Pathogen prevalence in ticks collected from the vegetation and livestock in Nigeria

Running title: Tick-borne pathogens in Nigeria

Anna L. Reye\textsuperscript{1*}, Olatunbosun G. Arinola\textsuperscript{2*}, Judith M. Hübschen\textsuperscript{1} and Claude P. Muller\textsuperscript{1*}

\textsuperscript{1} Institute of Immunology, Centre de Recherche Public de la Santé / National Public Health Laboratory, Luxembourg, Luxembourg

\textsuperscript{2} Department of Chemical Pathology and Immunology, College of Medicine, University of Ibadan, Ibadan, Nigeria

\textsuperscript{*} Both authors contributed equally to this work

\textsuperscript{*} Corresponding Author:

Prof. Dr. Claude P Muller
Institute of Immunology, Centre de Recherche Public de la Santé / National Public Health Laboratory
20A, rue Auguste-Lumiére
L-1950 Luxembourg, Luxembourg
Tel: +352 490604 220, Fax: +352 490686
Email: Claude.Muller@LNS.ETAT.LU

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

AEM Accepts, published online ahead of print on 10 February 2012
Downloaded from http://aem.asm.org/ on September 12, 2017 by guest
Abstract

Ticks are important disease vectors that can cause considerable economic losses by affecting animal health and productivity, especially in tropical and subtropical regions. In this study, we investigated the prevalence and diversity of bacterial and protozoal tick-borne pathogens in ticks collected from the vegetation and cattle in Nigeria by PCR. The infection rates of questing ticks were 3.1% for *Rickettsia* species, 0.1% for *Coxiella burnetii* and 0.4% for *Borrelia* species. Other pathogens like *Babesia*, *Theileria*, *Anaplasma* and *Ehrlichia* species were not detected in ticks from the vegetation. Feeding ticks collected from cattle displayed infection rates of 12.5% for *Rickettsia* species, 14% for *Coxiella burnetii*, 5.9% for *Anaplasma* species, 5.1% for *Ehrlichia* species and 2.9% for *Theileria mutans*. *Babesia* and *Borrelia* species were not detected in ticks collected from cattle. Mixed infections were found only in feeding ticks and mainly *Rickettsia* species and *Coxiella burnetii* were involved. The diversity of tick-borne pathogens in Nigeria was higher in feeding than in questing ticks, suggesting that cattle serve as reservoirs for at least some of the studied pathogens, in particular *C. burnetii*. The total estimated infection rates of herds of 20.6% for a *Rickettsia africæ*-like species, 27% for *Coxiella burnetii* and 8.5% for *Anaplasma marginale/centrale* suggest that these pathogens may have considerable implications for human and animal health.
Introduction

Ticks are important disease vectors that can cause considerable economic losses by affecting animal health and productivity, especially in tropical and subtropical regions (28, 32, 37). In Africa, the tick fauna is remarkably diverse with about fifty endemic tick species that are known to infest domestic animals (38). However, the highest impact on livestock health is caused by species belonging to only three genera, namely *Amblyomma*, *Hyalomma* and *Rhipicephalus* (28). Damage is either direct (skin lesions, impairment of animal growth) or indirect by transmission of a variety of pathogens (32). Major economical impact has been associated with the four tick-borne diseases anaplasmosis, heartwater, babesiosis and theileriosis, all of which are prevalent in Africa (4).

Bovine anaplasmosis is caused by the highly pathogenic species *Anaplasma marginale* sensu stricto and the naturally attenuated *A. marginale* subspecies *centrale* (2, 7). *Anaplasma* species are commonly detected in cattle and seroprevalence rates between 4.6% (Kenya) to 98% (South Africa) from different sub-Saharan countries are reported (4, 18, 26, 31). The causative agents of bovine babesiosis and theileriosis have been frequently detected in blood smears of cattle in Ghana, with prevalences as high as 97% for *Theileria mutans*, 87% for *Theileria velifera* and 61% for *Babesia bigemina* (4). Tick-borne human ehrlichiosis of varying severity are caused by *Ehrlichia chaffeensis* and *E. ewingii* (24). Several human pathogenic tick-borne *Rickettsia* species have been found in Africa including *Rickettsia conorii conorii*, *R. conorii caspia*, *R. africae*, *R. aeschlimannii*, *R. massiliae*, *R. akari* and *R. sibirica mongolotimonae* (8, 19, 27). Humans are frequently infected with *Rickettsia* species in Senegal, Burkina Faso, Cameroon, Mali and the Ivory Coast, where seroprevalence rates from 17-36% have been reported (16). *Coxiella burnetii* causes Q fever in humans and high serological prevalences have been reported from West African countries (17). Although transmission mainly occurs via contact with infected reservoir hosts (domestic goats, sheep...
and cows), C. burnetii can also be transmitted by ticks. The most important borrelial infection of humans in Africa is relapsing fever transmitted either by lice (louse-borne relapsing fever) or soft ticks (tick-borne relapsing fever, TBRF). TBRF is caused by at least 16 Borrelia species, of which Borrelia crocidura seems to be of increasing importance in West Africa (36). In Ghana, 15% of blood smears from cattle were positive for Borrelia species (4).

