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Original article

Prevalence, risk factors, and genetic diversity of veterinary important tick-borne pathogens in cattle from *Rhipicephalus microplus*-invaded and non-invaded areas of Benin

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ABSTRACT

Babesiosis, theileriosis, anaplasmosis, and heartwater are tick-borne diseases (TBD) that threaten livestock production in sub-Saharan Africa including Benin. This country has been faced with an invasion of *Rhipicephalus microplus*, a major vector for babesiosis, theileriosis, and anaplasmosis over the last decade. Yet, data on TBD and the impact of the invasive ticks are lacking, making risk level evaluation and disease control arduous. In this study, epidemiological features of *Babesia bovis*, *B. bigemina*, *Theileria* spp., *Anaplasma marginale* and *Ehrlichia ruminantium* infections in Benin cattle were investigated in *R. microplus*-invaded and non-invaded areas. Detection of pathogens was based on species-specific PCR assays and resulting data were used to identify risk factors. Genetic diversity and phylogenies were then evaluated using several markers. Out of 207 samples examined, 170 (82.1%), 109 (52.7%), 42 (20.3%) 24 (11.6%) and 1 (0.5%) were positive for *T. mutans*, *A. marginale*, *B. bigemina*, *B. bovis* and *E. ruminantium*, respectively. Animal gender (for *B. bovis*), exposure to *R. microplus* (for *B. bigemina* and *A. marginale*), animal age (for *B. bigemina* and *A. marginale*) and cattle breed and/or antiprotozoal treatment (for *T. mutants*) significantly modulated pathogen occurrence. In addition, *R. microplus* exposure was significantly related to co-infection patterns and cases of clinical theileriosis and/or anaplasmosis were recorded among cattle highly exposed to the tick. In the genetic characterization, *Theileria* spp. and *E. ruminantium* sequences were conserved. *Babesia* spp. and *A. marginale*, however, showed high sequence polymorphisms that indicate the presence of several strains and may be linked to *R. microplus* invasion. Taken together, these results ascertain the endemicity of tick-borne infections in Benin and suggest that the characteristics of *Babesia* spp. and *A. marginale* infections in *R. microplus*-invaded and non-invaded areas are different.

1. Introduction

Ticks are the most important ectoparasites of livestock in tropical and subtropical areas. They impair animal growth and cause serious lesions to skin and udders, but their major impact results from the transmission of pathogens which cause important diseases, generally termed tick-borne diseases (TBD) (Rajput et al., 2006; Stachurski, 2000). Babesiosis (caused by *Babesia bovis* and *B. bigemina*), theileriosis (caused by *Theileria* spp.), anaplasmosis (caused by *Anaplasma marginale*) and heartwater (caused by *Ehrlichia ruminantium*) are TBD, constituting pre-eminent livestock health and management problems in Africa (Jongejan and Uilenberg, 2004). Animals, once infected with the etiological agents of the above TBD, either develop asymptomatic infections or clinical diseases which result in morbidity, body weight, milk and sometimes animal losses (Bishop et al., 2004; Bock et al., 2004; Kocan et al., 2010; OIE, 2009). Cattle breeds indigenous to endemic areas often develop a state of “endemic stability”. This happens when relationships between hosts, pathogens, vectors, and environment are such that animal may become infected but apparent disease occurs rarely or not at all (Bock et al., 2004; Deem et al., 1996). Nevertheless,

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events associated with immunosuppression (e.g. malnutrition, pregnancy, lactation, concurrent infections) and/or high tick loads can disrupt the endemic stability and cause the onset of severe, life-threatening diseases (Bock et al., 2004; Kocan et al., 2010).

Benin, located in West Africa, is considered to be a TBD-endemic country. The estimated 2 million Benin cattle are mostly of indigenous breeds and reared under extensive grazing (Aregheore, 2009). They are permanently exposed to a tick fauna composed of 17 species (Madder et al., 2012; Vercruysse et al., 1982), several of which are vectors of important pathogens. Accordingly, *B. bovis, B. bigemina*, *T. mutans*, *A. marginale* and *E. ruminantium* have been reported in Benin cattle (Adehan et al., 2016b; Farougou et al., 2013, 2007b; Pangui and Salifou, 1992). Although infections with these pathogens are common, clinical diseases and animal fatalities related to TBDs are rare in Benin, suggesting an endemic stability. Accordingly, TBD are not found in the trypanosomosis-topped list of major causes of cattle losses in the country (Benin Livestock Department, 2010). Cattle breeders in Benin, like in other West African countries, rely on acaricide usage and manual removal to mitigate the impact of ticks and prevent the occurrence of TBDs (Adehan et al., 2016a; Adjou Moumouni, 2012; Stachurski, 2000). However, the effectiveness of the acaricides currently used in the country is threatened by the rapid expansion of acaricide-resistant (Adehan et al., 2016a) *Rhipicephalus* (*Boophilus) microplus* ticks. The tick is much feared for its invasiveness and ability to transmit *B. bovis, B. bigemina* and *A. marginale* (Walker et al., 2003). *R. microplus* was introduced in a southern district of Benin a decade ago through importation of live cattle from Brazil (De Clercq et al., 2012; Madder et al., 2012). Currently, it is well-established in the warm and humid southern and central region where it has outnumbered native *Rhipicephalus* (*Boophilus)* ticks (i.e *R. annulatus*, *R. decoloratus,* and *R. geigyi*). The hot and dry northern regions remain the only areas where the tick is not yet abundant (Biguezoton et al., 2016; De Clercq et al., 2015, 2012). Consequences of *R. microplus* invasion on the native tick fauna and tick control effectiveness in Benin are known. In contrast, due to the scarcity of comprehensive data on tick-borne pathogen occurrence, whether the invasive tick had or will have an effect on TBD epidemiology remains unclear. Therefore, the present study was carried out to document the characteristics of tick-borne pathogen infections in Benin cattle as a step towards mitigating the potential effects of the invasive tick. Cattle farms representative of the features of Benin cattle industry were surveyed in *R. microplus*-infested areas and in areas where the ticks may be found but in low numbers. The distribution of *B. bovis*, *B. bigemina*, *Theileria* spp., *A. marginale* and *E. ruminantium* infections in cattle, the related risk factors, pathogen genetic diversity and impact on animal health were assessed.

