

Zoonotic Pathogens Detected in Ticks in Kenyan Game Reserves

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Abstract

Little is known on tick-borne pathogens and their role in disease in game reserves in Kenya. Ticks were collected by sterile forceps from restrained cattle hide and placed into labeled falcon tubes. Ticks were screened for pathogens by High Resolution Melting (HRM) analysis and sequencing of specific RT-PCR products of *Anaplasma*, *Ehrlichia*, and *Rickettsia* species. A total of 317 ticks (281 adult ticks and 36 nymphs) comprising seven species were collected around the Tsavo National Reserve (TNR) in Taita Taveta County with *Amblyomma gemma* being the most commonly collected species (n = 135, 42.6%). From near Shimba Hill game reserve (SHNR), a total of 240 adult ticks were sampled, representing eight species, with again *Amblyomma gemma* being the most sampled species (n = 156, 65%). From Tsavo, a total of three pools of *Rhipicephalus appendiculatus* were positive for *Theileria parva*, two pools of *Rhipicephaline evertsi* for *Anaplasma platys* and one pool of *Amblyomma variegatum* nymphs for *Rickettsia africae*. *Rickettsia africae*, which causes African tick-bite fever, was detected in two pools of *Am. variegatum* and one pool of *Amblyomma gemma* collected near Shimba Hill game reserve. *Rickettsia* sp. and *Anaplasma* sp. were detected in *Am. gemma* and *Rh. evertsi* respectively. *Rickettsia aeschlimannii* was detected in a pool of *Am. gemma*. These findings highlight the risk of transmission of zoonotic pathogens to humans in regions with high human-wildlife interfaces. Of specific importance, we provide evidence of *R. aeschlimannii* in *A. gemma* for the first time, representing a potential new *R. aeschlimannii* vectors.

Keywords

High Resolution Melting, *Amblyomma gemma*, *Rickettsia africae*, *Rickettsia aeschlimannii*

1. Introduction

Tick-borne diseases are amongst neglected tropical diseases leading to high pre-

valence and distribution morbidity [1]. Lack of current information on the prevalence and distribution of tick-borne diseases and their vectors in sub-Saharan Africa make it difficult to assess their true impact [1]. Tick-borne pathogens include *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *E. canis*, *E. ruminantium*, and *Anaplasma phagocytophilum* [2]. Kyasanur Forest disease virus and Crimean-Congo hemorrhagic fever virus are some of viruses transmitted by ticks [3] [4]. The most important tick-borne diseases affecting livestock in Kenya includes theileriosis, anaplasmosis, babesiosis, and heartwater [5]. Omondi *et al.* [6] reported canine ehrlichiosis (*E. ruminantium*, *Ehrlichia canis*, and *Ehrlichia* sp), anaplasmosis (*Anaplasma ovis*, *Anaplasma platys* and *A. bovis*), and rickettsiosis (*Rickettsia aeschlimannii*) in ticks collected around Lake Victoria and Lake Baringo.

Encroachment of human settlements into the game areas by the pastoral communities has led to the emergence and re-emergence of tick-borne zoonotic diseases [7]. Other activities such as commercial ranching, and tourism increases the risk of pathogen transmission [8]. This has exposed the humans to the bite of the vectors [9]. Several tick-borne zoonotic pathogens have been detected in wildlife protected areas in Kenya [10] [11]. In order to curb the spread of these tick-borne pathogens the government must impose strict measure to prevent human encroachment into wildlife areas.

There is limited knowledge of circulating tick-borne pathogens of medical importance in their biological vectors at human-wildlife-livestock interface in Kenya due to limited resources. This study aimed at addressing the gap in knowledge on the zoonotic tick-borne pathogens circulating at human-wildlife-livestock interfaces in the Tsavo National Reserve (TNR) and the Shimba Hills National Reserve (SHNR), Kenya, by surveying the tick diversity and associated tick-borne pathogens.