Pathogens belonging to the genera of Anaplasma, Ehrlichia, Coxiella, Rickettsia, Babesia, Theileria and Borrelia have been reported in ticks from some West African countries. In Mali, Niger, Mauretania and Cameroon, feeding ticks from cattle were analysed for Rickettsia species and prevalence rates ranging from 7.4 to 75% were observed (21, 27). In Cameroon, the prevalence of Ehrlichia species in ticks removed from dogs was found to be 56% for E. chaffeensis, and 6% for E. canis (24). However, it is important that these studies on feeding ticks are complemented by pathogen prevalence studies in unfed (questing) ticks collected from the vegetation to estimate the risk of infection after tick bites during the next blood meal. So far, throughout West Africa only a single study investigated questing Amblyomma variegatum ticks from Burkina Faso for Ehrlichia ruminantium and reported a prevalence rate of 3.7% (1).

Thus, studies on tick-borne pathogens in ticks are fairly limited in West Africa. Here we present the first comprehensive study on the diversity of bacterial and protozoal tick-borne pathogens in questing and feeding ticks from Nigeria.
Materials and Methods

In 2009, questing and feeding ticks were collected in Oyo State, Southwestern Nigeria. The field collection sites were located within a 65 km radius of Ibadan, while the collection sites of feeding ticks were located within a 20 km radius. Questing ticks were collected from the vegetation at seven locations (Elepo, Alowo-nle, Fuleni, Orisunbare, Lanlate, Maya, Igbo-Ora) by cloth dragging and by direct hand-picking from their questing location. Collection sites included rain forest, derived savannah, shrubs and herbaceous (mainly graminoid) plant cover and displayed no notable topographical or climatic differences. Feeding ticks were obtained from 11 herds comprising of 1 to 13 cattle at four locations (Moniya, Alakia, Bodija, Mokola). Collection was performed throughout the year, but intensified in the wet season for questing ticks, especially in June and July (67.6%; 473/700). The collection of feeding ticks was most intense from January to March (80.9%; 110/136).

Ticks were stored in 70% ethanol at 4°C until further processing. For molecular analysis, 700 ticks from the vegetation (100 ticks per region) and 136 ticks from 62 cows were randomly selected and morphologically identified to the species level (38). The ticks were washed three times in phosphate buffered saline, rinsed with distilled water and dried on sterile filter paper before disruption and homogenization. Ticks were crushed individually in 300μl lysis buffer from the InviMag Tissue DNA Mini kit (Invitek, Berlin, Germany) using the TissueLyser II (Qiagen, Venlo, Netherlands) and DNA extraction was performed with the KingFisher 96 Magnetic Particle Processor (Thermo Scientific, Waltham, Massachusetts, USA) following the manufacturers’ instructions.

Detection PCRs were carried out using family-specific primers for members of the Rickettsiaceae and Piroplasmidae and species-specific primers for Coxiella burnetii as described before (references given in Table 1). The primers for the detection of Anaplasmataceae were modified to be genus specific for Anaplasma and Ehrlichia. The
Borrelia spp. primers were adapted to allow amplification of Relapsing Fever Group and Lyme Disease Spirochetes (Table 1). Primers directed against the flagellar B gene of Borrelia burgdorferi sensu lato group were used for further characterization of detected Borrelia species (Table 1). The resulting PCR amplicons of the right fragment size were either directly purified (Jet Quick PCR Purification Spin kit, Genomed, Loehne, Germany) or excised from a 1.5% agarose gel (QIAquick Gel Extraction Kit, Qiagen, Venlo, NL). Sequencing was performed as described before (30). Neighbour-joining phylogenetic analyses using the Kimura 2-parameter model with 1,000 bootstrap replicates and pairwise deletion were performed using MEGA v.4.0.2 software (13). Statistical analyses of differences in the prevalence rates between feeding and questing ticks were performed with Fisher’s exact test (in case of sampling number < 5) or Pearson's goodness of fit Chi-square (GFX) test (P values are given in Table 3 and 4).