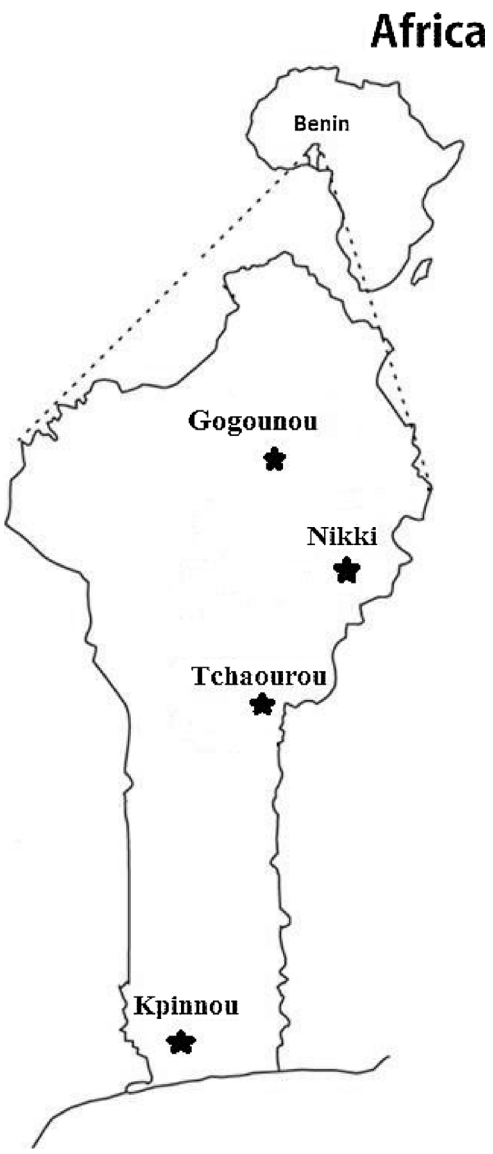

Fig. 1. Map of Benin showing the study areas. Cattle blood samples were collected in 4 different locations which are indicated by the black stars: Kpinnou (N 6.5681; E 1.781), Tchaourou (N 8. 5300; E 2.360), Nikki (N 9.5600; E 3.1230) and Gogounou (N 10.5035; E. 2.494).

2. Materials and methods

2.1. Study design

Cattle reared in four sites were investigated (Fig. 1). These include two *R. microplus*-infested localities namely Kpinnou and Tchaourou, and two North Benin localities namely Nikki and Gogounou where the tick may be found but in low numbers (Biguezoton et al., 2016; De Clercq et al., 2015, 2013, 2012).

In Kpinnou, the study population consisted of Brazilian Girolando cattle, while in Tchaourou, Nikki and Gogounou indigenous Borgou cattle were examined. The Girolando cattle belonged to Benin government's PAFILAV project (Milk and Meat Sectors Support Project). They were kept under a semi-extensive system characterized by a barn, free grazing on natural and artificial pastures and regular health care. The Borgou cattle were recruited in several herds and belonged to pastoralists (Tchaourou, Nikki, and Gogounou) and agro-pastoralists (only in Gogounou). Pastoralist cattle were managed according to Fulani herdsmen pastoral tradition. They were kept in open air park at night, grazed all the year on natural pastures and supplemented by forages cut from forests and crop residues. Agro-pastoralist cattle, however, were kept all day long in crop fields during the plowing season and when not in use were left to roam freely in the vicinity of farmers' houses or fed with crop residues. In each Borgou herd, 10% of animals were randomly selected and enrolled in the study.

2.2. Data and samples collection

Blood samples were collected from both male and female cattle of various ages in January-February 2015. Animal age was estimated by combining an examination of the teeth and horn rings as well as information provided by the owners. Each animal was then categorized as either young (≤ 4 years) or adult (> 4 years). Cattle were searched for the presence of ticks and cattle herders were interviewed on tick control measures and their efficacy. The health status of each animal (apparently healthy or sick) was determined by observation of its general appearance and when applicable, clinical signs and previous treatments were recorded. Approximately 5 ml of whole blood was collected from the jugular vein of each animal using EDTA-coated vacutainer tubes.