The *Rhipicephalus appendiculatus* tick vector of *Theileria parva* is absent from cape buffalo (*Syncerus caffer*) populations and associated ecosystems in northern Uganda

I. Obara 1 · N. Githaka 2 · A. Nijhof 1 · J. Krücken 1 · A. Nanteza 3 · D. Odongo 4 · D. Lubembe 4 · P. Atinmedi 5 · D. Mijele 6 · A. Njeri 2 · S. Mwaura 2 · G. Owido 2 · J. Ahmed 1 · P. H. Clausen 1 · R. P. Bishop 7

Received: 5 December 2019 / Accepted: 25 May 2020 / Published online: 4 June 2020
© The Author(s) 2020

**Abstract**

*Rhipicephalus appendiculatus* is the major tick vector of *Theileria parva*, an apicomplexan protozoan parasite that causes the most economically important and lethal disease of cattle in East and central Africa. The African cape buffalo (*Syncerus caffer*) is the major wildlife host of *T. parva* from southern Uganda and Kenya to southern Africa. We show herein that *R. appendiculatus* appears to be absent from the two largest national parks in northern Uganda. *Syncerus caffer* is common in both of these national parks, specifically Murchison Falls (MFNP) and Kidepo Valley (KVNP). We re-confirmed the previously reported absence of *T. parva* in buffalo sampled in the two northern parks based on RLB data using a nested PCR based on the *T. parva* p104 gene. By contrast, *T. parva*-infected *R. appendiculatus* ticks and parasite-infected buffalo were present in Lake Mburo (LMNP) in South central Uganda. This suggests that the distribution of *R. appendiculatus*, which is predicted to include the higher rainfall regions of northern Uganda, may be limited by additional, as yet unknown factors.

**Keywords** *T. parva* · East Coast fever · *R. appendiculatus* · Uganda · Cape buffalo

**Introduction**

The distribution of ixodid tick species, including *Rhipicephalus appendiculatus*, is believed to be determined primarily by climatic factors, combined with the availability of permissive mammalian hosts on which nymphal and adult ticks can feed to repletion. The distribution of arthropod vectors and wildlife hosts can have serious implications for disease epidemiology and the effectiveness of measures to control livestock pathogens. For example at the wildlife-livestock disease interface, transmission of *Theileria parva* to cattle involves the African cape buffalo (*Syncerus caffer*), the major mammalian wildlife host, that is asymptomatic when infected with *T. parva* by the ixodid tick *R. appendiculatus* (Norval et al. 1992; Young et al. 1973). Buffalo-derived *T. parva* induces a distinct clinical syndrome in cattle when compared to cattle transmissible parasites, characterised by low levels of schizont parasitosis and piroplasm parasitaemia, when transmitted to susceptible animals by *R. appendiculatus* (Norval et al. 1992).

Despite practical issues constraining production and delivery (reviewed by Di Giulio et al. 2009), an infection and treatment (ITM) vaccination procedure is currently the only practical means of providing immunity against *T. parva* in cattle. A large-scale production process for a specific incarnation of ITM known as the Muguga cocktail (Radley et al. 1975) has been refined over the past 20 years (reviewed by Patel et al. 2016). Despite repeated demonstration of efficacy in the
a dissected tick salivary gland was stained in Schiff’s reagent following fixation, and infection rates were estimated by counting the number of sporozoites in 70 female and 70 male ticks. Genomic DNA was extracted from the remaining salivary gland sub-sample using a DNaseasy Blood & Tissue kit (QIAGEN). For detection of parasite DNA in buffalo, blood samples were collected from the jugular vein of immobilized buffalo and transferred into heparinized tubes for subsequent DNA isolation. Venous blood was also collected from pastoralist Ankole cattle grazing adjacent to buffalo in LMNP at the same time as the buffalo were sampled, and in transhumant Zebu cattle populations from two locations close to KVNP in September 2017. DNA was extracted from heparinized venous blood using the Qiagen kit. In both tick and blood DNA, T. parva genomic DNA was detected using nested primers targeting the gene encoding the T. parva p104 antigen (Odongo et al. 2010).

