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RESEARCH ARTICLE

RESEARCH ON THE TICKS OF THE AGENTS RESPONSIBLE FOR RICKETTISIOSES AND ASSOCIATED INFECTIONS IN THE KINDIA AND MAMOU REGIONS (REPUBLIC OF GUINEA)

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ABSTRACT

Rickettsial and related infections are infectious, re-emerging, polymorphic, life-threatening and globally prevalent diseases. These pathologies prevail in Guinea in several forms with a variation of the etiological agents. The objective of this study was to identify the infectious agents responsible for rickettsial diseases and related diseases in the regions of Kindia and Mamou. The investigation work was carried out from January 6, 2016 to February 6, 2017. A total of 6 types of infectious agents were found by PCR, 2 at Mamou *Coxiella burnetti* and *Bartonella spp.* and 4 to Kindia *Coxiella burnetti*, *Borellia burgdorferi*, *Borellia burgdorferi*. The frequency of *Coxiella burnetti* rickettsiosis was 84.22% in the 2 target regions. For related infections the overall prevalence was 5.26% for rickettsial disease with Bartonella spp. 5.26% with Q Fever and 5.26% with Lyme *Borellia burgdorferi* disease. The vectors were overall *Ripicephalus decoloratus* (*Coxiella burnetti* and *Bartonella spp*. Rickettsiosis), *Amblyomma variegatum* (Crimean-Congo hemorrhagic fever). The presence of ticks on livestock is a factor in the spread of rickettsial diseases in regions in Guinea. Particular attention should be paid to these pathologies by the public and veterinary health services.

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INTRODUCTION

The emergence and re-emergence of many zoonotic diseases is common in many parts of the world (Parola, 2013). Most of these diseases are active and pose to humanity a significant threat. This is the case, especially rickettsial diseases, whose incidence is rising in many parts of the globe. These infectious diseases are the basis of outbreaks asking for this purpose, socio-economic impacts (Mokrani *et al*, 2012; Ngwamidiba *et al*., 2006; Renvoisé *et al*, 2012). Over the past 20 years, rickettsiologie experienced in the world, a boom. Several species of Rickettsia pathogens not seen for decades are now associated with human infections, and new species of Rickettsia indefinite pathogenicity continue to be detected or isolated ticks. Currently, the term refers Rickettsial three groups of diseases: rickettsial diseases caused by bacteria of the genus Rickettsia family Rickettsiaceae and includes fever group and typhus group.

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The ehrlichiosis and anaplasmosis follow and are caused by bacteria of the family Anaplasmataceae which was recently reorganized and scrub typhus caused Orientia tsutsugamushi (Parola *et al*., 2005; Renvoisé, and Raoult, 2009). For years, the diagnosis of rickettsial disease was confirmed almost exclusively by conventional serological methods. Advances in microbiological techniques have indeed contributed to the identification of novel microorganisms, to develop a new classification and the description of new species (Parola *et al*, 2005a;. Renvoisé and Raoult, 2009). *Rickettsia africae* is the agent of tick fever (hard or ixodidae) in the world and Africa, it affects all age groups. In 2018, it was recorded in Guadeloupe, in the Caribbean, the island of Reunion, South Africa, Ethiopia, Brazil and Uganda. During the same year, 14 cases were confirmed by PCR in China. Prevalence ranging from 3 to 15% were obtained in Pakistan, Japan and Australia. In Thailand, 80 people were confirmed by PCR including 34 children and 46 adults with mortality 2 to 5% (Parola, 2013). *R. prowaseckii*, Agent of classical typhus, known since ancient times and proved in the fifteenth century, reappears in the form of deadly outbreaks during conflict and displacement of people as in Burundi in 1993 and the Republic of Congo with over