Sequences are available at NCBI under accession numbers JN871727 - JN871848 for Rickettsia species, JN871849 - JN871863 for Coxiella burnetii, JN871864 - JN871869 for Anaplasma species, JN871870 - JN871872 for Borrelia species, JN871873 - JN871879 for Ehrlichia species and JN871880 - JN871883 for Theileria mutans.

Results

The 836 analysed ticks were comprised of four species. The predominant species on cattle were Rhipicephalus (Boophilus) annulatus (37.5%, n = 51) and Amblyomma variegatum (33.8%, n = 46), followed by Hyalomma impeltatum (14.7%, n = 20) and Rhipicephalus evertsi (14%, n = 19). From the vegetation, only Rh. evertsi (n = 700) was collected. Mainly adult ticks were collected from both environmental sources (males: 45.1%, females: 53.5%, nymphs: 1.4%).

Anaplasmataceae. Members of the Anaplasmataceae were detected in 11% (15/136) of ticks removed from cattle, with Anaplasma marginale subspecies being the most prevalent (53.3%;
Both *Ehrlichia ewingii* and *Ehrlichia chaffeensis* were detected in a single tick only (Figure 1A). In five ticks, not clearly identifiable *Ehrlichia* species with 99% sequence homology to *Ehrlichia ewingii* (3/5) or *Ehrlichia ruminantium* (2/5) were found. All four tick species were found to harbour *Anaplasma* species (*Rh. [Bo.] annulatus*: *A. marginale* ssp. *n = 7* and *E. ewingii* *n = 1*; *Hy. impeltatum*: *A. marginale* ssp. *n = 1*, *E. chaffeensis* *n = 1* and *E. ewingii* *n = 1*; *Am. variegatum*: *Ehrlichia* sp. *n = 1* and *Rh. evertsi*: *Ehrlichia* sp. *n = 3*). In total, 45.5% (5/11) of cattle herds were infected with *A. marginale* subspecies, 45.5% (5/11) with *Ehrlichia* sp., 9.1% (1/11) with *E. chaffeensis* and 9.1% (1/11) with *E. ewingii* (see also Table 2). In most cases, only one tick from one cow per herd was infected, except for *A. marginale* subspecies.

Ticks from the vegetation were not found to be infected with *Anaplasma* bacteria. However, two sequences with highest nucleotide similarity of 99% to an uncultured alpha proteobacterium (GenBank accession number AY254690) were recovered from two questing *Rh. evertsi* ticks collected in Lanlate (Figure 1A, Table 3).

*Rickettsiaceae*. *Rickettsia* species were detected in 12.5% (17/136) of ticks from cattle and in 3.1% (22/700) of ticks from the vegetation. In feeding ticks, a *Rickettsia africae*-like species (RAL) was predominant (82.4%; 14/17), all sequences of which showed a nucleotide homology of 99 to 100% to *Rickettsia africae*. *Rickettsia aeschlimannii* was the second predominant *Rickettsia* species (17.6%; 3/17). In at least one tick of each species, members of the *Rickettsiaceae* were detected: *Am. variegatum* (RAL, *n = 10*; *R. aeschlimannii*, *n = 1*), *Rh. (Bo.) annulatus* (RAL, *n = 1*; *R. aeschlimannii*, *n = 1*), *Rh. evertsi* (RAL, *n = 1*; *R. aeschlimannii*, *n = 1*) and *Hy. impeltatum* (RAL, *n = 2*). In 63.6% (7/11) of herds, RAL was detected in ticks. In most cases, more than one tick from more than one cattle per herd was positive and the estimated infection rate of cattle in positive herds ranged from 15.4 to 50% (Table 2). *R. aeschlimannii* was detected in 27.3% (3/11) of herds. Two nymphal
Am. variegatum ticks were infected with *Rickettsia* species, namely RAL (8.3%; 1/12) and *R. aeschlimannii* (8.3%; 1/12).

The predominant species *Rickettsia massiliae* was detected in 3% (21/700) of questing *Rh. evertsi* ticks from all locations (Table 3). In 0.1% (1/700) of ticks, a *Rickettsia* species belonging to the *Rickettsia rickettsii* group was detected (Figure 1B, Table 3).