Results and discussion

The distribution of ixodid ticks and T. parva infection in buffalo was analysed in MFNP and KVNP in northern Uganda, together with LMNP in South central Uganda. Tick sampling was undertaken in August 2016 (representing the wet season in northern Uganda and a dry season in Southern central Uganda) and again in January 2017 (representing a dry season in both southern and northern Uganda), and the batches of ticks collected at these sampling points were analysed independently. The following tick species were collected directly from buffalo or by dragging grasslands in which buffalo grazed in MFNP: Rhipicephalus evertsi (n = 116), Rhipicephalus pravus (n = 7), Amblyomma variegatum (n = 123), Amblyomma gemma (n = 63) and species within the genus Hyalomma (n = 89). The tick species collected from buffalo in KVNP, were Rhipicephalus evertsi (n = 6), Rhipicephalus pravus (n = 13), Amblyomma variegatum (n = 2) and Amblyomma lepidum (n = 4). However, the major tick vector of T. parva, R. appendiculatus, was absent from both parks. By contrast, among the ticks collected from LMNP in January 2017, the commonest species was R. appendiculatus (n = 479), although Amblyomma cohaerens (East Africa buffalo tick n = 31), R. evertsi (n = 3), R. pravus (24) and Hyalomma species (n = 13) were also confirmed as being present. It is noteworthy that R. appendiculatus and T. parva were absent from both MFNP and KVNP during the August wet season in northern Uganda, when tick numbers and parasite transmission intensity would be expected to be at their peak. While detection of both R. appendiculatus and T. parva in LMNP was during a relatively dry period when tick numbers would not be expected to be at their maximum, the R. appendiculatus morphology-based species identification was confirmed by mitochondrial COI sequence-
based genotyping (GenBank MN756033-MN756039). Of the six different COI haplotypes identified, five had previously been described in the Great Lakes region (Amzati et al. 2018) and one was novel. A total of 27 of the 140 adult *R. appendiculatus* ticks examined following salivary gland dissection were found to contain *T. parva*-infected acini, and this was confirmed by p104 PCR (data not shown). The identification of other tick species was by morphology only and not confirmed by COI gene analysis.

A nested p104-based PCR was applied to DNA extracted from buffalo blood samples (Odongo et al. 2010). No evidence of *T. parva* infection was detected using the nested PCR assay on buffalo-derived parasite samples from MFNP and KVNP in northern Uganda; however, 45% of the LMNP buffalo were positive. The values of prevalence of *T. parva* in LMNP suggested by the p104 PCR data were lower than those observed in the earlier RLB study in which all animals were infected (Oura et al. 2011). The reasons for this discrepancy are unclear and could be attributable to differences in methodology (use of p104 PCR relative to RLB), sample processing and preservation, seasonality of sampling or a combination of these. It may also be the case that since the Lake Mburo buffalo in this study were sampled in a low tick challenge season, the levels of parasite, which are known to fluctuate in carrier animals, were below the detection threshold. However, in the context of the current study, the key result

---

**Fig. 1** Map showing the sampling sites. The three national parks are indicated with red dots and the cattle sampling sites adjacent to the parks depicted as green dots.
was that the absence of *T. parva* from buffalo in MFNP and KVNPs and presence in LMNPs correlated with the distribution of the *R. appendiculatus* tick vector. Among 65 Zebu (*Bos indicus*) cattle sampled from two farms 20–30 km from KVNP, 9% (*n* = 6) were PCR positive for *T. parva*. Among 100 Ankole cattle from farms directly adjacent to LMNP, 56% were positive by p104 PCR. Further north in the Sudan, *T. parva* has been observed to be endemic in cattle, and the presence of the parasite and the vector in this country is thought to be at least partially mediated by extensive anthropogenic movement of livestock (Malak et al. 2012; Marcellino et al. 2015; Salih et al. 2007). In this context, it will be important to examine the *T. parva* infection status of cape buffalo in South Sudan, since ITM vaccination may in future be deployed in this region, and it is possible that buffalo-derived *T. parva* could breakthrough immunity induced in cattle as observed in Kenya. We hypothesise that the infected carrier cattle from the villages close to KVNP that were detected using p104 PCR were imported from the neighbouring Equatoria state in Sudan, rather than infected locally, given the absence of the *R. appendiculatus* vector. There is no evidence that these cattle had ever been associated with buffalo in KVNP.

