Multiple Pathogens Including Potential New Species in Tick Vectors in Côte d’Ivoire

Cyrille Bilé Ehounoud1,2,3, Kouassi Patrick Yao3, Mustapha Dahmani1, Yaba Louise Achi4, Nadia Amanzougaghene1, Adèle Kacou N’Douba5, Jean David N’Guessan3, Didier Raoult1,2, Florence Fenollar1,2, Oleg Mediannikov1,2*

1 Aix-Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, Faculté de médecine, Marseille cedex 05, France, 2 Campus International UCAD-IRD, Dakar, Senegal, 3 Felix Houphouet Boigny Université, UFR Sciences médicales, Côte D’Ivoire, 4 Ecole de spécialisation en Elevage de Bingerville, Côte D’Ivoire, 5 Felix Houphouet Boigny Université, UFR Sciences médicales, Côte D’Ivoire

* oleguss1@gmail.com

Abstract

Background

Our study aimed to assess the presence of different pathogens in ticks collected in two regions in Côte d’Ivoire.

Methodology/Principal Findings

Real-time PCR and standard PCR assays coupled to sequencing were used. Three hundred and seventy eight (378) ticks (170 Amblyomma variegatum, 161 Rhipicephalus microplus, 3 Rhipicephalus senegalensis, 27 Hyalomma truncatum, 16 Hyalomma marginatum rufipes, and 1 Hyalomma impressum) were identified and analyzed. We identified as pathogenic bacteria, Rickettsia africae in Am. variegatum (90%), R. microplus (10%) and Hyalomma spp. (9%), Rickettsia aeschlimannii in Hyalomma spp. (23%), Rickettsia massiliae in Rr. senegalensis (33%) as well as Coxiella burnetii in 0.2%, Borrelia sp. in 0.2%, Anaplasma centrale in 0.2%, Anaplasma marginale in 0.5%, and Ehrlichia ruminantium in 0.5% of all ticks. Potential new species of Borrelia, Anaplasma, and Wolbachia were detected. Candidatus Borrelia africana and Candidatus Borrelia ivorensis (detected in three ticks) are phylogenetically distant from both the relapsing fever group and Lyme disease group borreliae; both were detected in Am. variegatum. Four new genotypes of bacteria from the Anaplasmataceae family were identified, namely Candidatus Anaplasma ivorensis (detected in three ticks), Candidatus Ehrlichia urmitei (in nine ticks), Candidatus Ehrlichia rustica (in four ticks), and Candidatus Wolbachia ivorensis (in one tick).

Conclusions/Significance

For the first time, we demonstrate the presence of different pathogens such as R. aeschlimannii, C. burnetii, Borrelia sp., A. centrale, A. marginale, and E. ruminantium in ticks in Côte d’Ivoire as well as potential new species of unknown pathogenicity.
Author Summary

The management of febrile illnesses represents a veritable challenge in Sub-Saharan Africa. Until recently most of them were considered as malaria. However, it was shown that a large part of non-malarial febrile diseases in African rural regions (for instance, in Senegal) may be caused by tick-borne infections. Unfortunately, no data exist about the prevalence and incidence of tick-borne diseases in Côte d'Ivoire and their role in public health. We aimed to search for different pathogenic bacteria in ticks in order to understand if there is the background for tick-borne diseases. We detected pathogenic bacteria responsible for many infectious diseases like *Rickettsia* (spotted fevers), *Borrelia* (relapsing fevers), *Anaplasma*, *Ehrlichia* (ehrlichiosis and anaplasmosis) and *Coxiella burnetii* (Q fever). These findings suggested that, as in others sub-Saharan African countries, tick-borne disease may be considered as a health care problem in Côte d'Ivoire.

Introduction

Ticks are important vectors of many pathogens and are considered as the second biggest vectors of human and animal diseases after mosquitoes [1,2]. Many tick-borne bacterial emerging diseases such as spotted fevers, borrelioses, anaplasmoses, ehrlichioses, and Q fever have been described worldwide [3,4,5]. It was recently shown that in many tropical countries tick- and acari-borne infections play important role in human pathology. In Senegal, for instance, arthropod-borne borreliosis and rickettsiosis were identified in 16.3% of acute fevers recorded by rural dispensaries [6]. Acari-borne tsutsugamushi fever is one of the major causes of acute febrile morbidity in South-Eastern Asia [7]. Investigations of the vectors of tick-borne diseases are one of the main keys to controlling related morbidity [8].

Rickettsioses, caused by bacteria belonging to the spotted fever group (SFG) of the genus *Rickettsia*, are considered among the oldest known vector-borne zoonotic diseases [9]. The most common rickettsia in Africa is *Rickettsia africae*, the etiological agent of African tick-borne fever [10]. This disease has been reported with high seroprevalence in sub-Saharan African countries including Cameroon (11.9% - 51.8%) and Senegal (21.4% - 51%) [11,12]. *R. africae* has been detected by PCR in ticks in Mali, Niger, Burundi, and Sudan [13]. *Amblyomma hebraeum* and *Amblyomma variegatum* ticks are the main reservoirs and vectors of *R. africae* in Southeastern Africa and sub-Saharan Africa, respectively [9,14]. It was also reported in other species of *Amblyomma* such as *Amblyomma lepidum* in Djibouti [15] and *Amblyomma compressum* in the Democratic Republic of Congo and Liberia [16,17]. In Western Africa, *R. africae* has been detected in several *Rhipicephalus* ticks including *Rhipicephalus annulatus* in Guinea, Senegal, and Nigeria [12,16,18], *Rhipicephalus evertsi evertsi* in Senegal and Nigeria [12,18], *Rhipicephalus decoloratus* in Nigeria [19], *Rhipicephalus geigyi* in Liberia [16], and *Hyalomma* spp. ticks including *Hyalomma impeltatum* in Nigeria [18] and *Hyalomma marginatum rufipes* in Guinea [16] but not in Côte d'Ivoire, where a strain of *R. africae* has been isolated from *Am. variegatum* [20].

*Rickettsia aeschlimannii* is an agent of spotted fever which was first identified in a patient returning from Morocco [21]. In this country, it was first isolated from *Hyalomma marginatum marginatum* ticks [22]. *R. aeschlimannii* was also reported by PCR in other *Hyalomma* ticks including *H. marginatum rufipes* and *Hyalomma truncatatum* ticks collected from camels and cows in Egypt, Algeria, Sudan, and Tunisia [23]. In Western Africa, *R. aeschlimannii* was also detected in 15% to 95% of *H. marginatum rufipes* from Mali, Niger, Senegal and Nigeria [12,13,24] and in 6% to 7% of *H. truncatum* from Senegal [12] but not in Côte d'Ivoire.
Rickettsia massiliae is another SFG rickettsia. Since its description in 2005, *R. massiliae* infections in humans have been confirmed in Europe and South America [25,26,27]. It is associated with *Rhipicephalus* ticks. *R. massiliae* was found by PCR in *Rhipicephalus* spp. ticks including *Rhipicephalus* spp. from Côte d’Ivoire [28], *Rhipicephalus guilhoni* from Senegal [12], *Rhipicephalus senegalensis* from Guinea [16], and *Rhipicephalus everesi* from Nigeria [18].

Different borrelioses are caused by bacteria from the *Borrelia* genus. They are traditionally classified into the Lyme disease group and the relapsing fever group. The former is ecologically associated with hard ticks and is mostly found in the temperate northern hemisphere [29]. Relapsing fever group borreliae are mostly associated with soft ticks and found in subtropical regions worldwide [30,31]. In endemic regions, borrelioses may play an important role, for example in Slovakia [32]. Relapsing fever is one of the most common diseases in several African regions including Senegal [33,34] and east African countries [35]. It is caused by different *Borrelia* species such as *Borrelia hispanica*, *Borrelia duttonii*, and *Borrelia crocidurae*. *B. hispanica* was recently detected in 11.6% to 20% of *Ornithodoros* ticks from northern Africa [31,36]. *B. crocidurae* is responsible for tick-borne relapsing fever in West Africa. Its distribution in the south is thought to be limited by the 750 mm isohyets [37]. Neither this borrelia nor any other from the relapsing group has been reported in Côte d’Ivoire. A controversial study, based on molecular data, reported 30 cases of borreliosis in Togo but its epidemiology was not identified [38] and studies in neighboring countries did not confirm the presence of borreliosis in west tropical sub-Saharan Africa. In Ethiopia, *Borrelia* sp. was recently identified by PCR in 7.3% of *Amblyomma cohaerens* [39]. Phylogenetically, this *Borrelia* sp. was placed in an intermediate position between Lyme disease and relapsing fever groups.

