online xxx

Keywords:
18S SSU
Diversity
Next-generation sequencing
Species
Theileria
Theileria parva

ABSTRACT

Molecular classification and systematics of the *Theileria* is based on the analysis of the 18S rRNA gene. Reverse line blot or conventional sequencing approaches have disadvantages in the study of 18S rRNA diversity and a next-generation 454 sequencing approach was investigated. The 18S rRNA gene was amplified using RLB primers coupled to 96 unique sequence identifiers (MIDs). *Theileria* positive samples from African buffalo (672) and cattle (480) from southern Africa were combined in batches of 96 and sequenced using the GS Junior 454 sequencer to produce 825711 informative sequences. Sequences were extracted based on MIDs and analysed to identify *Theileria* genotypes. Genotypes observed in buffalo and cattle were confirmed in the current study, while no new genotypes were discovered. Genotypes showed specific geographic distributions, most probably linked with vector distributions. Host specificity of buffalo and cattle specific genotypes were confirmed and prevalence data as well as relative parasitemia trends indicate preference for different hosts. Mixed infections are common with African buffalo carrying more genotypes compared to cattle. Associative or exclusion co-infection profiles were observed between genotypes that may have implications for speciation and systematics: specifically that more *Theileria* species may exist in cattle and buffalo than currently recognized. Analysis of primers used for *Theileria parva* diagnostics indicate that no new genotypes will be amplified by the current primer sets confirming their specificity. *T. parva* SNP variants that occur in the 18S rRNA hypervariable region were confirmed. A next generation sequencing approach is useful in obtaining comprehensive knowledge regarding 18S rRNA diversity and prevalence for the *Theileria*, allowing for the assessment of systematics and diagnostic assays based on the 18S gene.

1. Introduction

The *Babesia* and *Theileria* are part of the phylum Apicomplexa (Order Piroplasmorida) (Adl et al., 2012). The *Theileria* are distinguished by their life cycle, where tick-transmitted sporozoites infect host leukocytes to form schizonts, which mature to merozoites that infect red blood cells to establish the piroplasm carrier stage (Uilenberg, 2006). Parasite morphology, host cell specificity, schizonts and piroplasm parasitemia levels, clinical disease presentation, serological and molecular methods, host and vector specificity have been used for classification and systematic purposes (Mans et al., 2015). Classification and diagnostics of the *Theileria* is important since they are the causative agents for a number of debilitating diseases in domestic and wild animals (Bishop et al., 2004), such as for example, *Theileria parva*, the causative agent of East Coast fever, Corridor disease and January disease in East and southern Africa.

A significant proportion of the systematic study of the Piroplasmorida comprise analysis of the 18S rRNA gene (Allsopp et al., 1994; Altay et al., 2007; Bhoora et al., 2009; Chae et al., 1999; Chansiri et al., 1999; Criado et al., 2006; Criado-Fornelio et al., 2004; Reichard et al., 2005). The reverse line blot (RLB) method (Gubbels et al., 1999), based on simultaneous detection of the 18S rRNA gene

* Corresponding author at: Parasites, Vectors and Vector-Borne Diseases, Agricultural Research Council-Onderstepoort Veterinary Institute, Onderstepoort 0110, South Africa.
E-mail address: mansb@arc.agric.za (B.J. Mans).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.ttbdis.2016.04.005
1877-959X/