This distribution of the *R. appendiculatus* vector is surprising since Murchison Falls and Kidepo Valley are both sufficiently humid and well vegetated, and hence climatically and ecologically suitable, for *R. appendiculatus* (Perry et al. 1990; Norval et al. 1992). *Rhipicephalus appendiculatus* ticks can be found up to 8000 ft above sea level in areas with an annual rainfall of over 500 mm (Norval et al. 1992). It is also possible that the current limits of *R. appendiculatus* distribution may have a biotic component, in addition to climate and vegetation, perhaps a result of predation pressure, or competition with a closely related species of tick. It is also important to note that *R. appendiculatus* ticks are under-represented in historical tick collections of wildlife in Uganda (Matthysse and Colbo 1987). The factors determining the distribution of *R. appendiculatus* merit further investigation, particularly since *T. parva* and *R. appendiculatus* are absent from West Africa (Norval et al. 1992) and most of central Africa (Silatsa et al. 2019), although buffalo occur throughout the region.

**Funding information** Open Access funding provided by Projekt DEAL. The work was supported by Deutsche Forschungsgemeinschaft (DFG) project number CL166/4-2.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** Sampling of buffalo employed standard procedures approved by the Uganda wildlife authority (UWA) and was performed by UWA staff. Cattle were sampled according to standard operating procedures of the Uganda Ministry of Agriculture, Livestock and Fisheries. The rabbits used for induction of sporogony in *T. parva*-infected ticks at ILRI Nairobi were handled according to a regularly implemented protocol approved by the institutional animal care and use committee (IACUC) which adheres to internationally recognised standards.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

**References**

Amzati GS, Pelle R, Muhigwa JB, Kanduma EG, Djioken A, Madder M, Kirschvink N, Marcusot T (2018) Mitochondrial phylogeography and population structure of the cattle tick *Rhipicephalus appendiculatus* in the African Great Lakes region. Parasit Vectors 11(1):329. https://doi.org/10.1186/s13071-018-2904-7

Bishop RP, Hemming JD, Morrison WJ, Weir W, Toye PG, Sitt T, Spooner PR, Musoke AJ, Skilton RA, Odongo DO (2015) The African buffalo parasite *Theileria sp.* (buffalo) can infect and immuno-mortalize leukocytes and encodes divergent orthologues of *Theileria parva* antigen genes. *International journal for parasitology*. Parasites and wildlife 4(3):333–342. https://doi.org/10.1016/j.ijppaw.2015.08.006

Birol G, Lynen G, Morzaria S, Oura C, Bishop R (2009) Live immunization against East Coast fever—status. Trends Parasitol 25(2): 85–92. https://doi.org/10.1016/j.pt.2008.11.007

Folmer O, Black M, Lutz R, Vrijenhock R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3(5): 294–299

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28(12):1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Leta S, De Clercq EM, Madder M (2013) High-resolution predictive mapping for *Rhipicephalus appendiculatus* (Acari: Ixodidae) in the Horn of Africa. Exp Appl Acarol 60(4):531–542

Malak AK, Mpoke L, Banak J, Muriuki S, Skilton RA, Odongo D, Sunter J, Kiara H (2012) Prevalence of livestock diseases and their impact on livelihoods in Central Equatoria State, Southern Sudan. Prev Vet Med 104(3–4):216–223. https://doi.org/10.1016/j.prevetmed.2011