100 000 cases notified (Parola *et al*., 2005). Rickettsial diseases are of great originality, combining pathologies both ancient and emerging diseases (Brouqui, 2008; Parola *et al*., 2003; Renvoisé and Raoult, 2009). Among the new rickettsial ago spotted fever in Japan (or "Oriental spotted fever") due to Rickettsia japonica, spotted fever due to Rickettsia Flinders islands Honei, fever Astrakhan due to R. conorii subsp. Caspia, typhus in Africa due to tick Rickettsia africae, chips spotted fever due to Rickettsia felis, extreme east of rickettsial disease caused by Rickettsia heilongjiangensis the TIBOLA "tick-borne lymphadenopathy" due to Rickettsia slovaca, the " lymphangitis - associated rickettsia "(LAR) due to Rickettsia sibirica subsp. mongolitimonae and unnamed rickettsial diseases caused aeschlimanii R., R. parkeri, R. helvetica, R. and R. massiliae marmionii (WHO, 2016 Parola *et al*., 2005b). Many other Rickettsia have been identified, but have not, at present, been implicated in human disease (Brouqui *et al*., 2004; Guiguen and Degeilh 2001; Renvoisé and Raoult, 2009). Q fever is a disease transmitted from animals to humans. It is caused by a microbe called Coxiella burnetii, which can live for months or even years in the soil and dust. Some animals such as cattle, sheep and goats can carry the germ that causes Q fever in the fabric of their reproductive systems; the uterus, placenta and the liquid product in the low-set. Infected animals also eliminate the microbe in their feces and milk (Boarbi and Mori, 2016 Sprong *et al*, 2012).

In recent years, in addition to conventional bacteriological methods of research, the use of cell culture techniques and molecular biology including Polymerase Chain Reaction (PCR), quantitative PCR (qPCR) and real-time PCR (RT-PCR) from samples of skin scabs, no doubt contributed to the accuracy of search results (Mouffok *et al*, 2011;. Renvoisé *et al*, 2012; Roux and Raoult, 1995). There are few studies on rickettsial diseases in Guinea. The first studies were conducted in the 80 at the Research Institute of Applied Biology of Guinea. The objective of this study was to identify the causative agents of rickettsial diseases with vectors such as ticks in areas of Kindia and Mamou.

MATERIAL AND METHODS

Study zone: This diagnostic study was carried out in the administrative regions of Mamou and Kindia (map). The investigative work was conducted in the period from 6 January 2016 to 6 February 2017. Mamou is a rugged area, consisting of plateaus forming the beginning of foutanien solid (ranging from 700 m to over 1000 m). In the south and east we go to a landscape of rolling hills and plateaus of lower altitude battleships (from 400 to 600 m). It is a very important activity with about 143,020 head of cattle, 37,295 head of sheep and 37,514 head of goats (anonymous national statistical records of 2000). Kindia a monotonous terrain, characterized mainly plains cut by between dewatered residual solid It more dissected terrain in the southwest includes valleys and some interior plains. The West and North Parties are characterized by mountains with an average altitude is 575 m. it was identified 12 3021 heads of cattle, 35 265 head of sheep, 32 500 head of goats (anonymous national statistical records of 2000).

Biological material: N'Dama cattle were grouped in village flocks considering uniformity of soil and climatic conditions.
A total of 10 village herds was selected in each region. In each village flocks, a sample was taken at random on which ticks pools were collected. Overall, 100 samples of ticks (Ixodidae or hard ticks) were obtained by region. This allowed total of 200 samples of ticks. The tubes containing the samples were identified on a debit card with the name of the animal, tattoo, dress, place, medium ticks collected per animal and owner. The samples packed in a cooler were transported to the laboratory of the Institute of Research in Applied Biology of Guinea (IRBAG) in Kindia.

Laboratory equipment: The material suitable for the real-time PCR was used (PCR Gene player -Rotor 6000 BIORAD, Oven, Ultra centrifuge microtubes, Consumables (Primers, Taq polymerase, nucleotide, etc.). Two primers for research of Rickettsia spp. were used: Rr190.70p Rr190.701n and amplifying a fragment of 630 bp OmpA gene encoding a 190-kD protein and RpCS.877p-RpCS.1273r amplifying a fragment of 396 bp glt gene encoding citrate synthase a (Bitam *et al*., 2006; Jordan *et al*, 2015. Roux, and Raoult, 1995).