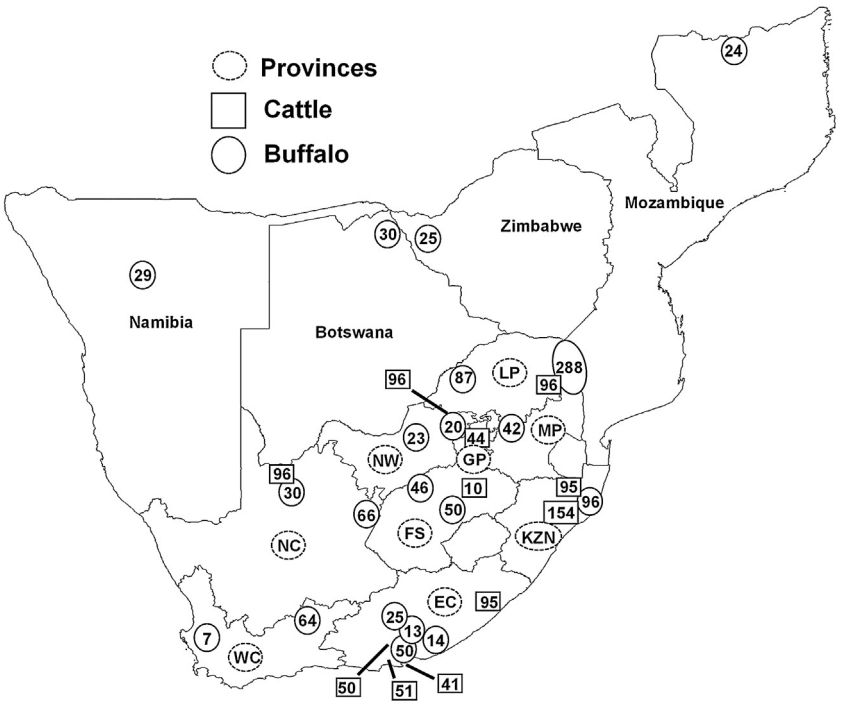

Fig. 1. Sampling sites for cattle and buffalo samples used in study. The total number of animals sampled per site are indicated in circles (buffalo) or rectangles (cattle). Provinces in South Africa are indicated in dotted circles and include Western Cape (WC), Eastern Cape (EC), Northern Cape (NC), Free State (FS), Kwa-Zulu Natal (KZN), North-West (NW), Gauteng (GP), Limpopo (LP) and Mpumalanga (MP). Refer to Table S1 to identify specific localities indicated in the map as well as numbers of positive animals per site.

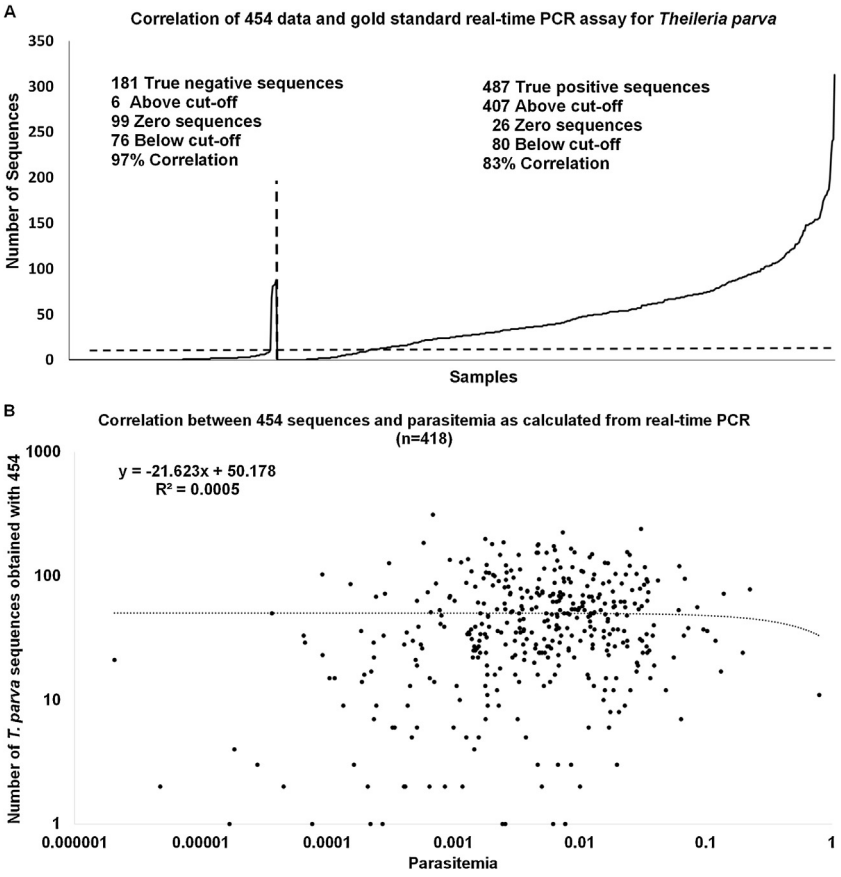

Fig. 2. Comparison of the 454 data and the hybridization PCR assay for *T. parva*. (A) Indicated are the expected true negative and true positive samples as determined with the hybridization PCR assay and the number of samples above or below the determined cut-off threshold (10 sequences) for the 454 data. The horizontal dashed line indicate the cut-off threshold and the vertical dashed line indicate the demarcation between true negative and positive samples. (B) Comparison of parasitemia and number of sequences obtained using 454 sequencing. Indicated are the R^2 value for the linear regression fit.

Please cite this article in press as: Mans, B.J., et al., Investigating the diversity of the 18S SSU rRNA hyper-variable region of *Theileria* in cattle and Cape buffalo (*Syncerus caffer*) from southern Africa using a next generation sequencing approach. Ticks Tick-borne Dis. (2016), http://dx.doi.org/10.1016/j.ttbdis.2016.04.005