All bacteria from the *Anaplasmataceae* family are intracellular mammal parasites, arthropods nematodes, and trematodes [40]. *Anaplasma centrale* and *Anaplasma marginale* are two etiological agents of bovine anaplasmosis in ruminants [41]. These species are distributed in tropical and subtropical regions of Africa and naturally infect cattle [42]. They were previously found by molecular biology in ticks in neighboring Mali [43]. These bacteria are often found in *Dermacentor*, *Rhipicephalus*, and *Amblyomma* ticks throughout the world [44]. *Ehrlichia ruminantium* is responsible for cowdriosis in ruminants with the *Amblyomma* genus ticks as a vector [45]. Cowdriosis induces mortality in ruminants in sub-Saharan Africa and in islands in the Caribbean where it causes serious losses to animal production [40]. *E. ruminantium* was previously identified in *Am. variegatum* in Burkina Faso but not in Côte d’Ivoire. No cases of human ehrlichiosis or anaplasmosis have been reported in Africa, but recently human pathogens such as *Anaplasma phagocytophilum* have been reported in Senegal and Algeria [46,47]. Bacteria from the *Wolbachia* genus of the *Anaplasmataceae* family are associated with arthropods and filarial nematodes. They are responsible for reproductive alterations in arthropods which are indirectly (via nematodes) associated with human pathogenesis [40].

Finally, Q fever is a zoonotic disease caused by *Coxiella burnetii*. This bacterium may cause severe infections such as chronic endocarditis and abortion [48,49]. It infects humans usually by a direct contact with domestic animals such as cattle, sheep, goats, and dogs [50]. It was previously reported in *Amblyomma*, *Rhipicephalus*, and *Dermacentor* ticks [43]. In Senegal, *C. burnetii* was detected in 0.8% to 14.2% of ticks including *Am. variegatum*, *Rhipicephalus* spp., *Hyalomma* spp., and *Ornithodoros sonrai* [51] and may play a role in Q fever epidemiology. In Côte d’Ivoire, the seroprevalence was estimated at 3.4% [52].

Although these diseases have emerged in many African countries, they remain neglected. In Côte d’Ivoire, little information is available about these diseases and their epidemiology. To date, the existence and/or prevalence of tick-borne associated pathogens remain poorly understood. Our study provides the first data screening for multiple tick-borne associated pathogens in Côte d’Ivoire.
**Materials and Methods**

**Ethics statement**

To perform this study, an approval of Côte d’Ivoire Ethics committee was received under the number N°86/MSLS/CNERN-dkn.

**Period, study area and tick collection**

The tick collection was conducted over a period ranging from October 30 to November 8, 2014. Ticks were manually collected from cattle in two regions of Côte d’Ivoire: Savannah and Bandama Valley (Fig 1, Table 1). In total, 378 ticks (304 adults and 74 nymphs) were collected.

![Map of Côte d’Ivoire showing the regions, cities and villages where the ticks were collected for our study.](https://doi.org/10.1371/journal.pntd.0004367.g001)
Table 1. Geographic coordinates of tick collection sites.

| City¹ or village² | Geographic coordinates | Species | Number (male/female/nymphs) | Total number |
|-------------------|------------------------|---------|-----------------------------|--------------|
| **Savannah region** |                        |         |                             |              |
| Kong¹             | 08°15N 05°07W          | Rh. microplus | 3/10/0                      | 13           |
| Ferké¹            | 09°55N 05°20W          | Rh. microplus | 1/4/0                       | 5            |
|                   |                        | H. impressum  | 1/0/0                       | 1            |
|                   |                        | H. truncatum  | 0/1/0                       | 1            |
| Kafolo-bac²       | 09°43N 04°39W          | Am. variegatum | 1/0/5                      | 6            |
|                   |                        | Rh. microplus | 3/8/1                      | 12           |
|                   |                        | H. truncatum  | 1/0/0                       | 1            |
| Torokinkenin²     | 08°84N 04°47W          | Am. variegatum | 2/2/1                      | 5            |
|                   |                        | Rh. microplus | 4/10/0                     | 14           |
| Téhini²           | 09°60N 03°67W          | Am. variegatum | 2/1/0                      | 3            |
|                   |                        | Rh. microplus | 1/4/1                      | 6            |
|                   |                        | H. marginatum rufipes | 0/1/0 | 1 |
| Doropo²           | 09°77N 03°40W          | Am. variegatum | 2/0/9                      | 11           |
|                   |                        | Rh. microplus | 0/7/0                      | 7            |
|                   |                        | H. marginatum rufipes | 2/2/0 | 4 |
|                   |                        | H. truncatum  | 0/2/0                       | 2            |
| Naissain²         | 09°39N 04°48W          | Am. variegatum | 0/1/4                      | 5            |
|                   |                        | Rh. microplus | 1/4/0                      | 5            |
| Sikolo²           | 09°43N 04°66W          | Am. variegatum | 0/0/4                      | 4            |
|                   |                        | Rh. microplus | 1/4/0                      | 5            |
| Fasselemon²       | 09°27N 04°52W          | Am. variegatum | 4/1/0                      | 5            |
|                   |                        | Rh. microplus | 1/4/0                      | 5            |
| Nafana²           | 09°18N 04°78W          | Am. variegatum | 1/0/0                      | 1            |
|                   |                        | Rh. microplus | 1/1/0                      | 2            |
| Tindara²          | 09°54N 04°74W          | Am. variegatum | 4/2/11                     | 17           |
|                   |                        | Rh. microplus | 0/12/0                     | 12           |
|                   |                        | H. truncatum  | 1/0/0                       | 1            |
| Laleraba²         | 10°13N 05°08W          | Am. variegatum | 4/1/0                      | 5            |
|                   |                        | Rh. microplus | 0/5/0                      | 5            |
|                   |                        | H. truncatum  | 7/3/0                       | 10           |
|                   |                        | H. marginatum rufipes | 1/2/0 | 3 |
| Hamdalaye²        | 09°98N 05°13W          | Am. variegatum | 10/6/3                     | 19           |
|                   |                        | Rh. microplus | 1/4/0                      | 5            |
|                   |                        | H. truncatum  | 7/2/0                       | 9            |
|                   |                        | H. marginatum rufipes | 2/1/0 | 3 |
| Monogon²          | 09°82N 04°92 W         | Am. variegatum | 2/3/0                      | 5            |
|                   |                        | Rh. microplus | 2/4/0                      | 6            |
|                   |                        | H. truncatum  | 1/2/0                       | 3            |
|                   |                        | H. marginatum rufipes | 2/3/0 | 5 |
| **Bandama Valley region** |                |         |                             |              |
| Dabakala¹         | 08°36N 04°41W          | Am. variegatum | 10/2/2                     | 14           |
|                   |                        | Rh. microplus | 2/4/0                      | 6            |
| Katiola¹          | 08°15N 05°07W          | Am. variegatum | 3/0/4                      | 7            |
|                   |                        | Rh. microplus | 1/4/0                      | 5            |
| Niakara¹          | 06°60N 05°29W          | Am. variegatum | 8/2/9                      | 19           |

(Continued)
from three cities and 12 villages in the Savannah region and three cities and seven villages in the Bandama Valley region (Table 1). Ticks were stored in 70% ethanol until morphological and molecular analyses in laboratory of URMITE, Marseille (France). The species and sex of the ticks were identified according to standard taxonomic keys for adult ticks [2].