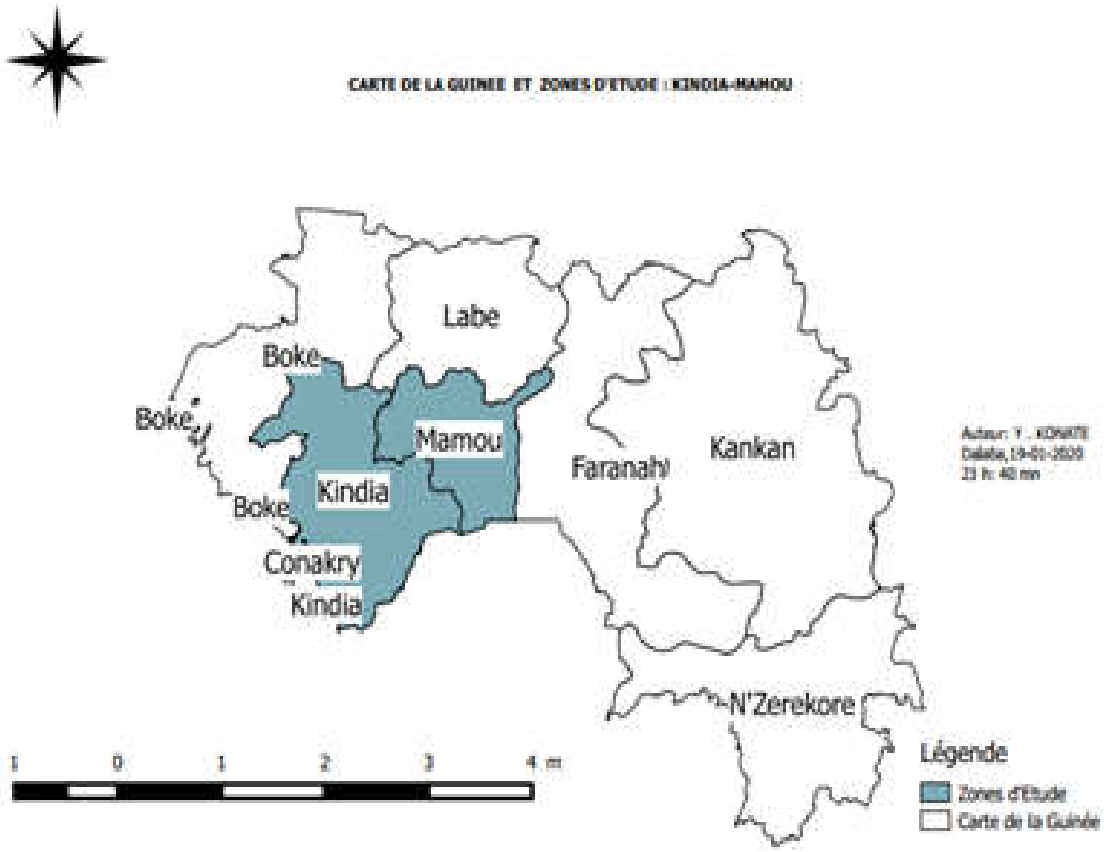
METHODS

Whole tick specimens were grouped into pools according to the origin. Washing with 1X saline phosphate buffered solution (PBS) was done. They were then crushed with a pestle in micro 100 of PBS. The DNA was extracted using a DNA Tissue purification kit (QIAGEN) following the standard manufacturer's recommended procedure. The classical scheme of realization of the PCR was followed. The DNA extracted from each tick was embedded in a classic cocktail gene amplification (PCR Polymerase Chain Reaction). PCR products were purified and the sequence of the amplified DNA fragments was obtained using an automated sequencer (ABI 310, Perkin-Elmer, Applied Biosystems Division). The sequences obtained were compared with corresponding sequences available in GenBank rickettsia (Bitam *et al*., 2006; Brouqui *et al*., 2004; Mouffok *et al*, 2011;. Renvoisé *et al*, 2012).

