Ticks and tick-borne pathogens infecting livestock and dogs in Tchicala-Tcholoanga, Huambo Province, Angola

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Abstract

The diversity of ticks and tick-borne pathogens (TBPs) infesting domestic animals in Tchicala-Tcholoanga, Angola in 2016 was investigated. Seventeen tick species were recorded, *Amblyomma pomposum* being the most abundant on cattle (40%), goats (38%) and sheep (35%); *Rhipicephalus turanicus* was the most abundant on dogs (46%). This study presents new records of *Haemaphysalis paraleachi, R. compositus, R. kochi* and *R. sulcatus* in Angola, the first georeferenced population of *Ha. leachi* in southern Africa, and the second record of *R. microplus* in Angola. Using the reverse line blot (RLB) hybridisation assay, 15 TBP species were detected in blood samples from cattle (n=88), goats (n=82), sheep (n=85) and dogs (n=85). The most frequently detected species were *Theileria velifera* in cattle (78%), *Theileria ovis* in sheep (80%), and *Babesia vogeli* in dogs (35%). Species-specific quantitative PCR assays detected *Babesia bigemina* in 43% (35/80) of blood samples of cattle, while *E. ruminantium* was detected in 4% (3/70) of blood samples and in 7% of *A. pomposum* ticks. *Anaplasma platys* was detected from cattle (18%) and sheep (6%) during RLB analysis. These findings constitute pioneering research in Angola.

Keywords: Ticks; reverse line blot; *Anaplasma*; *Babesia*; *Theileria*; Angola

1. Introduction

Ticks are important pathogen vectors that negatively impact the health of animal and human populations across the world, and are considered a major constraint to livestock production (Jongejan and Uilenberg 2004). In Africa, 40 tick species are recognised as a threat to domestic animal health (Walker et al. 2003). Species of major veterinary and economic importance belong to the genera *Amblyomma*, *Hyalomma* and *Rhipicephalus*, and are commonly associated with the transmission of parasites of the genera *Anaplasma, Ehrlichia*, *Babesia* and *Theileria* (Walker et al. 2003). Domestic ruminants and dogs are the most affected by these haemoparasites (Jongejan and Uilenberg 2004).

To understand the current distribution of ticks in Angola, it is important to consider the impact of major historical events, especially the armed conflict that affected the country for over three decades. It is believed that the war led to uncontrolled animal dispersion as well as a drastic reduction in livestock during the 1990s. Consequently, animals were imported from neighbouring countries, namely Botswana (Gomes 1993), Namibia and Zambia (A. Gomes, personal communication, 2015) and from overseas, particularly Brazil (A. José, personal communication, 2015). The livestock population in Angola comprises mainly cattle and goats, although sheep and pigs can be found in both traditional and commercial sectors. Dogs are frequently present in grazing areas, where they are used to herd livestock and to hunt. The first studies on ticks in Angola were conducted by Dias (1948) in various regions of the country. Subsequent studies were conducted by Serrano (1963), Dias (1983) and Gomes et al. (1994).

Despite the wide range of molecular tools currently available for the diagnosis of tick-borne diseases (TBDs), classical diagnostic assays, such as blood smears and serological tests, are practically the only methods previously used to detect haemoparasites in Angola. The aim of

this study was to update information on the diversity of ticks infesting livestock and dogs as well as tick-borne pathogens detected using DNA-based methods in Angola.

2. Material and Methods

The fieldwork for this cross-sectional study was conducted in four communes: Mbave (13 sampling sites), Sambo (13 sites), Samboto (11 sites) and Sede (12 sites) in Tchicala-Tcholoanga Municipality, Huambo Province, Angola (Figure 1). The annual rainfall is 1200–1600 mm during the wet season (October to April), and there is no rainfall during the dry season (May to September) (http://www.angola.climatemps.com/). The annual average temperature is 20°C. The main economic activity in the municipality is agriculture, and livestock production is based on indigenous breeds reared on small traditional farms.