### DNA extraction and real-time PCR

Total DNA from half of each tick was extracted using the EZ1 DNA tissue kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. DNA extracts were stored at +4°C until use. Bacterial DNA was initially detected using bacterial genus-specific or species-specific quantitative real-time PCRs (qPCRs) targeting: *Rickettsia* spp., *R. africae*, *R. aeschlimannii*, *R. massiliae*, *Borrelia* spp., *Anaplasmataceae* spp., *A. phagocytophilum*, *Bartonella* spp., *C. burnetii*, and *Spiroplasma* spp. (Table 2). Samples with a high discordance in the cycle threshold number (Ct) for *Rickettsia* spp. and *R. africae* (in all cases, low Ct for *Rickettsia* spp. and high Ct for *R. africae*) were subjected to specific qPCRs for two other rickettsial species: *R. aeschlimannii* and *R. massiliae* in order to identify possible co-infection. qPCRs were performed using a CFX 96 Real Time System (Bio-Rad, Marnes-la-Coquette, France) and the Eurogentec MasterMix Probe PCR kit (Eurogentec, Liège, Belgium). PCR tests were considered to be positive when the Ct was lower than 35 Ct [22]. In addition, two different specific qPCRs targeting two different sequences had to be positive in order to confirm the presence of a bacterium in the ticks. Positive controls (bacterial DNA) and negative controls (master mix or water) were used to validate the PCR runs.

### Table 1. (Continued)

| City or village | Geographic coordinates | Species | Number (male/female/nymphs) | Total number |
|----------------|------------------------|---------|-----------------------------|--------------|
| Darala²         | 08°44N 04°35W          | Am. variegatum | 2/1/0                      | 3            |
|                 |                        | Rh. microplus   | 1/4/0                      | 5            |
|                 |                        | Rh. senegalensis| 1/1/0                      | 2            |
| Ouandarama²     | 08°68N 04°39W          | Am. variegatum | 2/0/4                      | 6            |
|                 |                        | Rh. microplus   | 1/4/1                      | 6            |
|                 |                        | Rh. senegalensis| 0/1/0                      | 1            |
| Darakokaha²     | 08°27N 05°16W          | Am. variegatum | 1/0/0                      | 1            |
|                 |                        | Rh. microplus   | 1/4/0                      | 5            |
| N’golodougou²   | 09°15N 05°12W          | Am. variegatum | 4/2/0                      | 6            |
|                 |                        | Rh. microplus   | 2/7/0                      | 9            |
| Kolokaha²       | 08°97N 05°21W          | Am. variegatum | 2/2/0                      | 4            |
|                 |                        | Rh. microplus   | 1/4/0                      | 5            |
| Sépikaha²       | 08°91N 05°03W          | Am. variegatum | 1/0/9                      | 10           |
|                 |                        | Rh. microplus   | 2/4/2                      | 8            |
| Petionara²      | 08°47N 05°03W          | Am. variegatum | 72/29/69                   | 170          |
|                 |                        | Rh. microplus   | 34/122/5                   | 161          |
|                 |                        | Rh. senegalensis| 1/2/0                      | 3            |
|                 |                        | H. truncatum    | 17/10/0                    | 27           |
|                 |                        | H. marginatum rupes | 7/9/0                    | 16           |
|                 |                        | H. impressum    | 1/0/0                      | 1            |
| Total of ticks for the two regions | | | 132/172/74 | 378 |

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Standard PCR and sequencing

Most of samples which were considered positive by qPCRs were subsequently subjected to standard PCR. All samples which were positive using *Rickettsia* genus-specific but negative with *R. africae* qPCR were subjected to standard PCR to amplify a portion of the *ompA* gene. We also chose two positive ticks for *R. africae* by species to confirm the presence of *R. africae* by standard PCR. The primers used (190.70, 190.180, and 190.701) amplified a 632-bp fragment of the *Rickettsia ompA* gene [60]. For the identification of *Borrelia* species, primers targeting a portion of the *flaB* gene were used [33]. *Anaplasmataceae* spp. (*Anaplasma* spp., *Ehrlichia* spp., and *Wolbachia* spp.) were identified using Ana 212f and Ana 753r primers targeting a 500 bp portion of the 23S rRNA gene [47].

Table 2. Primers and probes used for real-time quantitative PCR in this study.

| Microorganisms | Targeted sequence | Primers f, r (5'-3') and Probes p (6FAM–TAMRA) | References |
|----------------|-------------------|------------------------------------------------|------------|
| *Rickettsia* spp. | *gltA* (RKND03) | f_GTGAATGAAAGATTACACTATTTAT | [53] |
|                 |                   | r_GTATCTTACATCATTCTAATAGC | |
|                 |                   | p_CTATTTGCGGTGCCTGCGGTTT | |
| *R. africae*    | poT15-dam2        | f_TGCAACAGGAAGCAGAAAC | [6] |
|                 |                   | r_CCTTCGGCAAACTCCTAC | |
|                 |                   | p_TGACGTCGATCCATACCGGA | |
| *R. aeschlimannii* | Intergenic spacer (RaescSca1) | f_AAAGAATGGATTTTCACGGCAGA | [12] |
|                 |                   | r_AACCGTAAACGTCCGTAAGC | |
|                 |                   | p_TGCGGAAATATGCGGTACGCAAGC | |
| *R. massiliae*  | Hypothetical protein | f_CCAACCTTTTTGTGTGTGA | [54] |
|                 |                   | r_TGCTGAGTCTGGACGACGACT | |
|                 |                   | p_CAGTGCTGCTTATACCGAAACCA | |
| *Anaplasma* spp. | 23S rRNA (TtAna) | f_TGACAGCCTACCTTTGTCA | [47] |
|                 |                   | r_TGGAGGACCGAACCTGTTTAC | |
|                 |                   | p_GGATTAGACCCAAACCA ACT | |
| *Anaplasma phagocytophilum* | apaG | f_TAAGCCGAGTTGGAAGATCA | [55] |
|                 |                   | r_CGCAACACCCATAAAAAA | |
|                 |                   | p_TGATGAAGCGGCTGCCGATCAG | |
| *Spiroplasma*   | rpoB              | f_GTGTTGGACCAAAACGGAATGGG | [55] |
|                 |                   | r_CCAAAATGGTTGTGGTGGG | |
|                 |                   | p_GCTAACCCTGCTTTAATGGG | |
| *Coxiella burnetii* | Insertion Sequence (IS1111) | f_CAAGAAACCTGATGCTGGC | [56] |
|                 |                   | r_CACAGAGCCACGGTGAAC | |
|                 |                   | p_CCAAGTTCGAAAACATGAGGCGT | |
|                 | (IS30A)           | f_GGCATGGCTACAGAATAGTCC | [57] |
|                 |                   | r_GGGATTAGAAATATACCTTGTGG | |
|                 |                   | p_CATGAAAGCGATTATTCAAAATCGTATGC | |
| *Bartonella* spp. | Internal transcribed spacer16S (BartoITS3) | f_GATGGCCGAGGGAGGTTC | [58] |
|                 |                   | r_GCCTGAGGAGACTGACCTC | |
|                 |                   | p_GCGGCGGCTGTAAGCGGTT | |
| *Borrelia* spp. | Internal transcribed spacer 16S RNA (Bor ITS4) | f_GGCCTCCGTGGTACCATCATCTA | [59] |
|                 |                   | r_CCGGGAGGGAGTGAATAGG | |
|                 |                   | p_TGCAAAGGAGCGCCTC | |
|                 | (Bor_16S)         | f_AGCGTTAAGCTGCTGATGTA | [34] |
|                 |                   | r_GCCTCCGTAGAGCTGGTGG | |
|                 |                   | p_CCGGCGGCTGAGGGTGAAC | |

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Standard PCR was performed on a ThermalCycler (Applied Biosystem, Paris, France). The reactions were carried out using the Hotstar Taq-polymerase (Qiagen), in accordance with the manufacturer’s instructions. The amplicons were visualized using electrophoresis on a 1.5% agarose gel stained with ethidium bromide and examined using an ultraviolet transilluminator. The PCR products were purified using a PCR filter plate Millipore NucleoFast 96 PCR kit following the manufacturer’s recommendations (Macherey–Nagel, Düren, Germany). The amplicons were sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) with an ABI automated sequencer (Applied Biosystems). The sequences which were obtained were assembled using ChromasPro software (ChromasPro 1.7, Technelysm Pty Ltd., Tewantin, Australia) and compared with those available in GenBank by NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Phylogenetic analysis

DNA sequences alignment was carried out using MEGA 6 (http://www.megasoftware.net/mega.php). We selected the Bayesian method [61] using TOPALi 2.5 software (Biomathematics and Statistics Scotland) to construct phylogenetic trees.