2. Materials and Methods

2.1. Study Area

Sampling approval was obtained from the Kenya's Directorate of Veterinary Services and Ministry of Health. Informed consent was obtained from the livestock's owner before sampling of the ticks at near Tsavo and Shimba Hills National Reserves, Kenya. The two selected human-wildlife-livestock interfaces were selected based on encroachment human settlement by the pastoral community and reported cases of tick-borne pathogens of economic and public health burden. The Tsavo National Park is a protected area in Kenya, and borders the Chyulu Hills National Park in Kenya and Mkomazi game reserve of Tanzania. The park is divided by the highway road that runs from Nairobi to Mombasa into Tsavo East national park (13,747 Km²) and Tsavo West national park (9065 Km²). The SHNR is a wildlife protected area in Kwale County. It is the largest forest area in east African and home to a variety of wildlife species.

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Institute Resources, 2007 [12];

<https://africaopendata.org/dataset/kenya-counties-shapefile> from openAfrica, 2015 [13]; and <https://gadm.org/data.html> from GADM, 2018 [14] (Figure 1).

2.2. Tick Collection and Identification

Sampling approval was obtained from the Kenya's Directorate of Veterinary Services and Ministry of Health. Informed consent was obtained from the livestock's owner before sampling of the ticks. Ticks were collected from restrained cattle hide using sterile forceps and placed into labeled falcon tubes. The tubes were then plugged with cotton swabs and transported in liquid nitrogen to the testing lab. They were stored at -80°C until when analyzed [6]. The ticks were identified to genus and/or species level as per the morphological keys [15] using sterile forceps, petri-dishes and gloves. Ticks were pooled according to species, sex and sampling sites into groups of 1 - 11 for adults and 1 - 20 for nymphs as described by Oundo *et al.* [10]. Representative of these morphologically identified ticks were molecularly identified.

3. Laboratory Procedure

3.1. DNA Extraction

Ticks were homogenized in a 1.5-ml sterile microcentrifuge tubes containing 750 mg of 2-mm yttria-stabilized zirconium using a handheld battery-operated