Figure 1. Map of Huambo Province in Angola showing the 11 municipalities, including Tchicala-Tcholoanga Municipality (shaded with dots), where a study on ticks and tick-borne pathogens was conducted. All four communes (Mbave, Sede, Sambo and Samboto) in Tchicala-Tcholoanga Municipality were sampled. Inset is a map of Angola showing the location of Huambo Province

In January 2016, ticks and blood samples were collected from cattle, goats, sheep and dogs of various ages and both sexes in the four communes. The month of January corresponds to the mid rainy season, which is the peak of adult tick activity for the species occurring in this region. Adult ticks were targeted because they are easier to identify to species level than their immature stages. Given our aim of determining the presence or absence of tick-borne pathogens (rather than prevalence), the required sample size was calculated using the formula below (Thrusfield 2007):

$$n = [1 - (1 - P1)^{\frac{1}{d}}][N - \frac{d}{2}] + 1$$

where n = required sample size; P1 = probability of finding at least one case in the sample (95% probability=0.95); N = population size or number of animals for each host species (considered as an infinite population, ∞); d = minimum number of animals affected in the population. The expected occurrence rate of tick-borne pathogens was assumed to be 15%, derived from experience in our laboratory, in which 15% is one of the lowest percentages of parasite occurrence in endemic areas. Therefore, ≥19 individuals of each host species was estimated to be sufficient (Cannon and Roe, 1982, cited in Thrusfield 2007) to detect tick-borne pathogens in the study area.

All visible ticks were manually collected from half of the body of five of the most infested animals of each host species in a commune. The total number of ticks collected from an animal represented a single tick collection. The ticks were identified to species level by stereomicroscopic examination using the keys published by Walker et al. (2003).

A total of 340 animals; 88 cattle, 82 goats, 85 sheep and 85 dogs were sampled and 4 ml of blood were collected from each animal. DNA was extracted from each blood sample using the QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany) and then stored at -20°C. The 340 blood samples were tested using reverse line blot (RLB) hybridisation assay as previously described (Nijhof et al. 2005). The positive controls were DNA extracted from *Anaplasma centrale* and *Babesia bovis* live blood vaccines (Onderstepoort Biological Products [OBP], Pretoria, South Africa), for the 16S rRNA and 18S rRNA PCRs respectively, while nuclease-free water was used as negative control. *Anaplasma*, *Ehrlichia*, *Babesia* and *Theileria* genus-specific probes and 36 species-specific probes, as outlined in Byaruhanga et al. (2016), and a probe specific for *Anaplasma platys* (Sirigireddy and Ganta 2005), were included on the RLB membrane.

Two quantitative real-time PCR (qPCR) assays were used for the detection of *Babesia bigemina* and *B. bovis* DNA from 88 blood samples from cattle, as described previously (Kim et al. 2007), except that the annealing temperatures were adjusted to 57°C for *B. bigemina* and 54°C for *B. bovis*. The positive controls were DNA extracted from *B. bigemina* and *B. bovis* live blood vaccines (OBP, Pretoria, South Africa). A TaqMan qPCR assay was conducted to amplify a 226 bp fragment of the conserved *pCS20* region of *Ehrlichia ruminantium* (Steyn et al. 2008) from 70 blood samples (cattle: 20; goats: 20; sheep: 20; dogs: 10). One hundred randomly-selected *Amblyomma pomposum* ticks (male and female) collected from apparently healthy ruminants were also analysed. DNA extraction from individual dissected ticks was performed using the DNeasy® Blood & Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol. The positive control was *E. ruminantium* Welgevonden genomic DNA, while nuclease-free water was used as the negative control. The near full-length parasite 16S rRNA gene for *Anaplasma*/*Ehrlichia* species was amplified, cloned and sequenced from

11 DNA samples (cattle: 6; sheep: 5) that showed *A. platys*-specific signals during RLB analysis. The amplification and sequence analyses were performed as described previously (Byaruhanga et al. 2018).

3. Results and Discussion

Five genera and 17 species of ticks were collected from domestic animals in Tchicala-Tcholoanga Municipality in Angola (Table 1). In previous surveys in Angola, Serrano (1963) and Dias (1983) recorded 34 and 19 tick species respectively. Subsequently, Gomes et al. (1994) recovered 13 species in a survey conducted in Huíla Province. *Haemaphysalis paraleachi, Rhipicephalus compositus, Rhipicephalus kochi* and *Rhipicephalus sulcatus* are new records for Angola and this is the second record for *Rhipicephalus microplus*.