Results

Of the 378 ticks identified, 170 Am. variegatum, 161 Rh. microplus, 3 Rh. senegalensis, 27 H. truncatum, 16 H. marginatum rufipes, and one H. impressum were analyzed. No A. phagocytophilum, Bartonella spp. and Spiroplasma spp. were detected in ticks. Rickettsia spp. was found in 187 of 378 ticks (49%); most of them, 174/378 (46%), were identified as R. philum (Table 3). To confirm the presence of R. philum positive ticks per species. The BLAST search of the sequences of R. philum, previously detected in Antigua (GenBank EU622980) and compared with those available in GenBank by NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

C. burnetti was detected in one tick (Table 3). Screening of all ticks for Borrelia spp. using qPCR, detected 16/378 (4%) positive ticks. We succeeded in amplifying a fragment of flaB gene and 16S rRNA sequence only in 4/378 (1%) ticks. A BLAST search showed that these sequences probably belong to an undescribed species, because only 87% (288/329 bp), 87% (287/328 bp), 97% (319/328 bp), and 87% (288/329 bp) similarities were observed with, respectively, the flaB gene of Borrelia duttonii (GenBank AB105132), Borrelia sp. IA-1 (GenBank EU492387), Borrelia sp. BrFlab (GenBank EF141022), and Borrelia sp. IA-1 (GenBank EU492387). The
The phylogenetic position of this *Borrelia* is shown in Fig 2. Because these potentially new species had not previously been isolated, we propose the provisional names *Candidatus* Borrelia africana for the genotype TCI22 and *Candidatus* Borrelia ivorensis for the genotypes TCI140 and TCI351. In a phylogenetic tree based on a 344 bp fragment of the *Borreliae flaB* gene, the sequences of *Candidatus* Borrelia africana and *Candidatus* Borrelia ivorensis are situated in the

### Table 3. Prevalence of positive ticks by PCR.

| Bacterium% (positives/tested) | *Am. variegatum* | *Rh. microplus* | *Rh. senegalensis* | *H. truncatum* | *H. marginatum* | *H. impressum* | Total |
|-------------------------------|------------------|-----------------|--------------------|----------------|-----------------|----------------|-------|
| *Rickettsia* spp.             | 90% (154/170)    | 10% (16/161)    | 33% (1/3)          | 15% (4/27)     | 69% (11/16)     | 100% (1/1)     | 49% (187/378) |
| *R. africae*                  | 90% (154/170)    | 10% (16/161)    | -                  | 4% (1/27)      | 12% (2/16)      | 100% (1/1)     | 46% (174/378) |
| *R. aeschlimannii*            | -                | -               | 11% (3/27)         | 44% (7/16)     | -               | -              | 2% (10/378)  |
| *R. massiliae*                | -                | -               | 33% (1/3)          | -              | -               | -              | 0.2% (1/378) |
| *C. burnetii*                 | 0.6% (1/170)     | -               | -                  | -              | -               | -              | 0.2% (1/378) |
| *Borrelia* spp.               | 6% (11/170)      | 2% (3/161)      | -                  | 4% (1/27)      | 6% (1/16)       | -              | 5% (16/378)  |
| *Borrelia* sp. genotype TCI301| -                | 0.6% (1/161)    | -                  | -              | -               | -              | 0.2% (1/378) |
| *Candidatus* Borrelia ivorensis | 1% (2/170)    | -                | -                  | -              | -               | -              | 0.5% (2/378) |
| *Candidatus* Borrelia africana | 0.6% (1/170)   | -                | -                  | -              | -               | -              | 0.2% (1/378) |
| *Anaplasma* spp.              | 12% (21/170)     | 24% (39/161)    | -                  | 11% (3/27)     | -               | -              | 16% (63/378) |
| *Anaplasma centrale*          | 0.6% (1/170)     | -                | -                  | -              | -               | -              | 0.2% (1/378) |
| *Anaplasma marginale*         | -                | 1% (2/161)      | -                  | -              | -               | -              | 0.5% (2/378) |
| *Candidatus* Anaplasma ivorensis | 2% (3/170)   | -                | -                  | -              | -               | -              | 0.8% (3/378) |
| *Ehrlichia* sp.               | 3% (6/170)       | 3% (5/161)      | -                  | 7% (2/27)      | -               | -              | 3% (9/378)  |
| *Candidatus* Ehrlichia rustica | 0.6% (1/170)   | 1% (2/161)      | -                  | 4% (1/27)      | -               | -              | 1% (4/378)  |
| *Candidatus* Ehrlichia urmiente | 3% (5/170)   | 2% (3/161)      | -                  | 4% (1/27)      | -               | -              | 2% (9/378)  |
| *Ehrlichia ruminantium*       | 1% (2/170)       | -                | -                  | -              | -               | -              | 0.5% (2/378) |
| *Candidatus* Wolbachia ivorensis | -                | 0.6% (1/161)    | -                  | -              | -               | -              | 0.2% (1/378) |
| *R. africae +*R. aeschlimannii | -                | -                | -                  | 12% (2/16)     | -               | -              | 0.5% (2/378) |
| *R. africae +*R. massiliae    | -                | -                | 33% (1/3)          | -              | -               | -              | 0.2% (1/378) |
| *R. africae +*C. burnetii     | 0.6% (1/170)     | -                | -                  | -              | -               | -              | 0.2% (1/378) |
| *R. africae +Borrelia sp.     | -                | 0.6% (1/161)    | -                  | -              | -               | -              | 0.2% (1/378) |
| *R. africae + Candidatus Borrelia Africana + Candidatus Borrelia ivorensis* | 2% (3/170) | - | - | - | - | - | 0.8% (3/378) |
| *R. africae + Anaplasma centrale* | 0.6% (1/170) | - | - | - | - | - | 0.2% (1/378) |
| *R. africae + Anaplasma marginale* | - | - | - | - | - | - | 0.2% (1/378) |
| *R. africae + Candidatus Anaplasma ivorensis* | 1% (2/170) | 0.6% (1/161) | - | - | - | - | 0.8% (3/378) |
| *R. africae + Candidatus Ehrlichia urmiente* | 1% (2/170) | - | - | - | - | - | 0.5% (2/378) |

= 0%; the name *Candidatus* is employed here for the new species because they are not isolated.

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Borrelia genus near Borrelia sp. from Ethiopian Amblyomma cohaerens (GenBank JX089967) and are closer to the relapsing fever group than to that of Lyme disease. As previously shown, Ethiopian Borrelia group together with these new genotypes to form a separate and

Fig 2. flaB gene-based phylogenetic analysis of the strains identified in this study. Phylogenetic tree highlighting the position of Borrelia sp. identified in the present study relative to borrelia type strains and uncultured borreliae. The flaB sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained from a Bayesian phylogenetic analysis with the HKY+Γ, JC+Γ and HKY+Γ substitution models for the first, second and third codons respectively. The GenBank accession numbers are indicated at the end. Sequences obtained in the present study are in bold. The numbers at the nodes are the bootstrap values obtained by repeating the analysis 100 times to generate a majority consensus tree. There were a total of 300 positions in the final dataset. The scale bar indicates a 10% nucleotide sequence divergence.