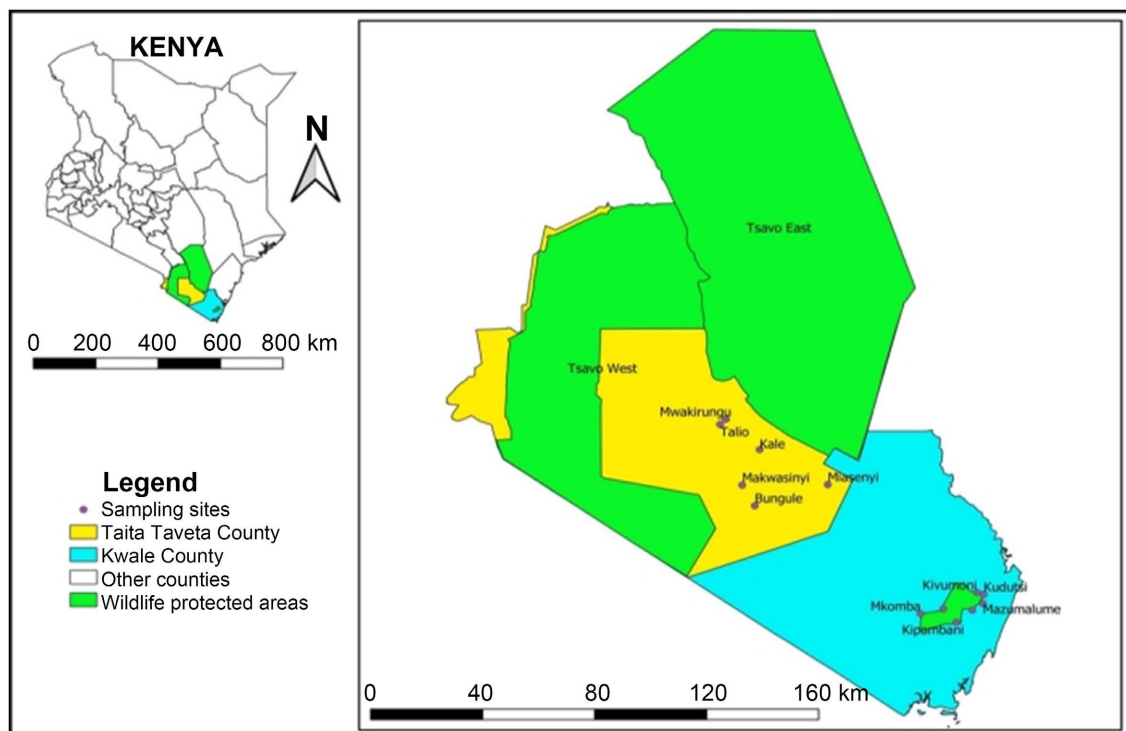


Figure 1. Map of ticks sampling locations near the TNR in Taita Taveta County, Kenya, and near the SHNR in Kwale County, Kenya. The map was prepared using common-license shapefiles in QGIS software. TNR, Tsavo National Reserve; SHNR, Shimba Hills National Reserve.

homogenizer oxide bead (Glen Mills, Clifton, NJ). DNA was extracted from the homogenate using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) as per the manufacturer's protocol. The concentration and purity of the DNA were evaluated using a Nanodrop spectrophotometer (Thermo Fisher Scientific Wilmington, USA) and extracts stored at -20°C .

3.2. Molecular Detection and Identification of Tick-Borne Pathogens

Tick-borne pathogens were screened using HRM and characterized by sequencing of specific RT-PCR products of *Anaplasma*, *Ehrlichia*, and *Rickettsia* using genus-specific primers (Table 1). For HRM, a 10 μl reaction volume was prepared containing a final concentration of 1 \times HOT FIREPol Eva-Green HRM mix (Solis BioDyne, Tartu, Estonia), 500 nM of the respective forward and reverse primers (Table 1), 100 ng of template DNA and 5 μl nuclease free water. DNA extracts from *Anaplasma phagocytophilum*, *Ehrlichia ruminantium*, and *Rickettsia africae* were used as positive controls while nuclease-free water was used as a negative control. Minimum infection rate was calculated using the following formula: [number of pathogen positive tick pools/total number of ticks of that species tested] \times 1000. The MIR assumes that only one tick is positive in a pool.

3.3. Data Analysis

Geneious software version 8.1.9 was used to edit and align all nucleotide sequences using the MAFFT plugin [16]. GenBank database using the Basic Local Alignment Search Tool (<https://www.ncbi.nih.gov/BLAST/>) was used in sequence identities. The map was prepared using QGIS software version 3.12.1 (QGIS) to show the sampling sites.

4. Results

4.1. Tick Diversity and Abundance

At near Tsavo National Reserve, a total of 317 (281 adult ticks and 36 nymphs) were collected, representing seven species (Table 2). *Amblyomma* ticks dominated the collections with *Amblyomma gemma* the most sampled species ($n = 135$, 42.6%). Other *Amblyomma* species sampled was *Amblyomma variegatum* ($n = 40$, 12.62%). Four species of *Rhipicephalus* ticks were collected including; *R. appendiculatus* ($n = 44$, 13.9%), *R. evertsi* ($n = 1$, 0.31%), *R. decoloratus* ($n = 5$, 1.6%), and *Rhipicephalus pulchellus* ($n = 91$, 28.7%). A single *Hyalomma* specimen was also collected (Table 2).

Near Shimba Hills game reserve 240 adult ticks were collected representing eight species (Table 2) and again *Amblyomma gemma* dominated the collection ($n = 156$, 65%). Other *Amblyomma* species sampled included; *A. lepidum* ($n = 5$, 2.1%), *A. variegatum* ($n = 15$, 6.3%). *Rhipicephalus* ticks included *R. appendiculatus* ($n = 18$, 7.5%), *R. evertsi* ($n = 6$, 2.5%), *R. decoloratus* ($n = 4$, 1.7%), *R. pulchellus* ($n = 34$, 14.2%). Two representatives of *Hyalomma* scupense were also collected ($n = 2$, 0.83%) (Table 2).

Table 1. PCR primers pairs used in the study.