Nine tick species; *A. pomposum, Haemaphysalis leachi, Rhipicephalus decoloratus, Rhipicephalus evertsi mimeticus, Rhipicephalus lunulatus, Rhipicephalus punctatus, R. sulcatus, Rhipicephalus turanicus* and *Rhipicephalus tricuspis* were present in all four communes. Twelve species were recovered in Sambo, 13 in Mbave and 15 species each in Samboto and Sede. There was a generally similar pattern of relative abundance of each tick species in each commune, for each host species. *Rhipicephalus microplus* (10 specimens) was only found in Sede in one sampled bovine.

Amblyomma pomposum was the most abundant species amongst cattle, sheep and goats (Table 1). Of the 15 species of *Amblyomma* that have been reported in Angola, *A. pomposum* is the most common, and is regarded as an important vector of *E. ruminantium*, the cause of heartwater in ruminants (Dias 1948; Serrano 1963; Gomes et al. 1994). Its distribution is restricted to Angola, western Zambia and the southern region of the Democratic Republic of

Table 1. Ticks collected from domestic ruminants and dogs in a cross-sectional study in Tchicala-Tcholoanga, Huambo Province, Angola

Tick species	Cattle (n=20)		Goats (n=20)		Sheep (n=20)		Dogs (n=20)	
	No. of ticks (%)	**No. of animals infested (%)**	**No. of ticks (%)**	**No. of animals infested (%)**	**No. of ticks (%)**	**No. of animals infested (%)**	**No. of ticks (%)**	**No. of animals infested (%)**
*A. pomposum**	617 (40.2)	20 (100)	52 (38.2)	14 (70)	127 (35.4)	16 (80)	8 (0.9)	6 (30)
Ha. Leachi[#]	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-	10 (1.1)	3 (15)
Ha. paraleachi[#]	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-	57 (6.1)	12 (60)
Hy. Truncatum	270 (17.6)	14 (70)	2 (1.5)	1 (5)	27 (7.5)	6 (30)	0 (0.0)	-
I. cavipalpus	7 (0.5)	3 (15)	1 (0.7)	1 (5)	0 (0.0)	-	13 (1.4)	4 (20)
R. compositus	12 (0.8)	5 (25)	0 (0.0)	-	1 (0.3)	1 (5)	0 (0.0)	-
*R. decoloratus**	466 (30.4)	18 (90)	3 (2.2)	3 (15)	34 (9.5)	10 (50)	4 (0.4)	2 (10)
R. evertsi evertsi	2 (0.1)	2 (10)	3 (2.2)	2 (10)	8 (2.2)	5 (25)	0 (0.0)	-
R. e. mimeticus	61 (3.9)	10 (50)	16 (11.8)	9 (45)	50 (13.9)	5 (25)	0 (0.0)	-
R. kochi	22 (1.4)	7 (35)	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
*R. lunulatus**	11 (0.7)	4 (20)	4 (2.9)	3 (15)	4 (1.1)	3 (15)	52 (5.6)	16 (80)
R. microplus	10 (0.7)	1 (5)	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-
*R. punctatus**	22 (1.4)	7 (35)	41 (30.2)	8 (40)	91 (25.4)	9 (45)	85 (9.1)	9 (45)
R. simus[#]	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-	6 (0.6)	4 (20)
R. sulcatus[#]	0 (0.0)	-	0 (0.0)	-	0 (0.0)	-	84 (9.0)	14 (70)
*R. tricuspis**	35 (2.3)	11 (55)	14 (10.3)	8 (40)	17 (4.7)	7 (35)	187 (20.0)	18 (90)
R. turanicus[#]	0 (0.0)	-	0 (0.0)		0 (0.0)	-	427 (45.8)	18 (90)
Total	**1535 (100)**		**136 (100)**		**359 (100)**		**933 (100)**	

*Tick species infesting all four animal species; [#]Tick species that were only collected from dogs.

A.= *Amblyomma*; Ha. = *Haemaphysalis*; Hy.= *Hyalomma*; R. = *Rhipicephalus*; I. = *Ixodes*

(no.) = number of animals infested or number of ticks collected; Percentage (%) of the total in parenthesis

Congo (Walker et al. 2003). Gomes and Neves (2018) found *R. microplus* in two provinces of Angola, and the present study records its presence on a bovine in a third locality. Additionally, this study reports the first georeferenced population of *Ha. leachi* in southern Africa since the reinstatement of *Haemaphysalis elliptica* as a valid species. *Rhipicephalus turanicus* was the most abundant tick species on dogs, and its possible role as a vector of *Babesia vogeli* and *Ehrlichia canis* should be evaluated.