Piromplasmdae. *Theileria mutans* was the only species of *Piromplasmdae* detected in ticks from Nigeria (Figure 1C). It was exclusively detected in feeding ticks and its prevalence ranged from 0.7% (1/136) in *Hy. impeltatum* to 2.2% (3/136) in *Rh. (Bo.) annulatus*. The infected ticks originated from three cattle of the same herd in Moniya (Table 2).

*Coxiella burnetii*. In 14% (19/136) of feeding ticks, *Coxiella burnetii* was detected (Figure 1D). Again, at least one tick of each species was found to harbour *C. burnetii* (*Am. variegatum*, n = 9; *Rh. (Bo.) annulatus*, n = 5; *Hy. impeltatum*, n = 2; *Rh. evertsi*, n = 3). In total, 63.6% (7/11) of herds were infested with ticks positive for *Coxiella* and in most cases more than one cattle per herd was involved (Table 2). Three *Am. variegatum* nymphs from different cattle of the same herd in Moniya and one from Bodija were found to harbour *C. burnetii* (33.3%, 4/12). The only *C. burnetii* infected questing tick (0.1%; 1/700) was collected in Orisunbare (Table 3).

*Borrelia* species. *Borrelia* species were only found in questing *Rh. evertsi* ticks (0.4%; 3/700) collected in Maya (Table 3). *Borrelia* species identification was not possible with the sequence obtained from the 16S rRNA gene, which showed a nucleotide homology of 99% to members of the *Borrelia burgdorferi* sensu lato complex (Figure 1E). Further characterization of the *Borrelia* species using primers directed against the flagellar gene was also unsuccessful. In addition, 16S rRNA sequences from unknown organisms were detected in nine DNA extracts from questing *Rh. evertsi*. Three of these sequences had highest nucleotide similarity of 94 to 97% to the soil bacterium *Conexibacter woesel*; the remaining six...
sequences formed a separate cluster with 83 to 85% nucleotide homology to C. woesei (Figure 1F).

Mixed Infections. All mixed infections (1.3%; 11/836) were detected in feeding ticks and were predominantly formed by RAL and C. burnetii (36.4%; 4/11) as well as T. mutans and A. marginale ssp. (18.2%; 2/11; see also Table 4). Pathogen combinations found only once were E. chaffeensis and C. burnetii, Ehrlichia sp. and C. burnetii, as well as Ehrlichia sp. and RAL. Two triple infections formed by C. burnetii, T. mutans and A. marginale ssp. as well as C. burnetii, RAL and Ehrlichia sp. were found. Tick species, in which multiple infections were detected, included Am. variegatum (n = 5), Hy. impeltatum (n = 3), Rh. (Bo.) annulatus (n = 2) and Rh. evertsi (n = 1). Mixed infections mainly occurred in adult ticks (90.9%), but one nymphal Am. variegatum was found to be coinfecte...
of the investigated pathogens are widespread throughout Africa and represent a threat to both human and animal health (4, 19-25, 27). As expected, the infection rate for most of the pathogens was significantly higher in feeding than in questing ticks (Table 2), suggesting that a number of these pathogens originated from the cattle blood ingested before tick collection rather than from transstadially maintained infections acquired during earlier blood meals. Therefore, the detection of pathogens in feeding ticks cannot establish vector competence whereas infected unfed ticks have at least maintained the pathogen transstadially. Although the latter are more likely to serve as potential vectors of live pathogens, *in vitro* experiments are required to confirm this. The fact that tick species diversity was much higher in feeding ticks may contribute significantly to the low prevalence rate of pathogens observed in questing ticks. It may well be that *Rh. evertsi* is a less suitable vector of pathogens than other tick species, although vector competence has been reported for *Babesia, Theileria* and *Anaplasma* species (38).

The only study on the prevalence of *C. burnetii* in ticks from Africa was conducted in Senegal, where 0.7 to 6.8% of feeding ticks from cattle were found to be infected (17). This rate is considerably lower than the 14% of feeding ticks that we found to be infected. Interestingly, *C. burnetii* was frequently detected in multiple ticks collected from the same cow. In addition, the infection rate of feeding *Rh. evertsi* was considerably higher (15.8%; 3/19) than in questing *Rh. evertsi* (0.1%; 1/700) and also the infection rate of feeding nymphal *Am. variegatum* ticks (33.3%; 4/12) was higher as compared to adults of the same species (14.7%; 5/34). These observations and the difference between infection rates in questing and feeding ticks (0.1% vs. 14%, p < 0.01) may be a reflection of the reservoir status of cattle for *C. burnetii*. In Nigeria, *C. burnetii* seems to represent a considerable risk factor for those in contact with cattle. In Senegal, where the prevalence of *C. burnetii* in cattle is relatively low (3.6%), seroprevalence rates in humans can be as high as 21.4 to 51% (12, 17), suggesting
even higher prevalence rates in Nigeria, where an estimated 27% (17/63) of cattle were infected. Thus, both ticks and cattle must be considered as a considerable source of Q fever and a significant threat to human health in the region.