Marcellino WL, Salih DA, Najera MN, Ndawa N, Araba A, El Hussein AM, Seitzer U, Ahmed JS, Bishop RP, Skilton RA (2015) The emergence of *Theileria parvus* in Jonglei State, South Sudan: confirmation using molecular and serological diagnostic tools. Transbound Emerg Dis 62(4):1229–1235. https://doi.org/10.1111/tbed.12495

Matthysse JG, Colbo MH (1987) The ixodid ticks of Uganda. Entomological Society of America, Maryland

Norval RAI, Perry BD, Young AS (1992) The epidemiology of *Theileria* in Africa. Academic, London
Nsubuga-Mutaka R (1999) ECF Immunisation in Uganda. In: Morzaria S, Williamson S (eds) Live vaccines for Theileria parva: deployment in Eastern, Central and Southern Africa. ILRI (International Livestock Research Institute), pp 26–29

Odongo DO, Sunter JD, Kiara HK, Skilton RA, Bishop RP (2010) A nested PCR assay exhibits enhanced sensitivity for detection of Theileria parva infections in bovine blood samples from carrier animals. Parasitol Res 106(2):357–365. https://doi.org/10.1007/s00436-009-1670-z

Oura CA, Tait A, Asiimwe B, Lubega GW, Weir W (2011) Haemoparasite prevalence and Theileria parva strain diversity in Cape buffalo (Syncerus caffer) in Uganda. Vet Parasitol 175(3–4):212–219. https://doi.org/10.1016/j.vetpar.2010.10.032

Patel E, Mwaura S, Kiara H, Morzaria S, Peters A, Toye P (2016) Production and dose determination of the Infection and Treatment Method (ITM) Muguga cocktail vaccine used to control East Coast fever in cattle.Ticks and Tick-Borne Diseases 7(2):306–314. https://doi.org/10.1016/j.tgbdis.2015.11.006

Perry BD, Lessard P, Norval RA, Kundert K, Kruska R (1990) Climate, vegetation and the distribution of Rhipicephalus appendiculatus in Africa. Parasitol Today 6(4):100–104

Radley DE, Brown CGD, Cunningham MP, Kirimi IM, Purnell RE, Young AS (1975) East coast fever: 3. Chemoprophylactic immunization of cattle using oxytetracycline and a combination of Theileria strains. Vet Parasitol 1:51–60

Salih DA, El Hussein AM, Seitzer U, Ahmed JS (2007) Epidemiological studies on tick-borne diseases of cattle in Central Equatoria State, Southern Sudan. Parasitol Res 101(4):1035–1044

Silatsa BA, Simo G, Githaka N, Mwaura S, Kamga RM, Oumarou F, Keambou C, Bishop RP, Dijkstra A, Kuiate JR, Njiokou F, Pelle R (2019) A comprehensive survey of the prevalence and spatial distribution of ticks infesting cattle in different agro-ecological zones of Cameroon. Parasit Vectors 12(1):489

Sitt T, Poole EJ, Ndambuki G, Mwaura S, Njoroge T, Omondi GP, Mutinda M, Mathenge J, Prettejohn G, Morrison WI, Toye P (2015) Exposure of vaccinated and naive cattle to natural challenge from buffalo-derived Theileria parva. Int J Parasitol Parasites Wildl 4(2):244–251

Walker AR, Bouattour A, Camicas JL, Estrada-Peña A, Horak IG, Latif AA, Pegram RG (2003) Ticks of domestic animals in Africa:a guide to identification of species. Bioscience Reports, Edinburgh

Young AS, Branagan D, Brown CG, Burridge MJ, Cunningham MP, Purnell RE (1973) Preliminary observations on a theilerial species pathogenic to cattle isolated from buffalo (Syncerus caffer) in Tanzania. Br Vet J 129(4):382–389

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.