RESULTS AND DISCUSSION

PCR identified two rickettsial species in areas of Mamou and Kindia (*Coxiella burnetii* Portage and Bartonella spp.) And 2 etiological agents of related infections (Coxiella burnetii Q fever in and Lyme disease to Borrelia burgdorferi). These organisms were isolated from Amblyomma variegatum Ripicephalus decoloratus and (Table 1, 2 and 3). In the region of Kindia, the prevalence of different types of infections are significantly different (p-value <0.05). Coxiella burnetii in rickettsial was the most common with a frequency equal to 80%. However, *Coxiella burnetii* Q fever and Lyme disease *Borrelia burgdorferi* to appear in the same proportions, with a prevalence of 10%.

The PCR results showed two types of infectious agents, *Coxiella burnetii* and namely Bartonella spp., All these germs were isolated in ticks Ripicephalus decoloratus. Frequencies significantly different (p-value <0.05). The species *Coxiella burnetii* was the most frequent with 92.86%. *Coxiella burnetii infection* was most rependue in 2 regions with a frequency of 84.22%, There are 95% chance that the true value of the frequency of *Coxiella burnetii* in both areas either in interval [60.42 to 96.62]. There is a significant difference from the standpoint frequency between the agents responsible for various infections (p-value <0.001). The etiological agents of Lyme disease *Borrelia burdorferi a*nd



Map of the investigation area

Table 1. Positive PCR obtained for the region of Kindia

Serial No.	Types of infections	Number of positive	Frequency (%)	95%	p-value	vectors
1	Coxiellaburnetii Rickettsiosis to	8	80	[44.39 to 97.48]	0.0006	*Ripicephalusdecoloratus and AmblyommaVariegatum*
2	Coxiellaburnetii Q fever	1	10	[0.25 to 44.50]		*AmblyommaVariegatum*
3	Lyme disease Borreliaburgdorferi	1	10	[0.25 to 44.50]		*AmblyommaVariegatum*
	Total	10	100			
n = 100 samples						

Table 2. Positive PCR obtained for the region of Mamou

No. order	Types of infections	Number positive	Frequency (%)	95%	p-value	vectors
1	rickettsiosis to *Coxiellaburnetii*	13	92.86	[66.13 to 99.82]	3.2 * 10-5	*Ripicephalusdecoloratus*
2	rickettsiosis to *Bartonella spp.*	1	7.14	[0.18 to 33.87]		*Ripicephalusdecoloratus*
Total		14	100			

n = 100 samples

Table 3. Summary rickettsial porting in 2 regions

No.	Types of infections	Number of positive	Percentage (%)	95%	p-value	vectors
1	rickettsiosis to *Coxiellaburnetii*	21	84,22	[60.42 to 96.62]	<0.001	*Rhipicephalusdecoloratus* and AmblyommaVariegatum
2	rickettsiosis to *Bartonella spp.*	1	5.26	[0.13 to 26.03]		*Rhipicephalusdecoloratus* and AmblyommaVariegatum
3	Coxiellaburnetii Q fever	1	5.26	[0.13 to 26.03]		*AmblyommaVariegatum*
4	Lyme diseaseBorreliaburgdorferi	1	5.26	[0.13 to 26.03]		*AmblyommaVariegatum*
	Total	19	100			