Eight *Anaplasma marginale/centrale* positive ticks were collected and the estimated prevalence in cattle was 9.5%, which is higher than the prevalence found in cattle blood smears (1.9%) from Nigeria (11). Based on the detection PCR used in the present study, it was not possible to differentiate between the highly pathogenic bovine *A. marginale* sensu stricto and the naturally attenuated *A. marginale* subsp. *centrale*, which is sometimes used as a vaccine (7). As the cattle in this study were not vaccinated, they must have been naturally infected with either one of these subspecies, but the risk of severe disease cannot be estimated.

*Theileria mutans*, the causative agent of benign bovine theileriosis, was detected in four *Rh. annulatus* and *Hy. impeltatum* ticks removed from three cows of the same herd in Moniya. This pathogen was not detected in any questing *Rh. evertsi* ticks from the seven field locations, suggesting that this tick species may not be a competent vector or that *T. mutans* has a limited distribution in Nigeria. It seems that in Nigeria, the estimated prevalence rate of cattle (4.8%; 3/63) is comparable to that of cattle blood smears from within the country (3.3%) (11). Interestingly, it is much lower than in Ghana, where 97% of cattle blood smears were found to be positive for *T. mutans* (4). However, all cattle came from an area within a 30 km radius, thus also reflecting only a focal prevalence of *T. mutans* in few cattle herds.

Interestingly, *Babesia* species were not detected in any of the analysed ticks, although reports on the seroprevalence of *Babesia bigemina* (29.4%) and *Babesia bovis* (14.1%) in Nigeria exist (3). However, this study was performed in Northern Nigeria in the 1980s, suggesting geographical as well as temporal differences in the distribution of *Babesia* species.
Different SFG *Rickettsia* species have been reported in ticks from cattle in Mali (16.2%), Niger (16.3%), Mauretania (0%) and Cameroon (74.7%). We detected *R. massiliae* and a member of the *R. rickettsii* group only in questing ticks (p < 0.05). This is compatible with the minor role of vertebrates in the perpetuation and survival of *R. massiliae* (15). In contrast, RAL and *R. aeschlimannii* were only detected in feeding ticks, indicating a potential role of cattle as hosts. The estimated prevalence rate of RAL in cattle from the same herd was high (15.4 to 50%), either supporting the suspected role of cattle as a reservoir or reflecting a high transovarial transmission rate of RAL in the local tick population. Further studies are warranted to unambiguously identify the involved *Rickettsia* species and clarify the life cycle of RAL.

Different *Borrelia* species have been described in Africa, most of which are transmitted by soft ticks (36). Ticks of the genus *Rhipicephalus* are known to transmit *Borrelia theileri* to cattle, causing bovine borreliosis. The 16S rRNA sequences of the *Borrelia* species detected in this study differed at least in three nucleotide positions from all known *Borrelia* sequences. These new sequences form a separate cluster within the *Borrelia burgdorferi* s.l. group, possibly belonging to a so far unknown *Borrelia* species. Unfortunately, further characterization based on another gene was unsuccessful.

Several sequences from unknown bacteria were obtained in the *Anaplasmataceae* and *Borrelia* detection PCRs. As these sequences showed highest similarities to soil bacteria, they were most likely derived from bacteria from the outside rather than the inside of the ticks.

We also report here for the first time mixed infections in feeding ticks from Western Africa involving mainly RAL and *C. burnetii*. Mixed infections involving *C. burnetii* may originate either from subsequent blood meals, co-feeding events, or feeding on coinfected hosts. In mixed infections involving *Rickettsia* species also transovarial transmission may play a role (15). Coinfections with multiple pathogens may complicate clinical diagnosis and treatment.
In the Northern hemisphere, symptoms of Lyme Borreliosis have been reported to be more diverse, intense and persistent in patients coinfected with either human anaplasmosis or babesiosis (34). As treatment with an additional antimicrobial is necessary for a Borreliosis/Babesiosis coinfection, misdiagnosis may lead to prolonged illness of patients (34). For other tick-borne human pathogens, including spotted fever group rickettsiae and *Coxiella burnetii*, the impact of coinfections on the severity of symptoms has not yet been assessed. No such information exists on coinfections in cattle, although coinfections with severe symptoms have been reported in dogs (9, 33).

