Experimental and Applied Acarology (2018) 76:243–248
https://doi.org/10.1007/s10493-018-0311-6

In search of the vector(s) of *Babesia rossi* in Nigeria: molecular detection of *B. rossi* DNA in *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae) ticks collected from dogs, circumstantial evidence worth exploring

Joshua Kamani[1] · Ping-Jun Chung[2] · Chung-Chan Lee[2] · Yang-Tsung Chung[2]

Received: 29 June 2018 / Accepted: 2 October 2018 / Published online: 8 October 2018

Abstract
The brown dog tick *Rhipicephalus sanguineus* (sensu lato) (Acari: Ixodidae) has a cosmopolitan distribution, is a proven vector of a host of pathogens with emerging evidence incriminating it in the transmission of some others. Specifically it is reputed as the main vector of *Babesia vogeli* whereas the southern African yellow dog tick *Haemaphysalis elliptica*, long considered to be *H. leachi*, is apparently the only proven vector of *B. rossi*, since the resurrection of the separate species *H. elliptica* as a member of the *leachi*-group by Apanaskevich et al. However, recent epidemiological surveys conducted in Nigeria show higher prevalence of *B. rossi* than *B. vogeli* infection in dogs most of whom were infested with *R. sanguineus* and rarely with ticks of the *H. leachi* group. The discrepancy between tick distribution and *Babesia* spp. prevalent in dogs stimulated us to investigate the possible role of *R. sanguineus* (s.l.) in the natural transmission of *B. rossi*. Out of a total of 66 tick samples identified morphologically and molecularly as *R. sanguineus* collected from dogs manifesting clinical signs of tick-borne diseases, eight (12%) were positive in nested PCR for *Babesia* sp. DNA. Sequencing results for these amplified products showed that all of the 18S rDNA sequences (693 bp) were identical to each other, and bore 99.3–99.9% identities with those from other *B. rossi* isolates accessible in GenBank. None of the ticks harbored the DNA of *B. vogeli* or *B. canis*. The possible implications for the detection of *B. rossi* DNA in *R. sanguineus* (s.l.) ticks collected from dogs in the epidemiology of *B. rossi* infection of dogs in Nigeria is highlighted.

Keywords *Babesia rossi* · Vector · *Rhipicephalus sanguineus* (s.l.) · *Haemaphysalis elliptica* · Dog · Nigeria · PCR

✉ Joshua Kamani
mshelizakj@gmail.com

[1] Parasitology Division, National Veterinary Research Institute, PMB 01, Vom, Plateau State, Nigeria

[2] Department of Veterinary Medicine, College of Veterinary Medicine, National Chung Hsing University, Taichung, Taiwan

Introduction

Babesia canis (sensu lato) is a tick-borne hemoprotozoan parasite that induces anemia, fever, jaundice, hemoglobinuria, and sometimes fatal symptoms in dogs (Boozer and Macintire 2003). The pathogen belongs to the Piroplasmidae and there are three described subspecies, *B. canis canis*, *B. canis vogeli* and *B. canis rossi* (Uilenberg et al. 1989). They are each transmitted by a different tick species, *Dermacentor reticulatus*, *Rhipicephalus sanguineus* (s.l.) and *Haemaphysalis elliptica* (previously lumped with *Haemaphysalis leachi*), respectively (Uilenberg 2006; Apanaskevich et al. 2007). There are also differences in the geographical distribution, antigenicity and pathogenicity to dogs of each subspecies (Schetters et al. 1997). *Babesia canis* (sensu stricto) is found in the Palaearctic region coinciding with the distribution of its vector tick *D. reticulatus*, *B. vogeli* has a global distribution similar to that of its vector *R. sanguineus* (s.l.) throughout tropical, subtropical and Mediterranean areas, and *B. rossi* is thought to be restricted to sub-Saharan Africa (Irwin 2009; Solano-Gallego and Baneth 2011; Jongejan et al. 2018). Genotyping has confirmed the existence of three separate species (Zahler et al. 1998; Carret et al. 1999), *B. canis*, *B. vogeli* and *B. rossi* (Allsopp and Allsopp 2006; Solano-Gallego and Baneth 2011). *B. rossi* is known to be the most pathogenic of the three species and causes the most severe disease manifestations in canine hosts (Jacobson 2006; Penzhorn 2011).