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well-supported (bootstraps 100) branch on the phylogenetic tree situated between Lyme disease and relapsing fever clusters, albeit closer to the latter. We also identified *Borrelia* sp. (genotype TCI301) in *Rh. microplus* which was almost identical to *Borrelia* sp. previously identified in the same ticks in Brazil (GenBank EF141022).

Sixty-three ticks were positive using qPCR targeting the 23S rRNA of *Anaplasmataceae*. Only 39 DNA samples were positive using qPCR were successfully amplified in standard PCR. A possible explanation may consist of the lower sensitivity of standard PCR compared to qPCR. After sequencing, we obtained good quality sequences for only 22 samples (22/378; 6%). We suggest that the poor sequence quality may be explained by co-infection by two or more species belonging to the *Anaplasmataceae* family. We have identified one case of *A. centrale* in *Am. variegatum* (100% identity with the *A. centrale* strain Israel, NR_076686), and two cases of *E. ruminantium* in *Am. variegatum* (100% identity with the *E. ruminantium* strain Welgevonden, NR_077000). We have identified *A. marginale* in two *Rh. microplus* (100% of homology with *A. marginale* strain Florida, NR_0765879). Finally, for all remaining sequences, Blast analysis shows a homology score of under 92% which means that these sequences are likely to

Fig 3. 23S rRNA based phylogenetic analysis of strains identified in this study. Phylogenetic tree highlighting the position of *Anaplasma* sp, *Ehrlichia* sp and *Wolbachia* sp identified in the present study relative to *Anaplasma*, *Ehrlichia* and *Wolbachia* type and uncultured strains. The 23S rRNA sequences were aligned using MEGA 6 and phylogenetic inferences were obtained from a Bayesian phylogenetic analysis with the HKY standard model.

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correspond to new species. After the construction of a phylogenetic tree (Fig 3), we propose that the status of Candidatus is applied to an uncultured species but not formally recognized by the International Code of Nomenclature of Bacteria [62]. The result shows three cases of Anaplasma: Candidatus Anaplasma ivorensis related to A. phagocytophilum identified in ticks, two in Am. variegatum, and one in Rh. microplus. The three sequences have one to two SNP (single nucleotide polymorphism) between them. In one Rh. microplus, a potential new Wolbachia sp., Candidatus Wolbachia ivorensis, was identified, closely related to the Wolbachia endosymbiont of Cimex lectularius (GenBank AP013028). We also identified two groups of sequences corresponding to new Ehrlichia spp. which cluster in two clades. Indeed, in four cases (one Am. variegatum, two Rh. microplus, and one H. truncatum), we identified Candidatus Ehrlichia rustica in the sub-group of Ehrlichia chaffeensis. In nine ticks (five Am. variegatum, three Rh. microplus and one H. truncatum), we detected Candidatus Ehrlichia urmitei that was previously observed by our team in Rh. bursa ticks collected in the Bacque area of France (M. Dahmani, personal communication) (Fig 3). Candidatus Ehrlichia urmitei forms an independent and well-supported clade situated between the E. ruminantium clade and that of Ehrlichia muris (Fig 3).

Finally, 15 co-infections (15/378; 4%) were detected by qPCR. All 15 co-infections involved the presence of R. africae. In Am. variegatum, ten co-infections (10/15; 66%) were observed with R. africae plus another pathogen such as Coxiella burnetii (1/170; 0.6%), A. centrale (1/170; 0.6%), A. marginale (1/170; 0.6%), Candidatus Borrelia Africana, Candidatus Borrelia ivorensis (3/170; 2%), Candidatus Anaplasma ivorensis (2/170; 1%), or Candidatus Ehrlichia urmitei (2/170; 1%) as well as H. marginatum rufipes with R. africae plus R. aeschlimannii (2/16; 12%) and in Rh. senegalensis with R. africae plus R. aeschlimannii (1/3; 33%) (Table 3). The access numbers of the sequences of all the potential new species deposited in GenBank are summarized in Table 4.

| Sequences type                      | Gene       | Ascension number |
|-------------------------------------|------------|------------------|
| Candidatus Borrelia africana TCI22  | flaB       | KT364343         |
| Candidatus Borrelia ivorensis TCI140| flaB       | KT364344         |
| Candidatus Borrelia ivorensis TCI351| flaB       | KT364346         |
| Borrelia sp. genotype TCI301        | flaB       | KT364345         |
| Candidatus Borrelia africana TCI22  | 16S rRNA   | KT364339         |
| Candidatus Borrelia ivorensis TCI140| 16S rRNA   | KT364340         |
| Candidatus Borrelia ivorensis TCI351| 16S rRNA   | KT364341         |
| Borrelia sp. TCI301                 | 16S rRNA   | KT364342         |
| Candidatus Anaplasma ivorensis TCI50| 23S rRNA   | KT364326         |
| Candidatus Anaplasma ivorensis TCI94| 23S rRNA   | KT364327         |
| Candidatus Anaplasma ivorensis TCI149| 23S rRNA   | KT364328         |
| Candidatus Wolbachia ivorensis TCI113| 23S rRNA  | KT364329         |
| Candidatus Ehrlichia rustica TCI141 | 23S rRNA   | KT364330         |
| Candidatus Ehrlichia rustica TCI145 | 23S rRNA   | KT364331         |
| Candidatus Ehrlichia rustica TCI167 | 23S rRNA   | KT364332         |
| Candidatus Ehrlichia rustica TCI238 | 23S rRNA   | KT364333         |
| Candidatus Ehrlichia urmitei TCI148 | 23Sr RNA   | KT364334         |
| Candidatus Ehrlichia urmitei TCI230 | 23S rRNA   | KT364335         |
| Candidatus Ehrlichia urmitei TCI106 | 23S rRNA   | KT364336         |
| Candidatus Ehrlichia urmitei TCI127 | 23S rRNA   | KT364337         |
| Candidatus Ehrlichia urmitei TCI166 | 23S rRNA   | KT364338         |

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Discussion

Domestic animal resources supply some 30% of total human food and agricultural production requirements. They are particularly vital to subsistence and economic development in developing countries as they continually provide essential food products, draught power and manure for crop production and generate income as well as employment for most of the rural poor [63]. However, livestock-associated ticks are often reservoirs or vectors of human vector-borne diseases [18]. Intensification of livestock farming is one cause of the abundance of various vectors and tick-borne diseases. In recent years, the spectrum of tick-borne diseases infecting animals has increased; many of these diseases, such as rickettsioses, borrelioses, Q fever, anaplasmoses, and ehrlichioses, are gaining increasing attention from clinicians and veterinarian [4]. Advances in the development of molecular biology tools facilitate the detection of new bacteria [4,64].

Rickettsioses have been identified in humans, animals and ticks which are considered to be the main vectors of such pathogens as R. africae, R. aeschlimannii, and R. massiliae in sub-Saharan Africa [9]. In our study, rickettsial DNA was found in 49% of ticks collected from cattle. For the first time, the presence of R. aeschlimannii in ticks in Côte d’Ivoire is shown. This study provides evidence of R. aeschlimannii infection in 23% of Hyalomma ticks including H. marginatum rufipes (44%) and H. truncatum (11%). R. aeschlimannii has not been observed in other tick species. These data support the theory that the Hyalomma genus is a main vector and reservoir of R. aeschlimannii. It was previously reported in 45% to 51% of H. marginatum rufipes and 6% to 7% in H. truncatum collected from cows, donkeys, sheep, goats and horses in Senegal [12]. These data are comparable to those of our study. The high prevalence of R. africae (90%) in Am. variegatum can be explained by the high transovarial and trans-stadial transmission rates (100%) and a filial infection rate (93%) that was previously demonstrated in Am. variegatum [20]. This result shows that this tick species acts as a vector but also as a reservoir for R. africae in Côte d’Ivoire. R. africae was recently detected in other tick genera including Rhipicephalus and Hyalomma [12,18,19,57]. In our study, the prevalence of R. africace is 10% in Rh. microplus and 9% in Hyalomma spp., which is lower than in co-fed Am. variegatum, suggesting that these ticks are probably not the competent vectors for R. africace. This bacterium likely infects Rh. microplus and Hyalomma spp. during co-feeding. The first report of the presence of R. massiliae in Côte d’Ivoire was in Rhipicephalus spp. [28]; this is comparable to the detection of R. massiliae in a Rh. senegalensis tick found in our study.