The diversity of tick-borne pathogens in Nigeria was higher in feeding than in questing ticks, suggesting that cattle serve as reservoirs for at least some of the studied pathogens, in particular *Coxiella burnetii*. Investigations on the implications for human and animal health as well as on the economic impact of these infections are warranted to determine the cost benefit of vaccination of ruminants against *A. marginale marginale*, *C. burnetii* or ticks. Other preventive measures like removal of feeding ticks and the concerted use of acaricides also need to be assessed.
Acknowledgements

The authors want to thank the staff of the Department of Chemical Pathology and Immunology of the University of Ibadan and others for assisting in the tick collection as well as livestock traders and abattoir staff for allowing access to cattle.

This work was funded by the Ministry of Cooperation of the Grand-Duchy of Luxembourg and the Centre de Recherche Public-Santé. A.L. Reye was supported by a fellowship from the Bourse Formation Recherche and the Aides à la Formation Recherche of the Fonds National de la Recherche Luxembourg.
References


sanguineus
ticks from kennel-confined dogs in Limbe, Cameroon. Exp. 

W. McBride. 2007. Ehrlichia species in Rhipicephalus sanguineus ticks 
in Cameroon. Vector Borne Zoonotic Dis. 7:221-227.

borne diseases in smallholder farming systems in the western-Kenya 

2001. Detection and identification of spotted fever group Rickettsiae and 

Importance of ticks and their chemical and immunological control in 

Tickborne pathogen detection, Western Siberia, Russia. Emerg. Infect. 
Dis. 11:1708-1715.

Prevalence and seasonality of tick-borne pathogens in questing Ixodes 

Parasitol. 175:331-342.


33. Suksawat, J., Y. Xuejie, S. I. Hancock, B. C. Hegarty, P. 
Nilkumhang, and E. B. Breitschwerdt. 2001. Serologic and molecular 
evidence of coinfection with multiple vector-borne pathogens in dogs 

727.

Nguyen, T. Yamaguchi, H. Fukushi, N. Nagaoka, M. Akilyama, K. 

Tick-borne relapsing fever imported from West Africa: diagnosis by 
quantitative buffy coat analysis and in vitro culture of Borrelia 

37. Vesco, U., N. Knap, M. B. Labruna, T. Avsic-Zupanc, A. Estrada-
Pen, A. A. Guglielmine, G. H. Bechara, A. Gueye, A. Lakos, A. 
Grindatto, V. Conte, and D. De Meneghi. 2011. An integrated 
database on ticks and tick-borne zoonoses in the tropics and subtropics