	Target gene	Primer name	Primer sequence (5'-3')	Amplicon Size (bp)	Reference
<i>Rickettsia</i> spp.	16S rRNA	Rick-F1	GAACGCTATCGGTATGCTTAACACA	364	[15]
		Rick-R2	CATCACTCACTCGGTATTGCTGGA		
	ompB	ompB 2788	AAACAATAATCAAGGTAAGTGT	856	[16]
		ompB 3599	TACTTCCGGTTACAGCAAAAGT		
<i>Ehrlichia</i> spp.	16S rRNA	<i>Ehr</i> 16S F	CGTAAAGGGCACGTAGGTGGACTA	200	[17]
		<i>Ehr</i> 16S R	CACCTCAGTGTCAGTATCGAACCA		
		<i>Ehr</i> J V F	GCAACCCTCATCCTTAGTTACCA	300	[18]
		<i>Ehr</i> J V R	TGTTACGACTTCACCCTAGTCAC		
<i>Anaplasma</i> spp.	16S rRNA	<i>Ana</i> 16S F	GGGCATGTAGGCGGTTTCGGT	112 - 200	[17]
		<i>Ana</i> 16S R	TCAGCGTCAGTACCGGACCA		
		<i>Ana</i> J V F	CGGTGGAGCATGTGGTTTAATTC	300	[18]
		<i>Ana</i> J V R	CGRCGTTGCAACCTATTGTAGTC		
<i>Theileria and Babesia</i> spp.	18S rRNA	RLB-F	GAGGTAGTGACAAGAAATAACAATA	450	[19]
		RLB-R	TCTTCGATCCCCTAACTTTC		

Table 2. Abundance and diversity of tick species collected in near Tsavo and Shimba Hills National Reserves, Kenya.

Species	Near Tsavo				Near Shimba Hills				t-test	P value
	Nymphs	Males	Females	Total %	Nymphs	Males	Females	Total %		
<i>A. gemma</i>	5	39	96	135	0	59	97	156		
<i>A. lepidum</i>	0	0	0	0	0	3	2	5		
<i>A. variegatum</i>	7	22	18	40	0	7	8	15		
<i>H. scupense</i>	0	0	0	0	0	0	2	2		
<i>Hyalomma</i> spp	0	0	1	1	0	0	0	0		
<i>R. appendiculatus</i>	14	13	31	44	0	11	7	18		
<i>R. evertsi</i>	0	1	0	1	0	2	4	6		
<i>R. decoloratus</i>	2	3	2	5	0	1	3	4		
<i>R. pulchellus</i>	8	22	69	91	0	23	11	34	1.096	0.305

4.2. Tick-Borne Pathogens Identified

At near Tsavo National Reserve (TNR) in Taita Taveta County, a total of three pools of *Rhipicephaline appendiculatus* were positive for *Theileria parva* (GenBank accession Number OL451869-OL451871), two pools of *Rhipicephaline evertsi* for *Anaplasma platys* (GenBank accession Number OL451873-OL451874) and one pool of *Amblyomma variegatum* nymphs for *Rickettsia africae* (GenBank accession Number OL466919) as shown in **Table 3**.

At Shimba Hills, *Rickettsia africae* was detected in two pools (GenBank accession Number OL466921-OL466922) of *A. variegatum* and one pool of *A. gemma*. *Rickettsia sp.* (GenBank accession Number OL466920) and *Anaplasma sp.* (GenBank accession Number OL451872) were detected in pools of *A. gemma*

Table 3. Tick-borne pathogens identified.

Site	Tick-borne pathogens	species	No. of infected pools/% of total tested	Minimum infection rate
Near Tsavo	<i>Theileria parva</i>	<i>R. appendiculatus</i>	3	9.46
National Reserve	<i>Anaplasma platys</i>	<i>R. evertsi</i>	2	6.3
	<i>Rickettsia africae</i>	<i>A. variegatum</i> (nymphs)	1	3.15
Near Shimba Hills	<i>Rickettsia africae</i>	<i>A. variegatum</i>	2	8.3
National Reserve		<i>A. gemma</i>	1	4.16
	<i>Rickettsia sp.</i>	<i>A. gemma</i>	1	4.16
	<i>Anaplasma sp.</i>	<i>R. evertsi</i>	1	4.16
	<i>R. aeschlimannii</i>	<i>A. gemma</i>	1	4.16

and *R. evertsi* respectively. *Rickettsia aeschlimannii* (GenBank accession Number OL466924) was detected in a pool of *A. gemma*.