C. burnetii infections have been also reported as being between 0.7% and 6.8% in ticks from cattle in western African countries [51] but not in Côte d’Ivoire where the seroprevalence of C. burnetii was estimated to be 3% [52]. Here, we show for the first time the presence of C. burnetii in Côte d’Ivoire, although only in one tick. Most Borrelia species such as B. hispanica, B. duttonii, and B. crocidurae detected in Africa, are related to soft ticks. Their main vectors are Ornithodoros spp. [65]. To date, Borrelia sp. was identified only once in an African hard tick, Am. cohaerens, in Ethiopia [39]. It has been also reported that Rhipicephalus spp. transmits Borrelia theileri to cattle, causing bovine orf infections [18]. In Côte d’Ivoire, we show that Am. variegatum were infected by three potential new Borrelia and Rh. microplus by one potential new Borrelia. The sequences of Borrelia sp. (genotype TC1301) were identical to 99% of those of Borrelia sp. found in engorged Rhipicephalus sp. ticks collected from horse in Brazil (EF141022). Phylogenetic analysis showed that Borrelia sp. TC1301 is classified in the relapsing fever group, close to B. crocidurae and B. hispanica, two etiological agents of relapsing fever in Africa [26,31]. Blast analysis of the flaB gene showed that these new Candidatus Borrelia ivoricensis and Candidatus Borrelia africanae borreliae were significantly different to all other borreliae, except for this Borrelia sp in Am. cohaerens in Ethiopia [39]. These potential new Borrelia
form a new clade between the clades of Lyme disease borreliae and relapsing fever borreliae. Thus, this is the first time that *Borrelia* species have been detected in Côte d’Ivoire and the first time their presence has been confirmed in hard ticks in Africa.

Bacteria from the *Anaplasmataceae* family were previously known to be pathogens of veterinary importance. However, in the three last decades, many human pathogens have been identified in this family [66]. Recently, based on the *rrl* gene, our team developed new tools to identify most bacteria belonging to *Anaplasmataceae* family [47]. These tools combine a qPCR followed by a standard PCR then sequencing, and have been used successfully to amplify DNA from bacteria belonging to *Anaplasma* spp., *Ehrlichia* spp., *Neorickettsia* spp., and *Wolbachia* spp. available in our laboratory [67]. We have successfully amplified *Anaplasmataceae* DNA in 6% of our ticks. For the first time, we have demonstrated the presence of *A. marginale*, *A. centrale*, *E. ruminantium*, and potential novel *Ehrlichia*, *Anaplasma*, and *Wolbachia* spp. in ticks in Côte d’Ivoire. *A. marginale* was observed in *Am. variegatum* and *Rh. microplus*. To the best of our knowledge, *A. marginale* has never been reported in Africa in *Rh. microplus*. The first report of its presence in Côte d’Ivoire was in 2007, but the exact route of its introduction into this region has not yet been determined [68]. A recent study indicated that the majority of the *Rhipicephalus* (ex-*Boophilus*) spp. collected and identified from farms around Azaguié (Côte d’Ivoire) are *Rh. microplus* (96%) [69]. *A. centrale* is a species closely related to *A. marginale*; this naturally attenuated strain has been used as a live vaccine to prevent severe diseases due to *A. marginale senso stricto* strains for 100 years [70]. We identified this species in *Am. variegatum*. To the best of our knowledge, *A. centrale* has never previously been detected in these ticks. The potential of *Am. variegatum* to transmit *A. centrale* needs further investigation. *E. ruminantium* was previously described in *Am. variegatum* which is invasive to cattle attaches to the hooves and cattle remain standing, particularly in the rainy season [71,72]. Recent phylogenetic analyses of *Am. variegatum* from Kenya, Mali, Burkina Faso, Ethiopia and the Caribbean show low genetic diversity within this population, suggesting an westward expansion of these ticks and supporting east-west genetic separation, with Caribbean genetic sequences being associated with and often identical to West African haplotypes. The data suggest that *Am. variegatum* reached West Africa from Zambia [73]. We have also identified three potential new species, *Candidatus* Anaplasmivaorensis, *Candidatus* Ehrlichia urmitiei, and *Candidatus* Ehrlichia rustica. The detection of these potential new species has limitations, as not all previously described species are already molecularly characterized. Indeed, such species as *Anaplasma caudatum*, *Anaplasma bovis*, and *Anaplasma mesaeterum* [74] are incompletely characterized with no strain available and no or few genes sequenced, so the detection of a ‘new’ genotype may, in fact, be the re-discovery of an old, incompletely characterized species. Further studies are required to clarify whether these new genetic variants represent a new species. The other potential new ehrlichiae was closely related to *Ehrlichia* sp. amplified from *Rh. bursa* in France. Interestingly, this new species was amplified from two different regions in the world and from different species of ticks (*Rhipicephalus*, *Amblyomma*, and *Hyalomma* spp).

Finally, it is reported that ticks are often co-infected following a blood meal from a co-infected host [75,76]. Recently, mixed infections were reported for the first time in West Africa in feeding ticks and caused mainly by *Rickettsia* spp. and *C. burnetii* [18]. In Côte d’Ivoire, for the first time we show multiple co-infections in ticks. These co-infections systematically involved *R. africae*. To date, no human cases of anaplasmoses, ehrlichioses, borrelioses, rickettsioses or co-infections have been reported in Côte d’Ivoire. However, these diseases are still little known by clinicians and laboratory diagnostic is lacking in most cases. It is important to continue to study the epidemiological data of such emerging pathogens which may be the source of disease complications in both animals and humans. We provide evidence and demonstrate the endemicity of these different bacteria in the studied regions that have the same
characteristics agro-ecological and climatic. Furthermore, these diseases could be a cause of death of unknown origin in rural areas in Côte d'Ivoire [77].

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**Author Contributions**

Conceived and designed the experiments: KPY FF OM. Performed the experiments: CBE KPY MD YLA NA AKN JDN OM. Analyzed the data: CBE KPY MD DR FF OM. Contributed reagents/materials/analysis tools: CBE KPY YLA AKN JDN. Wrote the paper: CBE DR FF OM.

**References**

1. Bechah Y, Socolovschi C, Raoult D. Identification of rickettsial infections by using cutaneous swab specimens and PCR. Emerg Infect Dis. 2011; 17(1):83–6. doi: 10.3201/eid1701.100854 PMID: 21192860.

2. Walker AR, Bouattour A, Camicas J, Estrada-Peña A, Horak IG, Latif AA, et al. Ticks of Domestic Animals in Africa: A Guide to Identification of Species. Published by Bioscience Reports, Edinburgh, United Kingdom. 2003. http://www.biosciencereports.pwp.blueyonder.co.uk/.

3. Pérez-Eid C. Emergence of tick-borne diseases in temperate countries. Ann Biol Clin (Paris). 2004; 62(2):149–54. PMID: 15047466.

4. Dantas-Torres F, Chomel BB, Otranto D. Ticks and tick-borne diseases: a One Health perspective. Trends Parasitol. 2012; 28(10):437–46. doi: 10.1016/j.pt.2012.07.003 PMID: 22902521.

5. Robyn MP, Newman AP, Amato M, Walawander M, Kothe C, Nerone JD, et al. Q Fever Outbreak Among Travelers to Germany Who Received Live Cell Therapy -United States and Canada. 2014. MMWR Morb Mortal Wkly Rep. 2015; 2; 64(38):107. doi: 10.15585/mmwr.mm6438a3 PMID: 26421460.

6. Sokhna C, Mediannikov O, Fenollar F, Bassene H, Diatta G, Tall A, et al. Point-of-care laboratory of pathogen diagnosis in rural Senegal. PLoS Negl Trop Dis. 2013; 7(1):e1999. doi: 10.1371/journal.pntd.0001999 PMID: 23350001.