Figure legend

Figure 1

Neighbour-Joining phylogenetic trees based on (A) a 263 nucleotide (nt) fragment of the 16S rRNA gene of Anaplasmataceae (nt 246466 - 246728 of CP001079.1), (B) a 339 nt fragment of the 17-kDa gene of Rickettsiaceae (nt 1194686 - 1195024 of CP000766.2), (C) a 226 nt fragment of the 18S rRNA gene of Piroplasmidae (nt 656 - 881 of HQ184411.1), (D) a 317 nt fragment of the htpB gene of Coxiella (nt 273435 - 273751 of CP000733.1), (E) a 323 nt fragment of the 16S rRNA gene of Borrelia species (nt 444099 - 443777 of CP002228.1) and (F) a 321 nt fragment of the 16S rRNA gene of Conexibacter wheesi (nt 834 - 1151 of NR_028979.1). Nigerian sequences are named with their unique identifier, tick species, geographic location and biological source. Compressed clusters containing sequences from Nigeria are marked with an asterisk. Only bootstrap values above 70 are shown.
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Primer name</th>
<th>Primer orientation</th>
<th>Target gene</th>
<th>5'-3' Sequence</th>
<th>Reference</th>
<th>[Primer] (µM)</th>
<th>[MgCl2] (mM)</th>
<th>Annealing temperature (°C)</th>
<th>Elongation time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaplasmataceae</td>
<td>EHR1</td>
<td>forward</td>
<td>16S rRNA</td>
<td>GAAMAAACGCTGGCCGCAAG</td>
<td></td>
<td>0.4</td>
<td>2</td>
<td>63</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>newEHR2*</td>
<td>reverse</td>
<td>16S rRNA</td>
<td>CAGCTTCTCAAGCTTCAGTC</td>
<td>(29)</td>
<td>0.8</td>
<td>2</td>
<td>59</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>EHR3</td>
<td>forward</td>
<td>16S rRNA</td>
<td>TGCTAGGAATCTCTGAGTA</td>
<td></td>
<td>0.8</td>
<td>2</td>
<td>59</td>
<td>45</td>
</tr>
<tr>
<td>Rickettsiaceae</td>
<td>Rr17k.1p</td>
<td>forward</td>
<td>17-kDa</td>
<td>TTTACAAAATTCTAAAAACCAT</td>
<td></td>
<td>0.8</td>
<td>2</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Rr17k.539n</td>
<td>reverse</td>
<td>17-kDa</td>
<td>TCAATTCACAACTTCGATT</td>
<td></td>
<td>0.8</td>
<td>2</td>
<td>54</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Rr17k.90p</td>
<td>forward</td>
<td>17-kDa</td>
<td>GCTCTTGAAACTTCATGTT</td>
<td>(10)</td>
<td>0.8</td>
<td>2</td>
<td>54</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Rr17k.539n</td>
<td>reverse</td>
<td>17-kDa</td>
<td>TCAATTCACAACTTCGATT</td>
<td></td>
<td>0.8</td>
<td>2</td>
<td>54</td>
<td>45</td>
</tr>
<tr>
<td>Piroplasmidae</td>
<td>BI1</td>
<td>forward</td>
<td>18S rRNA</td>
<td>GTCTTCTGAACTTGATG</td>
<td></td>
<td>0.8</td>
<td>3</td>
<td>61</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>BN2</td>
<td>reverse</td>
<td>18S rRNA</td>
<td>TAGTTTAGGTTAGAGACTACG</td>
<td></td>
<td>(5)</td>
<td></td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Coxiella</td>
<td>Q5</td>
<td>forward</td>
<td>htpB</td>
<td>GGCGGCGAGTACACACCC</td>
<td></td>
<td>0.4</td>
<td>1.5</td>
<td>58</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Q3</td>
<td>reverse</td>
<td>htpB</td>
<td>GCCAACCTCAATTAGGCCC</td>
<td></td>
<td>0.4</td>
<td>1.5</td>
<td>58</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Q6</td>
<td>forward</td>
<td>htpB</td>
<td>TGCTGCACTGACACCCCA</td>
<td></td>
<td>0.8</td>
<td>2</td>
<td>56</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Q4</td>
<td>reverse</td>
<td>htpB</td>
<td>TCAAGCTCAAGCTGATG</td>
<td>(35)</td>
<td>0.8</td>
<td>2</td>
<td>56</td>
<td>30</td>
</tr>
<tr>
<td>Borrelia species</td>
<td>newLB*</td>
<td>forward</td>
<td>16S rRNA</td>
<td>GTAAACGAGTCACACTTGTTG</td>
<td></td>
<td>(14)</td>
<td>0.4</td>
<td>2</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>newLB*</td>
<td>reverse</td>
<td>16S rRNA</td>
<td>TGCCCCCTACACCGCACTCT</td>
<td></td>
<td>(14)</td>
<td>0.4</td>
<td>2</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Outer1</td>
<td>forward</td>
<td>flaB gene</td>
<td>AAAGAATGGCAAGCTCATC</td>
<td></td>
<td>0.8</td>
<td>2</td>
<td>59</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Outer2</td>
<td>reverse</td>
<td>flaB gene</td>
<td>GCATTCTATTCTAAGCTGATG</td>
<td></td>
<td>0.8</td>
<td>2</td>
<td>59</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Inner1</td>
<td>forward</td>
<td>flaB gene</td>
<td>ACAATTCAGTGACACAGAGTGCTA</td>
<td></td>
<td>0.8</td>
<td>2</td>
<td>59</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Inner2</td>
<td>reverse</td>
<td>flaB gene</td>
<td>GAAGGTCTCTAGCAGTGTCTGCTG</td>
<td></td>
<td>0.8</td>
<td>2</td>
<td>59</td>
<td>30</td>
</tr>
</tbody>
</table>

* Primer sequence modified
TABLE 2. Origin and number of herds, numbers of cattle and feeding ticks analysed and prevalence of detected pathogens. Asterisks mark total prevalence rates in ticks which differ significantly from those observed in ticks from vegetation (p < 0.05).