Using RLB, 15 haemoparasite species were detected from the 340 DNA samples from cattle, goats, sheep and dogs (Table 2). However, genus-specific signals without species-specific signals were also observed. *Theileria mutans*, *Theileria velifera* and *Theileria* sp. (sable) were the most commonly detected species in cattle (>70% each). *Theileria mutans* and *T. velifera* are transmitted by *Amblyomma hebraeum, Amblyomma lepidum* and *Amblyomma variegatum* (Walker et al. 2003). Considering that these ticks are absent in the study region, the current findings suggest that *A. pomposum* is also a vector. *Theileria velifera* has previously been recorded in Angola (Kubelová et al. 2012). *Theileria* sp. (sable) is a parasite of sable antelope (Stoltsz and Dunsterville 1992, cited by Nijhof et al. 2005), and reports of widespread occurrence of the parasite in cattle require DNA sequencing studies, to confirm circulation of the pathogen in cattle populations, and to rule out cross-reactions between *Theileria* sp. (sable) and *T. velifera* RLB probes. The infection rate of *Anaplasma marginale* (28%) (Table 2) is lower than that found in Bié Province in Angola (38%) (Kubelová et al. 2012), using conventional PCR. *Anaplasma platys* which is not a known pathogen of cattle, was also detected. *Theileria ovis* was the most abundant haemoparasite species in sheep (80%) (Table 2). Although *T. ovis* is considered a haemoparasite of negligible pathogenic importance, it was implicated in massive sheep losses in Pakistan (Durrani et al. 2011). It is transmitted by several tick species including *Amblyomma* spp., *Hyalomma* spp. and *Rhipicephalus* spp., all of which

were found on sheep in this study. The detection of *T. ovis* in dogs in the present study was unexpected, and, to our knowledge, has not been previously reported. Thus, further investigation is needed. The detection of *A. platys*, *Theileria bicornis*, *B. bovis* and *Babesia rossi* in sheep (Table 2) was unforeseen. Of the 82 blood samples from goats, only two were positive for tick-borne parasites (Table 2). In contrast, 100% of goats examined in the neighbouring Bié province were infected with *Anaplasma ovis* using PCR (Kubelová et al. 2012). The most frequently occurring haemoparasite species in dogs was *B. vogeli* (35%) followed by *B. rossi* (24%). In contrast, Cardoso et al. (2016) reported *A. platys* and *Hepatozoon canis* as the most frequent parasite species in dogs in Luanda. Furthermore, infection rates with *B. vogeli* in our study were considerably higher than those recorded by Cardoso et al. (2016) in Luanda (5.8%). The detection of both *E. canis* and *B. vogeli* in dogs, in the absence of *Rhipicephalus sanguineus*, indicates a potential vector role for *R. turanicus.* However, our findings do not exclude the possibility of the presence of *R. sanguineus*. *Babesia rossi* is known to be transmitted by *Ha. elliptica*. In this study, the occurrence of *B. rossi* may be attributed to the presence of *Ha. leachi,* whose role together with *Ha. paraleachi* as possible vectors of *B. rossi* needs to be investigated. The detection of *T. ovis* in seven dogs is considered unusual.

Of the 80 blood samples from cattle that were tested using the *B. bigemina*-specific qPCR, 35 (44%) were positive for *B. bigemina* DNA. The relatively high proportion of *B. bigemina* infections corresponds with the occurrence of the competent vector, *R. decoloratus*, the second most frequently collected tick from cattle (90% infestation rate). No *B. bovis* was detected from cattle using the species-specific qPCR, consistent with the low occurrence of *R. microplus*. *Ehrlichia ruminantium* was not detected using RLB, due to its relatively low sensitivity (Byaruhanga et al. 2016). Therefore, qPCR was performed to ascertain infection rates and

Table 2. Proportions of blood samples from domestic ruminants and dogs from Tchicala Tcholohanga municipality, province of Huambo, Angola found positive for tick-borne pathogens, using the reverse line blot hybridisation assay