Canine babesiosis has a high prevalence in Nigeria with molecular studies confirming the presences of all the three species (Sasaki et al. 2007; Kamani et al. 2010, 2013, Adamu et al. 2014). These studies reported higher prevalence of *B. rossi* (2–38%) than *B. vogeli* (0.3–1%). Curiously, several studies on the ectoparasites of dogs in Nigeria have reported *R. sanguineus,* the vector of *B. vogeli*, as the predominant tick species infesting dogs and not *H. elliptica*, the vector of *B. rossi* (Kamani et al. 2013; Adamu et al. 2014). In fact, some studies did not find *Haemaphysalis* spp. ticks on the dogs examined but found them positive for *Babesia* spp. (Okoli et al. 2006; Adamu et al. 2012; Opara et al. 2017). These findings raise the question as to whether *R. sanguineus* is involved in the natural transmission of *B. rossi* in dogs as well. Recently *H. hystricis* was incriminated as a novel vector for *B. gibsoni* in Taiwan by first amplifying the DNA of the pathogen in the tick followed by the demonstration of its transovarian passage to the tick progenies (Jongejan et al. 2018). Therefore, the aim of this study was to detect and characterize the DNA of *B. rossi* in *R. sanguineus* ticks removed from dogs as a preliminary step in the evaluation of its possible involvement in the epidemiology of this infection in Nigeria.

Materials and methods

A total of 66 ticks were collected from 31 dogs manifesting clinical signs of tick-borne diseases that were referred to veterinary hospitals in Jos, the capital city of Plateau State of Nigeria, from January to June 2011. The living ticks were immersed in 70% ethanol and kept at − 20 °C until processing. Morphological identification was done using a binocular compound microscope and standard taxonomic keys for adult ticks (Walker et al. 2003). After identification, the DNA was isolated from individual ticks by using the QIAamp DNA Miniprep kit (Qiagen) according to the manufacturer's instructions.

The oligonucleotide primers used for amplification and sequencing of the 18S ribosomal RNA gene (rDNA) of canine babesial species were designed using primer design

software (Primer Select, DNASTAR, Madison, WI, USA) and related sequence information available in the GenBank database: *B. rossi* (accession numbers L19079 and DQ111760), *B. vogeli* (HQ148663 and AY072925), *B. canis* (AY072926), and *Babesia gibsoni* (JQ910685). The primers B18S-F1 (5′-CGGTGAAACTGCGAATGGCT-3′) and B18S-R1 (5′-TAACCAGCGCTAGTTAGCAGG-3′), B18S-F2 (5′-ATTACCCAATCCCGACACGGG-3′) and B18S-R2 (5′-TGTCTGGACCTGGTGAGTTTC-3′) were used respectively, in a nested PCR for the first and second amplifications targeting the 693 bp of the 18S ribosomal DNA (18S rDNA) gene of *Babesia* spp.

For the confirmation of the morphological identification of ticks, primers TQ16S-1F (5′-CTGCTCAATGATTTTTTAAATTGCTGTGG-3′) and TQ16S-2R (5′-ACGCTGTTATCCCTAGAG-3′) were used for the amplification of the 320-bp of the tick mitochondrial 16S rDNA (Black and Piesman 1994; Nava et al. 2012).

Polymerase chain reaction (PCR) was performed in a total volume of 25 µl containing 2 µl of extracted DNA, 0.2 mM deoxynucleoside triphosphates (dNTPs), 0.625 U Hotstartaq DNA Polymerase (Qiagen), and 12.5 pmol of each primer in the reaction buffer provided by the manufacturer (Qiagen). The reaction conditions were one cycle of 5 min at 95 °C, 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 90 s at 72 °C each, followed by an extension step for 5 min at 72 °C.

The resulting PCR products were electrophoresed on a 1.2% agarose gel stained with ethidium bromide to check the size of amplified fragments by comparison to a DNA molecular weight marker (1 kb Plus DNA Ladder, Promega). In each case, the single amplified product of the expected size was column purified using the QIAquick PCR Purification Kit (Qiagen) and directly sequenced by using an ABI PRISM 3730 capillary sequencer (Applied Biosystems) and the Dye Terminator Cycler Sequencing Kit (Applied Biosystems). The representative nucleotide sequence for the partial region of the 18S rDNA of *B. rossi* was deposited in GenBank under accession number JN982342. The partial sequences of mitochondrial 16S rRNA gene from ticks were deposited in GenBank under accession numbers from JN982352 to JN982359.

The BLAST program (http://www.ncbi.nlm.nih.gov/BLAST) was used for the comparison and the analysis of sequence data obtained in this study with those previously deposited in GenBank.