5. Discussion

Detection of tick-borne zoonotic diseases at the human-wildlife-livestock interfaces of Shimba Hills National Reserve and the Tsavo National Reserve, gives an insight into the possibility of tick-borne zoonotic diseases spilling over from the wildlife to livestock and humans. Most of the tick species identified in this study have been incriminated as the vectors of tick-borne diseases of public health and veterinary concern [17].

Rickettsia africae is a zoonotic tick-borne pathogen which causes African tick bite fever in humans and manifests with headache, fever, rash, myalgia and skin lesion at the site of tick bite [11]. It is often misdiagnosed at the clinical setting because it shares symptoms with common febrile illnesses including malaria. This calls for robust surveillance and inclusions of *R. africae* in hospitals and other clinical settings. In this study, *R. africae* was detected in *A. gemma* and *A. variegatum* ticks. These findings are consistent with those that confirm the circulation of *R. africae* among the *Amblyomma* species [10] [11] [18]. Further, *R. africae* was detected in nymphs of *A. variegatum* ticks collected from cattle.

Rickettsia aeschlimannii is also considered to be a zoonotic tick-borne pathogen which has been reported in patients travelling from Africa [19] and it is understood that migratory birds play an important role in the epidemiology of *R. aeschlimannii* by passive transportation of *R. aeschlimannii*-infected ticks from one region to another [20]. This pathogen has been detected in *Hyalomma* ticks in Kenya [6], which are thought to serve as reservoirs for *R. aeschlimannii* [21]. However, in the present study, *R. aeschlimannii* was detected in *A. gemma*. According to our knowledge this is the first time *R. aeschlimannii* has been detected in *A. gemma*.

Uncharacterized *Rickettsia sp.* was detected in *Am. variegatum* ticks. Although its pathogenicity was unknown, it can be a public health threat in future

[19]. Many known *Rickettsia* species of public health concern today, were not initially considered to be harmful to humans. This species needs further characterization with additional markers such as *ompA*, *sca4*, 17kDa [10].

Anaplasma platys is a TBP infecting dogs which is transmitted by brown dog ticks (*Rhipicephalus sanguineus s.l.*) [22]. The pathogen has been reported also to infect humans causing mild headache, lethargy and myalgia [23] [24]. In the current study, *Anaplasma platys* was detected in *R. evertsi*.

Theileria parva is a TBP of veterinary importance which causes East Coast Fever in cattle [15]. In this current study, *Theileria parva* was detected in *R. appendiculatus* ticks. These findings were consistent with those of Oundo *et al.* [10]. The cattle get infected by coming into close proximity to buffaloes which are natural reservoir for *T. parva* [25].

6. Conclusion

The detection of *Rickettsia africae*, *Rickettsia aeschlimannii*, and *Anaplasma platys* gives an insight into the possibility of transmission of zoonotic tick-borne pathogens from wildlife to humans at human-wildlife-livestock interfaces. Since persons infected with these pathogens present with fever, it is paramount to include tests that can diagnose these infections in clinical settings.

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Data Availability Statement

Sequences obtained in this study have been deposited in the GenBank database under the following accession numbers: OL466919-OL466924 (*Rickettsia* 16S rRNA), OL451872-OL451874 (*Anaplasma* 16S rRNA), and OL451869-OL451871 (*Theileria* 18S rRNA).

Author's Contributions

Conceptualization: MNC, MWM

Data curation: All authors.

Formal analysis: All authors.

Investigation: SKG

Methodology: All authors

Project administration: SKG

Resources: SKG

Supervision: MNC, MWM

Validation: MNC, MWM

Visualization: SKG, MNC, MWM

Writing-original draft: All authors.

Writing-review & editing: All authors.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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