Tick-borne pathogen	Number and percentage of positive samples			
	Cattle (n=88)	Sheep (n=85)	Goats (n=82)	Dogs (n=85)
Anaplasma centrale	11 (12.5)	2 (2.4)	2 (2.4)	-
Anaplasma marginale	25 (28.4)	1 (1.2)	-	-
Anaplasma bovis	1 (1.1)	-	-	-
Anaplasma platys	16 (18.2)	5 (5.9)	-	-
Anaplasma sp. Omatjenne	22 (25)	4 (4.7)	-	-
Ehrlichia canis	-	-	-	1 (1.2)
Babesia bigemina	3 (3.4)	-	-	-
Babesia bovis	-	1 (1.2)	-	-
Babesia rossi	-	1 (1.2)	-	20 (23.5)
Babesia vogeli	-	-	-	30 (35.3)
Theileria sp. (sable)	63 (71.6)	46 (54.1)	-	-
Theileria mutans	65 (73.9)	-	-	-
Theileria velifera	69 (78.4)	-	-	-
Theileria bicornis	-	4 (4.7)	-	-
Theileria ovis	-	68 (80)	-	7 (8.2)

whether *A. pomposum* is involved in the transmission. Of the 70 blood samples from cattle, goats, sheep and dogs tested using *E. ruminantium*-specific *pCS20* qPCR, only three tested positive for the pathogen. Of the 100 *A. pomposum* DNA samples tested, seven were positive to *E. ruminantium*. Our *E. ruminantium* detection rate from *A. pomposum* ticks (7%) is consistent with several other studies in *A. hebraeum, A. variegatum* and *A. lepidum* (Muramatsu et al. 2005). This is the first study to determine *E. ruminantium* infection rates in *A. pomposum*. *Anaplasma platys* was detected in cattle (18%) and sheep (6%), and confirmed by 16S rRNA sequence analysis. Of the three near full-length 16S rRNA gene sequences (1249 bp) obtained from three cattle samples, two sequences (MN401149 and MN401150) were 97.8 to 98.0% identical to *A. platys* sequences from domestic dogs in South Africa (MK814421, query cover 100%) and Cuba (KX792089, query cover 100%), and 98% identical to *Anaplasma* sp. Omatjenne (U54806). The pathogen has been reported worldwide, mostly from dogs, causing canine cyclic thrombocytopenia. However, variants of *A. platys* have been implicated as causes of disease in cattle and humans (Sykes and Foley 2014).

The majority of haemoparasites detected in this study are usually associated with the 17 tick species identified, but for some pathogens such as *A. centrale* (usually transmitted by *Rhipicephalus simus*), *E. canis* and *B. vogeli* (usually transmitted by *R. sanguineus*), their usual tick vectors were not found. This points to the possibility that these pathogens may be transmitted by tick vectors other than those which have been previously reported. However, the presence of established biological tick vectors of these pathogens may not be ruled out. A limitation of this study is that ticks were collected from only 20 animals of each host species, and only from Tchicala-Tcholoanga Municipality in Huambo Province. Although this may not provide a comprehensive comparison of the spatial and host-specific distribution of tick species in Angola, the findings contribute to the knowledge of the occurrence of ticks and tick-borne pathogen species in the country. The findings provide reference for future research in Angola, involving a larger animal sample and with pathogen DNA sequencing to determine the prevalence, distribution and diversity of the common pathogens.

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Declarations

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Conflicts of interest/Competing interests

The authors declare that there is no conflict of interest and no competing interests.

Ethics approval

The research was approved by the Animal Ethics Committee (AEC) of the Faculty of Veterinary Science, University of Pretoria, South Africa (number: V053-16), and followed the AEC guidelines for study design, handling and care of study animals, collection of blood and tick samples and relevant laboratory procedures. Permission to perform the research was granted by the Department of Agriculture, Forestry and Fisheries, Republic of South Africa (Reference number: 12/11/1/1/9).

Consent to participate

Not applicable

Consent for publication

All the authors listed for this publication have agreed on the submission for publication.

Availability of data and material

Raw data can be provided on request.

Code availability

Not applicable

Authors' contributions

Gourgélia Sili: Writing – Original Draft, Investigation, Methodology, Visualisation, Data curation. **Charles Byaruhanga**: Writing – Original Draft, Investigation, Visualisation, Formal analysis, Methodology, Data curation. **Ivan Horak**: Resources, Writing – Review & Editing, Investigation, Validation. **Helena Steyn**: Methodology, Investigation. **Mamohale Chaisi**: Methodology, Investigation, Writing – Review & Editing. **Marinda C. Oosthuizen**: Project administration, Conceptualisation, Writing – Review & Editing, Supervision. **Luís Neves**: Project administration, Conceptualisation, Funding acquistion, Writing – Review & Editing, Supervision.

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