Results

A total of 66 ticks were collected and all of them were morphologically determined as *R. sanguineus* adults. All tick samples were subjected to DNA extraction followed by a nested PCR amplification with the 18S rDNA-gene. Among 66 samples examined, 8 (12.1%) were positive for *Babesia* spp. Sequencing results for those amplicons showed that all 18S rDNA sequences of 693 bp were identical to each other and bore 99.3–99.9% identities with the corresponding sequences of *B. rossi* from South Africa (GenBank Accession No. L19079), eastern Sudan (DQ111760), and Nigeria (AB303071-75), 95.4% identity with that of *B. canis* (AY072926), and 94.8% identity with that of *B. vogeli* (AY072925).

To verify the previous morphological identification of the ticks from which DNAs were derived at molecular level, those *B. rossi*-positive DNA samples were further analyzed with a PCR using species-specific primers based on the tick mitochondrial 16S rDNA. BLAST analyses of the resulting sequences of PCR amplicons (320 bp) from all samples confirmed their affiliation of *R. sanguineus* with 98.2–99.1% identities with *R. sanguineus* from USA

(AF081829) (Black and Roehrdanz 1998; Nava et al. 2012). In contrast, these sequences bore only 83.1–83.6% similarities with that of *H. elliptica* (HM068958).

Discussion

Babesia parasites are naturally transmitted only by ticks, and in the case of canine babesiosis there is very strong vector specificity (Uilenberg 2006). As a consequence, the prevalence of babesiosis is dependent on the presence of the tick vector in the environment. For a long time it was widely accepted that *H. elliptica* (previously regarded as synonymous with *H. leachi*), the southern African yellow dog tick, is the only tick species that can transmit *B. rossi* to dogs (Schetters et al. 2009; Schoeman 2009; Penzhorn 2011; Matijatko et al. 2012). In surveys conducted in Nigeria, however, the prevalence of 2–38% was observed for *B. rossi* compared to 0.3–1.0% *B. vogeli* but the predominant tick species recovered from the infected dogs was *R. sanguineus*, and *Haemaphysalis* spp. ticks (*H. elliptica or H. leachi*) was rarely detected (Sasaki et al. 2007; Kamani et al. 2013; Adamu et al. 2014). Similarly, several other studies attest to the cosmopolitan occurrence of *R. sanguineus* and the rarity of *Haemaphysalis* spp. on dogs in Nigeria (Okoli et al. 2006; Adamu et al. 2012; Aquino et al. 2016; Opara et al. 2017). The disparity therefore between the high prevalence of *B. rossi* infection of dogs and the rarity of its acclaimed *Haemaphysalis* spp. vector in Nigeria may be a pointer to the possible involvement of other vector(s) in the epidemiology of this disease. Therefore, the detection of *B. rossi* DNA in *R. sanguineus* in this study coupled with increasing reports incriminating it in the transmission of more pathogens prompt us to probe the possible role of this catholic tick in the epidemiology of *B. rossi* in Nigeria.

The tick *R. sanguineus* accounts for the 10% of 867 known tick species implicated to transmit different pathogens (Jongejan and Uilenberg 2004). They are known vectors of pathogens such as *B. vogeli*, *B. gibsoni*, *Ehrlichia canis*, *Anaplasma platys*, *Hepatozoon canis*, *Mycoplasma haemocanis*, and *Rickettsia conorii* in domestic and wild animals in spite of their relatively low anthropophily (Palmas et al. 2001; Dantas-Torres 2008). In the present study, *B. rossi* DNA was detected in 12% of *R. sanguineus* ticks collected from dogs presenting clinical signs compatible with tick-borne disease. Ordinarily, the detection of pathogen DNA in an engorged tick removed from a host should not be used as a proof of vector competence. It could be that the tick ingests the pathogen from its host. But this may not always be the case as shown in an earlier study where 258 ticks remove from dogs in Nigeria were screened for various pathogens. None of the ticks examined was positive for *Babesia* spp. DNA even though their hosts were infected with *Babesia* spp. and DNA of other pathogens, *Ehrlichia*, *Hepatozoon*, *Anaplasma* and *Rickettsia* spp. were detected in them (Kamani et al. 2013). Therefore, the finding in the present study deserves some consideration under the prevailing scenario where the disease is prevalent but its acclaimed vector is rare. Although the absence of evidence is not evidence of absence, the fact remains that ticks of the *H. leachi*-group are rarely found on or are absent from dogs in Nigeria. Thus the detection of *B. rossi* DNA in *R. sanguineus* (s.l.) in the present study should be considered as a strong circumstantial evidence suggestive of the possible role of this tick in the epidemiology of *B. rossi* in Nigeria. More so, *Babesia* spp. could be transmitted transstadially or transovarially (Uilenberg 2006; Chauvin et al. 2009).