7. Mayxay M, Sengvilaiapasuouth O, Chanthongthip A, Dubot-Pérès A, Rolain JM, Parola P, et al. Causes of Fever in Rural Southern Laos. Am J Trop Med Hyg. 2015; 93(3):517–20. doi: 10.4269/ajtmh.14-0772 PMID: 26149859.

8. Ghosh L, Nagar G. Problem of tics and tick-borne diseases in India with a special emphasis on progress in tick control research: a review. J Vector Borne. 2014; 51 (4): 259–70. PMID: 25540956.

9. Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, et al. Update on tick-borne rickettsioses around the world: a geographic approach. Clin Microbiol Rev. 2013; 26 (4):657–702. doi: 10.1128/CMR.00032-13 PMID: 24092850.

10. Kelly PJ, Beati L, Mason PR, Matthewman LA, Roux, Raoult D, et al. *Rickettsia africae* sp. nov., the etiological agent of African tick bite fever. Int J Syst Bacteriol. 1996; 46 (2):611–4. PMID: 8934912.

11. Ndip LM, Fokam EB, Bouyer DH, Ndip RN, Titanji JP, Walker DH, et al. Detection of *Rickettsia africae* in patients and ticks along the coastal region of Cameroon. Am J Trop Med Hyg. 2004; 71:363–6. PMID: 15381820.

12. Mediannikov O, Diatta G, Fenollar F, Sokhna C, Trape JF, Raoult D. Tick-borne rickettsioses, neglected emerging diseases in rural Senegal. PLoS Negl Trop Dis. 2010; 4(9). pii: e821. doi: 10.1371/journal.pntd.0000821 PMID: 20856858.

13. Parola P, Inokuma H, Camicas JL, Brouqui P, Raoult D. Detection and identification of spotted fever group Rickettsiae and *Ehrlichiae* in African ticks. Emerg Infect Dis. 2001; 7(6):1014–7. PMID: 11747731.

14. Parola P. Rickettsioses in sub-Saharan Africa. Ann N Y Acad Sci. 2006; 1078:42–7. PMID: 17114679.

15. Socolovschi C, Matsumoto K, Marie JL, Davoust B, Raoult D, Parola P. Identification of *Rickettsia*, *Uganda* and *Djibouti*. Emerg Infect Dis. 2007; 13 (10):1508–10. doi: 10.3201/eid1310.070078 PMID: 18257998.
24. Svihrova V, Hudeckova H, Jesenak M, Schwarzova K, Kostanova Z, Cizenar M, et al. Lyme borreliosis—erraticus in ticks and fleas from the Democratic Republic of the Congo. Ticks Tick Borne Dis. 2012; 3(5–6):371–3. doi: 10.1016/j.ttbdis.2012.10.015 PMID: 23137572.

25. Mediannikov O, Diatta G, Zolia Y, Balde MC, Kohar H, Trape JF, et al. Tick-borne rickettsiae in Guinea and Liberia. Tick Borne Dis. 2012; 3(1):43–8. doi: 10.1016/j.ttbdis.2011.08.002 PMID: 22309858.

26. Reye AL, Arinola OG, Hubschen JM, Muller CP. Pathogen prevalence in ticks collected from the vegetation and livestock in Nigeria. Appl Environ Microbiol. 2012; 78(8):2562–8. doi: 10.1128/AEM.06686-11 PMID: 22327584.

27. Ogo NI, de Mera IG, Galindo RC, Okubanjo OO, Inuwa HM, Agbede RI, et al. Molecular identification of Borrelia in ticks from camels (Camelus dromedarius) in Nigeria, West Africa. Med Vet Entomol. 2015; 29(2):205–8. doi: 10.1111/mve.12116 PMID: 23697545.

28. Raoult D, Fournier PE, Abboud P, Caron F. First documented human Rickettsia aesculianii infection. Emerg Infect Dis. 2002; 8:748–9. PMID: 12095451.

29. Parola P, Paddock CD., Raoult D. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev. 2005, 18(4): 719–56. PMID: 16223955.

30. Kernif T, Djerbouh A, Mediannikov O, Ayach B, Rolain JM, Raoult D, et al. Rickettsia aeschlimannii in Hyalomma dromedarii ticks from sub-Saharan Algeria. Ticks Tick Borne Dis. 2012; 3(5–6):377–9. doi: 10.1016/j.tbbdis.2012.10.013 PMID: 23164496.

31. Kamani J, Baneth G, Apanaskevich DA, Mumcuoglu KY, Harrus S. Molecular detection of Rickettsia aeschlimannii in Hyalomma spp. ticks from camels (Camelus dromedarius) in Nigeria, West Africa. Med Vet Entomol. 2015; 29(2):205–9. doi: 10.1111/mve.12094 PMID: 25565180.

32. Parola P, Socolovschi C, Jeanjean L, Bitam I, Fournier PE, Sotto A, et al. Warmer weather linked to tick attack and emergence of severe rickettsioses. PLoS Negl Trop Dis. 2008; 2(11):e338. doi: 10.1371/journal.pntd.0000338 PMID: 19015724.

33. Garcia-Garcia JC, Portillo A, Nunez MJ, Santibanez S, Castro B, Oteo JA. A patient from Argentina infected with Rickettsia massiliae. Am J Trop Med Hyg. 2010; 82(4):691–2. doi: 10.4269/ajtmh.2010.09–0663 PMID: 20348520.

34. Cascio A, Torina A, Valenzise M, Blanda V, Camarda N, Bombaci S, et al. Scalp escharan neck lymphadenopathy caused by Rickettsia massiliae. Emerg Infect Dis. 2013; 19(5):836–7. doi: 10.3201/eid1905.121169 PMID: 23697545.

35. Berrelha J, Briolant S, Muller F, Rolain JM, Marie JL, Pagés F, et al. Rickettsia felis and Rickettsias massiliae in Ivory Coast, Africa. Clin Microbiol Infect. 2009; 15 Suppl 2:251–8. doi: 10.1111/j.1469-0691.2008.02278.x PMID: 19546811.

36. Trape JF, Diatta G, Arnathau C, Bitam I, Sarthi M, Belghyti D, et al. The epidemiology and geographic distribution of relapsing fever borreliosis in West and North Africa, with a review of the Ornithodoros erraticus complex (Acari: Ixodida). PLoS One. 2013; 8(11):e78473. doi: 10.1371/journal.pone.0078473 eCollection2013. PMID: 24223812.

37. Vial L, Diatta G, Tall A, Ba el H, Bouganali H, Durand P, et al. Incidence of tick-borne relapsing fever in West Africa: longitudinal study. Lancet. 2006; 368(9529):37–43. PMID: 16815378.

38. Parola P, Ryelandt J, Mangold AJ, Mediannikov O, Guglielmonne AA, Raoult D, et al. Relapsing fever Borrelia in Ornithodoros ticks from Bolivia. Ann Trop Med Parasitol. 2011; 105(5):407–11. doi: 10.1179/1364859411Y.0000000021 PMID: 21929883.

39. Cutler SJ, Bonilla EM, Singh RJ. Population structure of East African relapsing fever Borrelia spp. Emerg Infect Dis. 2010; 16(7):1076–80. doi: 10.3201/eid1607.091085 PMID: 20587177.

40. Sarthi M, Garnier, Boudebouch N, Bouattour A, Rihani A, Hassar M, et al. Borrelia hispanica relapsing fever, Morocco. Emerg Infect Dis. 2009; 15(10):1626–9.
37. Trape JF, Godeluck B, Diatta G, Rogier C, Legros F, Albergel J, et al. The spread of tick-borne relapsing fever in West Africa and its relationship to sub-Saharan drought. Am J Trop Med Hyg. 1996; 54(3):289–93. PMID: 8600768.

