



## Short communication

*Babesia canis* and *Babesia rossi* co-infection in an untraveled Nigerian dog

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## ABSTRACT

A sexually intact 6-month-old female Alsatian dog was presented to the Veterinary Clinic of the National Veterinary Research Institute, Vom, Plateau State, Nigeria, for the following complaints: anorexia, hemoglobinuria, fever, tick infestation and general malaise. Microscopy revealed piroplasms with a wide range of sizes (1–5 µm in length) in red blood cells, raising a suspicion of a co-infection with two or more *Babesia* species. Specific PCR assays for canine *Babesia* spp. and DNA sequencing revealed the presence of *Babesia canis* and *Babesia rossi* co-infection. This study constitutes the first report of co-infection with *B. canis* and *B. rossi* in the West African sub-region and the first report of autochthonous *B. canis* on the African continent. Practitioners should be aware of potential changes in the species/sub-species of *Babesia* causing canine babesiosis in this region.

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## 1. Case report

A sexually intact 6-month-old female Alsatian dog was presented to the Veterinary Clinic of the National Veterinary Research Institute, Vom, Plateau State, Nigeria for the following complaints; anorexia, hemoglobinuria, fever (39.6 °C), tick infestation and general malaise. A complete blood count revealed a regenerative anemia (packed cell volume (PCV) was 28%, reference range: 39–58%) with 522,000 reticulocytes/µl (reference range: <60,000/µl) a marked thrombocytopenia ( $22 \times 10^3$  platelets/µl, reference range:  $190\text{--}468 \times 10^3$  platelets/µl) and a neutrophilic leukocytosis with a regenerative left shift (Segmented neutrophils  $17.4 \times 10^3$ /µl, band neutrophils  $2.8 \times 10^3$ /µl, immature granulocytes  $2.3 \times 10^3$ /µl). Microscopy revealed

numerous piroplasms (9–14% parasitemia) with a wide range of sizes (1–5 µm in length), raising a suspicion of a co-infection with two or more *Babesia* species. There was anisocytosis, macrocytosis and polychromasia consistent with a regenerative anemia and there were only mild toxic changes in the white blood cells. Serum biochemical profile and urinalysis were not performed.

An engorged female *Rhipicephalus sanguineus* tick was removed and a hemolymph smear was positive for *Babesia* kinetes. Treatment was instituted using diminazene aceturate (Berenil, Intervet: 5 mg/kg subcutaneously). The dog was bathed using diazinon at recommended dose which is a standard topical acaricide treatment used in this region. A whole blood sample was sent to North Carolina State University for molecular testing.

Total DNA was extracted and partial 18S rRNA genes (amplicon of ≈1500 base pairs) were amplified as previously described (Birkenheuer et al., 2003). Direct sequencing of this amplicon was performed (MCLab, San Francisco, CA, USA), but a detailed analysis of the

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chromatogram revealed the presence of more than one sequence (i.e. multiple peaks at each position). Species-specific PCR reactions targeting *B. vogeli*, *Babesia canis*, *Babesia rossi* and *B. gibsoni* were then performed as previously described (Birkenheuer et al., 2003). *B. canis* and *B. rossi* reactions were positive and no amplicons were detected for *B. vogeli* or *B. gibsoni*. Amplicons were sequenced directly in both directions and found to be identical to *B. canis* and *B. rossi*, respectively.

Two weeks after treatment, the dog's PCV was 43% and no piroplasms were detected by microscopy. Two months after the initial follow up the PCV was 38% and piroplasms were seen. It is not clear whether or not the piroplasms detected during the dog's final visit were due to a treatment failure or re-infection. Unfortunately samples from that visit were not available for molecular characterization.

Diagnosis of canine babesiosis in the West African sub-region is mainly based on microscopic examination of Giemsa stained blood smears. In our practice canine babesiosis is common and most cases have been presumed to be due to either *B. canis* or *B. gibsoni* infection based on microscopic examination, but co-infection is rarely suspected or detected. One molecular survey only found *B. rossi* and *B. vogeli* in Nigeria (Sasaki et al., 2007). In the present case we assumed the dog was infected with both *B. canis* and small *B. gibsoni* based on organism size and morphology. The molecular identification of *B. canis* and *B. rossi* co-infection highlights the difficulty and limitations of light microscopy for the accurate identification of piroplasms.

Co-infection with multiple *Babesia* spp. has only been rarely reported. Co-infection with *B. rossi* and *B. vogeli* and triple infection with *B. rossi*, *B. vogeli* and *Ehrlichia canis* has been reported in South African dogs (Matjila et al., 2008). In some scenarios co-infection with two or more vector borne diseases result in worse disease. The subjective impression was that this case was not "atypical" and there was not any appreciable difference in the severity of disease. The accurate identification of co-infections is important because each *Babesia* species/sub-species carries a different prognosis and may require a different treatment. Matjila et al. (2004) reported that *B. rossi* is the most prevalent canine piroplasm in South Africa and concur with earlier suggestion of Uilenberg et al. (1989) that *B. canis vogeli* parasites may be present in large parts of tropical and sub tropical region, but *B. canis* infections have not been reported or suspected in Africa. The source of the *B. canis* infection in this case remains unclear. *Dermacentor reticulatus* is believed to be the primary tick vector for *B. canis* in Europe although there is some evidence that *R. sanguineus* may also be a competent vector for *B. canis* (Hauschild and Schein, 1996; Lori et al., 2010). The dog did not have a history of travel to Europe and *Dermacentor* sp. ticks were not detected during physical examination. The dog's dam was from Jos, Nige-

ria which is just north of Vom. The littermates were sold to other individuals and were not available for testing. The dog in this report was kept in a wooden kennel during the day and allowed to roam during the night as a security dog and the immediate environment is generally overgrown with grasses. Though situated in the tropical zone, a higher altitude means that Plateau State has a near temperate climate with an average temperature of between 18 and 22 °C. It has an average height of 1200 m (4000 ft) above sea level and average annual rainfall of 1400 mm (56").

*Dermacentor* spp. have been reported in the Northern region of the African continent, but not in Nigeria (<http://wwwold.icttd.nl/php/search.php>). The presence of *B. canis* on the African continent has clinical implications for practitioners. While *B. canis* is not considered to be as virulent as *B. rossi* it is typically believed to be more virulent than *B. vogeli*. These differences in virulence may result in practitioners seeing "atypical" cases of babesiosis compared to range of signs and laboratory abnormalities they have become accustomed to seeing. Additionally, there are commercially available vaccines against *B. canis* that may be useful to practitioners in this area if *B. canis* becomes prevalent in this region. Both are subunit vaccines. One contains soluble parasite antigens from *B. canis* and the other contains soluble parasite antigens from *B. canis* and *B. rossi*. This study constitutes the first report of co-infection with *B. canis* and *B. rossi* in the West African sub-region and the first report of *B. canis* on the African continent. Practitioners should be aware of potential changes in the species/sub-species of *Babesia* causing canine babesiosis in this region.

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