Considering that *R. sanguineus* is the most widespread arthropod tick throughout the tropics and subtropics because of its specialized feeding on domestic dogs (Walker et al. 2003), we thought that the finding present herein deserve further investigations to elucidate

the epidemiological implications on *B. rossi* infections in dogs globally. Currently *B. rossi* is mainly confined to sub-Saharan Africa (Solano-Gallego and Baneth 2011) with few sporadic cases occurring in areas outside the African continent (Fritz 2010; Allison et al. 2011; Chomel 2011). Therefore, the circumstantial evidence from this report highlights a potential risk for transmission of *B. rossi* to dogs living in other geographical areas in the world via the spread of the infested *R. sanguineus* ticks. Further epidemiological surveillance and controls are necessary to prevent such a possibility. If possible, *B. rossi* should be included in the routine diagnosis of dogs with compatible clinical signs and hematological abnormalities in those countries, especially in regions with suitable environmental conditions supporting the presence of *R. sanguineus* ticks.

In conclusion, this study confirms the presence of *B. rossi* DNA in *R. sanguineus* ticks in Nigeria where *B. rossi* infection is prevalent, but the acclaimed vector tick, *H. elliptica* is rarely reported. We therefore ask, is this tick involved in the transmission of *B. rossi* to dogs in Nigeria? Based on prevailing evidences, the scientific community is inclined to answer negatively. However, there is the need to provide an evidence based data before accepting or dismissing the idea. In the interim, while experimental transmission studies might not be feasible, the use of molecular methods to determine the transovarial passage of *B. rossi* in *R. sanguineus* will be the next step in answering this question.

Acknowledgements We are grateful to the staff, Parasitology Division, National Veterinary Research Institute, Vom for technical support. This work was financially supported in part by the Taiwanese government through the research grant to National Chung Hsing University (NCHU-CC98116).

References

Adamu NB, Adamu JY, Salisu L (2012) Prevalence of ecto-, endo- and haemoparasites in slaughtered dogsin Maiduguri, Nigeria. Rev Méd Vét 163:178–182

Adamu M, Troskie M, Oshadu DO, Malatji DP, Penzhorn BL, Matjila PT (2014) Occurrence of tick-transmitted pathogens in dogs in Jos, Plateau State, Nigeria. Parasite Vectors 7:119

Allison RW, Yeagley TJ, Levis K, Reichard MV (2011) *Babesia canis rossi* infection in a Texas dog. Vet Clin Pathol 40:345–350

Allsopp MT, Allsopp BA (2006) Molecular sequence evidence for the reclassification of some *Babesia* species. Ann N Y Acad Sci 1081:509–517

Apanaskevich DA, Horak IG, Camicas JL (2007) Redescription of *Haemaphysalis* (*Rhipistoma*) *elliptica* (Koch, 1844), an old taxon of the *Haemaphysalis* (*Rhipistoma*) *leachi* group from East and southern Africa, and of *Haemaphysalis* (*Rhipistoma*) *leachi* (Audouin, 1826) (Ixodida, Ixodidae). Onderstepoort J Vet Res 74:181–208

Aquino LC, Kamani J, Haruna AM, Paludo GR, Hicks CA, Helps CR, Tasker S (2016) Analysis of risk factors and prevalence of haemoplasma infection in dogs. Vet Parasitol 221:111–117

Black WC 4th, Piesman J (1994) Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. Proc Natl Acad Sci USA 91:10034–10038

Black WC 4th, Roehrdanz RL (1998) Mitochondrial gene order is not conserved in arthropods: prostriate and metastriate tick mitochondrial genomes. Mol Biol Evol 15:1772–1785

Boozer AL, Macintire DK (2003) Canine babesiosis. Vet Clin North Am Small Anim Pract 33:885–904

Carret C, Walas F, Carcy B, Grande N, Précigout E, Moubri K, Schetters TP, Gorenflot A (1999) *Babesia canis canis*, *Babesia canis vogeli*, *Babesia canis rossi*: differentiation of the three subspecies by a restriction fragment length polymorphism analysis on amplified small subunit ribosomal RNA genes. J Eukaryot Microbiol 46:298–303

Chauvin A, Moreau E, Bonnet S, Plantard O, Malandrin L (2009) *Babesia* and its hosts: adaptation to long-lasting interactions as a way to achieve efficient transmission. Vet Res 40:37

Chomel B (2011) Tick-borne infections in dogs: an emerging infectious threat. Vet Parasitol 179:294–301