38. Nordstrand A, Bunikis I, Larsson C, Tsgobe K, Schwans TG, Nilsson M, et al. Tick-borne relapsing fever diagnosis obscured by malaria. Togo. Emerg Infect Dis. 2007; 13(1):117–23. PMID: 17370524.

39. Mediannikov O, Abdissa A, Socolovschi C, Diatta G, Trape JF, Raoult D, et al. Detection of a new Borrelia species in ticks taken from cattle in Southwest Ethiopia. Vector Borne Zoonotic Dis. 2013; 13(4):266–9. doi: 10.1089/vbz.2011.0874 PMID: 23421894.

40. Pruneau L, Moumène A, Meyer DF, Marcelino I, Lefrançois T, Vachiéry N, et al. Understanding Anaplasmataceae pathogenesis using “Omics” approaches. Front Cell Infect Microbiol. 2014; 4:86. doi: 10.3389/fcimb.2014.00086. eCollection 2014 PMID: 25072029.

41. Rymaszewska. A, Grenda S. Bacteria of the genus Anaplasma and its relationship to sub-Saharan drought. Am J Trop Med Hyg. 1996; 54(3):289–93. PMID: 8600768.

42. Aubry P, Geale DW. A review of bovine anaplasmosis. Transbound Emerg Dis. 2011; 58(1):1–30. doi: 10.1111/j.1865-1682.2010.01173.x PMID: 21040509.

43. Raoult D, Parola P. Molecular tools in the epidemiology of tick-borne borrelial diseases. Ann Biol Clin (Paris). 2001. 59(2):177–82. PMID: 11282521.

44. Georges M. Garry, Berger’s of Manual of Systematic Bacteriology: The proteobacteria. Second Edition. Volume two; 2005. pp. 120-122.

45. Allsopp BA. Natural history of Ehrlichia ruminantium. Vet Parasitol. 2010; 167(2-4):123–35. doi: 10.1016/j.vetpar.2009.09.014 PMID: 19836892.

46. Djiba ML, Mediannikov O, Mbengue M, Thiongane Y, Molez JF, Seck MT, et al. Survey of Anaplasmataceae bacteria in sheep from Senegal. Trop Anim Health Prod. 2013; 45(7):1557–61. doi: 10.1007/s11250-013-0399-y PMID: 23353269.

47. Dahmani M, Bernard D, Mohamed SB, Fenollar F, Raoult D, Mediannikov O. Development of a new PCR-based assay to detect Anaplasmataceae and the first report of Anaplasma phagocytophilum and Anaplasma platys in cattle from Algeria. Comp Immunol Microbiol Infect Dis. 2015; 38:39–46. doi: 10.1016/j.cimid.2015.02.002 PMID: 25748051.

48. Maurin M, Raoult D. Q fever. Clin Microbiol Rev. 1999; 12(4):518–33. PMID: 10515901.

49. Kazar J. Coxiella burnetii infection. Ann N Y Acad Sci. 2005; 1063:105–14. PMID: 16481501.

50. Cooper A, Hedlefs R, Ketheesan N, Govan B. Serological evidence of Coxliella burnetii infection in dogs in a regional centre. Aust Vet J. 2011; 89(10):385–9. doi: 10.1111/j.1751-0813.2011.00819.x PMID: 21933165.

51. Mediannikov O. Coxiella burnetii in humans and ticks in rural Senegal. PLoS Negl Trop Dis. 2010; 4(4):e654. doi: 10.1371/journal.pntd.0000654 PMID: 20386603.

52. Tissot-Dupont H, Brouqui P, Faugere B, Raoult D. Prevalence of antibodies to Coxiella burnetii, Rickettsia conorii, and Rickettsia typhi in seven African countries. Clin Infect Dis. 1995; 21(5):1126–33. PMID: 8589132.

53. Rolain JM, Shlul H, Maurin M, Raoult D. Evaluation of antibiotic susceptibilities of three rickettsial species including Rickettsia felis by a quantitative PCR DNA assay. Antimicrob Agents Chemother. 2002; 46(9):2747–51. PMID: 12183224.

54. Socolovschi C, Frederic P, Raoult D. Rickettsia felis in Aedes albopictus mosquitoes, Libreville, Gabon. Emerg Infect Dis. 2012; 18(10):1687–9. doi: 10.3201/eid1810.120178 PMID: 23017437.

55. Subramanian G, Sekevova Z, Raoult D, Mediannikov O. Multiple tick-associated bacteria in Ixodes ricinus from Slovakia. Ticks Tick Borne Dis. 2012; 3(5–6):406–10. doi: 10.1016/j.ttbdis.2012.10.001 PMID: 23182274.

56. Rolain JM, Raoult D. Molecular detection of Coxiella burnetii in blood and sera during Q fever. QJM. 2005; 98(8):615–7; author reply 617–20. PMID: 16027172.

57. Mediannikov O, Trape JF, Diatta G, Parola P, Fournier PE, Raoult D. Rickettsia africae, Western Africa. Emerg Infect Dis. 2010; 16(3):571–3. doi: 10.3201/eid1603.090346 PMID: 20202453.

58. Raoult D, Roblot F, Rolain JM, Besnier JM, Loulergue J, Bastides, et al. First isolation of Bartonella alsatica from a valve of a patient with endocarditis. J Clin Microbiol. 2006; 44(1):278–9. PMID: 16390990.

59. Bottleau E, Verbruggen E, Aubry C, Socolovschi C, Vlieghie E. Meningoencephalitis complicating relapsing fever in traveler returning from Senegal. Emerg Infect Dis. 2012; 18(4):697–8. doi: 10.3201/eid1804.111771 PMID: 22469185.
60. Mura A, Socolovschi C, Ginesta J, Lafrance B, Magnan S, Rolain JM, et al. Molecular detection of spotted fever group rickettsiae in ticks from Ethiopia and Chad. Trans R Soc Trop Med Hyg. 2008; 102 (9):945–9. doi: 10.1016/j.trstmh.2008.03.015 PMID: 18440576.

61. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 2003; 19 (12):1572–4. PMID: 12912839.

62. Murray RG, Stackebrandt E. Taxonomic note: implementation of the provisional status Candidatus for incompletely described procaryotes. Int J Syst Bacteriol. 1995; 45 (1):186–7 I. PMID: 7857801.

63. Ayalew W, Rege JEO, Getahun E, Tibbo M, Mamo Y. Delivering Systematic Information on Indigenous Animal Genetic Resources—the development and prospects of DAGRIS. Animal Genetic Resources Group, International Livestock Research Institute. P, O Box 5689. 2003. Addis Ababa, Ethiopia.

64. Doudier B, Olano J, Parola P, Brouqui P. Factors contributing to emergence of *Ehrlichia* and *Anaplasma* spp. as human pathogens. Vet Parasitol. 2010; 167(2–4):149–54. doi: 10.1016/j.vetpar.2009.09.016 PMID: 19836890.

65. Parola P, Raoult D. Ticks and tick-borne bacterial diseases in humans: an emerging infectious threat. Clin Infect Dis. 2001; 32(6):897–928. PMID: 11247714.

66. Dahmani M, Loudahi A, Mediannikov O, Fenollar F, Raoult D, Davoust B. Molecular detection of *Anaplasmataceae* ticks found in West Africa. Exp Appl Acarol. 2007; 43(3):233–4. PMID: 17929178.

67. Beati L, Patel J, Lucas-Williams H, Adakal H, Kanduma EG, Tembo-Mwase E, et al. Phylogeography and demographic history of *Amblyomma variegatum* (Fabricius) (Acari: Ixodidae), the Tropical Bont Tick. Vector Borne Zoonotic Dis. 2012; 12(6):514–25. doi: 10.1089/vbz.2011.0659 PMID: 22446720.

68. Koné S, Fürst T, Jaeger FN, Esso EL, Baïkor N, Kouadio KA, et al. Causes of death in the Taabo health and demographic surveillance system, Côte d’Ivoire, from 2009 to 2011. Glob Health Action. 2015; 8.27271. doi: 10.3402/gha.v8i2.27271. eCollection2015 PMID: 25959772.