<table>
<thead>
<tr>
<th>Herd</th>
<th>Ticks (T/C)</th>
<th>Cattle</th>
<th>Inf. ticks (%)</th>
<th>Pot. Inf. cattle (%)</th>
<th>Inf. ticks (%)</th>
<th>Pot. Inf. cattle (%)</th>
<th>Inf. ticks (%)</th>
<th>Pot. Inf. cattle (%)</th>
<th>Inf. ticks (%)</th>
<th>Pot. Inf. cattle (%)</th>
<th>Inf. ticks (%)</th>
<th>Pot. Inf. cattle (%)</th>
<th>Inf. ticks (%)</th>
<th>Pot. Inf. cattle (%)</th>
<th>Inf. ticks (%)</th>
<th>Pot. Inf. cattle (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alakia</td>
<td>9 (1-2)</td>
<td>8</td>
<td>(11.1)</td>
<td>(12.5)</td>
<td>-</td>
<td>-</td>
<td>(11.1)</td>
<td>(12.5)</td>
<td>(1)</td>
<td>(12.5)</td>
<td>-</td>
<td>(100)</td>
<td>-</td>
<td>(100)</td>
<td>-</td>
<td>(100)</td>
</tr>
<tr>
<td>Aleshinloye</td>
<td>1 (1)</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bodija 1</td>
<td>7</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(11.1)</td>
<td>(14.3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bodija 2</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bodija 3</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moniya 1</td>
<td>13</td>
<td>1</td>
<td>(3)</td>
<td>(3.7)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(3)</td>
<td>(25)</td>
<td>(3)</td>
<td>(25)</td>
</tr>
<tr>
<td>Moniya 2</td>
<td>13</td>
<td>1</td>
<td>(3)</td>
<td>(3.7)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(3)</td>
<td>(25)</td>
</tr>
<tr>
<td>Moniya 3</td>
<td>11</td>
<td>2</td>
<td>(1-2)</td>
<td>(2.2)</td>
<td>(2.2)</td>
<td>(9.1)</td>
<td>-</td>
<td>-</td>
<td>(2.2)</td>
<td>(9.1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moniya 4</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moniya 5</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moniya 6</td>
<td>6</td>
<td>2</td>
<td>(1-2)</td>
<td>(2.2)</td>
<td>(2.2)</td>
<td>(9.1)</td>
<td>-</td>
<td>-</td>
<td>(2.2)</td>
<td>(9.1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>136</td>
<td>63</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>14</td>
<td>13</td>
</tr>
</tbody>
</table>

A. marginale/centrale; E. chaffeensis; E. ewingii; Ehrlichia sp.; R. aeschlimannii; RAL, Rickettsia africae-like species; T, Theileria; C, Coxiella; Inf., Infected; Pot., Potentially; -, not detected (0%).
### TABLE 3. Names of locations, numbers of questing ticks analysed and prevalence of detected pathogens. Asterisks mark total prevalence rates in ticks which differ significantly from those observed in ticks from cattle (p < 0.05).

<table>
<thead>
<tr>
<th>Location</th>
<th>Ticks</th>
<th>alpha proteobacterium (%)</th>
<th>R. massiliae (%)</th>
<th>RRG (%)</th>
<th>Borrelia sp. (%)</th>
<th>unknown bacterium (%)</th>
<th>C. burnetii (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alowo-nle</td>
<td>100</td>
<td>4 (4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Igbo Ora</td>
<td>100</td>
<td>2 (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fuleni</td>
<td>100</td>
<td>1 (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maya</td>
<td>100</td>
<td>2 (2)</td>
<td>-</td>
<td>1 (3)</td>
<td>2 (2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lanlate</td>
<td>100</td>
<td>2 (2)</td>
<td>-</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Elape</td>
<td>100</td>
<td>2 (2)</td>
<td>-</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Orisunbare</td>
<td>100</td>
<td>2 (2)</td>
<td>-</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>700</td>
<td>0.3 (8)</td>
<td>21 (3)*</td>
<td>1 (0.1)</td>
<td>3 (0.4)</td>
<td>9 (1.3)</td>
<td>1 (0.1)*</td>
</tr>
</tbody>
</table>

R., *Rickettsia*; RRG, *Rickettsia rickettsii* group; C., *Coxiella*; -, not detected (0%).

### TABLE 4. Mixed infections in ticks feeding on cattle and pathogen species involved. Ticks from the vegetation were not found to harbour multiple pathogens.

<table>
<thead>
<tr>
<th>Pathogen species involved in mixed infections</th>
<th>Ticks feeding on cattle (%)</th>
<th>Potentially infected cattle (%)</th>
</tr>
</thead>
</table>

R., *Rickettsia*; RRG, *Rickettsia rickettsii* group; C., *Coxiella*; -, not detected (0%).