Dantas-Torres F (2008) The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): from taxonomy to control. Vet Parasitol 152:173–185

Fritz D (2010) A PCR study of piroplasms in 166 dogs and 111 horses in France (March 2006 to March 2008). Parasitol Res 106:1339–1342

Irwin PJ (2009) Canine babesiosis: from molecular taxonomy to control. Parasite Vectors 2(Suppl. 1):S4

Jacobson LS (2006) The South African form of severe and complicated canine babesiosis: clinical advances 1994–2004. Vet Parasitol 138:126–139

Jongejan F, Uilenberg G (2004) The global importance of ticks. Parasitology 129:S3–S14

Jongejan F, Su B-L, Yang H-J, Berger L, Bevers J, Liu P-C, Fang J-C, Cheng Y-W, Kraakman C, Plaxton N (2018) Molecular evidence for the transovarial passage of *Babesia gibsoni* in *Haemaphysalis hystricis* (Acari: Ixodidae) ticks from Taiwan: a novel vector for canine babesiosis. Parasite Vectors 11:134. https://doi.org/10.1186/s13071-018-2722-y

Kamani J, Sannusi A, Dogo GI, Tanko TJ, Egwu OK, Agbadu ET, Ogo NI, Sarah K, Onovoh E, Shamaki D, Lombin LH, Catto V, Birkenheuer AJ (2010) *Babesia canis* and *Babesia rossi* co-infection in an untraveled Nigerian dog. Vet Parasitol 173:334–335

Kamani J, Baneth G, Mumcuoglu KY, Waziri NE, Eyal O, Guthmann Y, Harrus S (2013) Molecular detection and characterization of tick-borne pathogens in dogs and ticks from Nigeria. PLoS Negl Trop Dis 7:e2108. https://doi.org/10.1371/journal.pntd.0002108

Matijatko V, Torti M, Schetters TP (2012) Canine babesiosis in Europe: how many diseases? Trends Parasitol 28:99–105

Nava S, Mastropaolo M, Venzal JM, Mangold AJ, Guglielmone AA (2012) Mitochondrial DNA analysis of *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae) in the Southern Cone of South America. Vet Parasitol 190:547–555

Okoli IC, Okoli CG, Opara M (2006) Environmental and multi-host Infestation of the brown dog tick, *Rhipicephalus sanguineus* in Owerri, South-East Nigeria. Vet Arh 76(1):93–100

Opara MN, Adewumi TS, Mohammed BR, Obeta SS, Simon MK, Jegede OC, Agbede RIS (2017) Investigations on the haemoprotozoan parasites of Nigerian local breed of dogs in Gwagwalada Federal Capital Territory (FCT) Nigeria. Res J Parasitol 12:1–7

Palmas C, Bortoletti G, Conchedda M, Contini C, Gabriele F, Ecca AR (2001) Study on immunobiology in ectoparasites of public health interest: *Rhipicephalus sanguineus*. Parassitologia 43(Suppl. 1):29–35

Penzhorn BL (2011) Why is Southern African canine babesiosis so virulent? An evolutionary perspective. Parasite Vectors 5:51–56

Sasaki M, Omobowale O, Tozuka M, Ohta K, Matsuu A, Nottidge HO, Hirata H, Ikadai H, Oyamada T (2007) Molecular survey of *Babesia canis* in dogs in Nigeria. J Vet Med Sci 69:1191–1193

Schetters TP, Moubri K, Précigout E, Kleuskens J, Scholtes NC, Gorenflot A (1997) Different *Babesia canis* isolates, different diseases. Parasitology 115:485–493

Schetters TP, Moubri K, Cooke BM (2009) Comparison of *Babesia rossi* and *Babesia canis* isolates with emphasis on effects of vaccination with soluble parasite antigens: a review. J S Afr Vet Assoc 80:75–78

Schoeman JP (2009) Canine babesiosis. Onderstepoort J Vet Res 76:59–66

Solano-Gallego L, Baneth G (2011) Babesiosis in dogs and cats: expanding parasitological and clinical spectra. Vet Parasitol 181:48–60

Uilenberg G (2006) Babesia: a historical overview. Vet Parasitol 138:3–10

Uilenberg G, Franssen FF, Perié NM, Spanjer AA (1989) Three groups of *Babesia canis* distinguished and a proposal for nomenclature. Vet Q 11:33–40

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

Zahler M, Schein E, Rinder H, Gothe R (1998) Characteristic genotypes discriminate between *Babesia canis* isolates of differing vector specificity and pathogenicity to dogs. Parasitol Res 84:544–548