N = 200 samples

Coxiella burnetii Q fever were less rependues with a frequency of 5.26%. Only two species of ticks Rhipicephalus and Amblyomma decoloratus Variegatum found carriers of these germs. In this study, the infection rate of C. burnetii in ticks collected from regions of Kindia and Mamou substantially ranged from 80% [CI 44.39 to 97.48] to 92.86% [CI 66 , from 13 to 99.82]. These percentages are significantly higher than those obtained in other parts of the world. A study was conducted in Ethiopia from 2011 to 2014 to study the presence and genotypes of Coxiella burnetii using molecular methods in ticks collected from domestic animals has shown that ticks were tested for C. burnetii by reacting quantitative polymerase chain in real time (qPCR) targeting two different genes, followed by a sequence of typed several spacings (STDs). An overall prevalence of 6.4% (54/842) of C. burnetii was recorded. C. burnetii has been detected in 28.6% (14/49) of Amblyomma gemma, 25% (31/124) of Rhipicephalus pulchellus, 7.1% (1/14) of Hyalomma marginatum rufipes, 3.2% (2/62) Am. variegatum, 3.1% (4/128) Am. cohaerens, 1.6% (1/63) of Rh. Praetextatus and 0.6% (1/153) of Rhipicephalus (Boophilus) decoloratus. Comprehensive significantly higher frequencies of C. burnetii DNA were observed in Am gemma and Rh pulchellus than other tick species (Mantel - Haenszel, MH, P <0.0001)... The overall incidence of C. burnetii was significantly higher (MH, P <0.0001) in ticks of southeastern districts Arero, Moyale and Yabelo than other districts. This study demonstrated the presence of 18 MST genotype of C. burnetii in ticks in the districts of southeastern and genotype STD 20 in ticks in the central districts (Parola *et al*., 2005a). 0001) in ticks district of southeastern Arero, Moyale and Yabelo than other districts. This study demonstrated the presence of 18 MST genotype of C. burnetii in ticks in the districts of southeastern and genotype STD 20 in ticks in the central districts (Parola *et al*., 2005a). 0001) in ticks district of southeastern Arero, Moyale and Yabelo than other districts. This study demonstrated the presence of 18 MST genotype of C. burnetii in ticks in the districts of southeastern and genotype STD 20 in ticks in the central districts (Parola *et al*., 2005a).

Other studies have shown the isolation of species Richettsia spp. who have not been found also found in this study, is the case of Rickettsia aeschlimannii isolated from the Hyalomma marginatum ticks collected in 1997 in Morocco, this bacterium was considered a pathogen spotted fever (Parola *et al*, 2005a;. Sarih *et al*., 2008). In Zimbabwe, it was reported that Coxiella burnetii has been detected by PCR in the tick Hyalomma marginatum rufipes, Egypt in H. impeltatum (Amanda *et al*., 2006; Loftis *et al*., 2006), Senegal in H. marginatum rufipes ticks H. truncatum and Rhipicephalus evertsi (Mediannikov *et al*, 2010; Parola *et al*, 2013). In Peru, Bartonellosis and rickettsial diseases have been frequently reported in 2013, Bartonella spp. was detected in 17 pools of ticks (6 C. felis, P. irritans 9, 1 1 C. canis and P. humanus). Also, Rickettsia spp. was detected in 76 pools of ticks (62 C. felis, 10 P. irritans, 2 P. humanus 2 and C. canis) (G Caceres *et al*., 2013). In Thailand and Vietnam, researching rickettsial and related diseases of work has to isolate Ehrlichia spp., Strain EBm52, which was obtained from Boophilus microplus ticks collected from cattle (Parola, *et al* ., 2003). From the perspective of infectious disease, the first cases of rickettsial infections have been reported in North Africa by Conor & Bruch in 1910 and in South Africa by Mc Naught Sant'Anna in 1911 then in 1912. The Mediterranean spotted fever was considered the only known rickettsial disease in Africa (Boillat and Greub, 2007). However, new cases of rickettsial diseases caused by Rickettsia africae affiliated group of spotted fever or "Spotted Fever Group (SFG)" described in the Ivory Coast and Zimbabwe (Sambou, 2012; Graf *et al*., 1981). This pathogen R. africae was identical to isolated Rickettsia strains in Amblyomma variegatum ticks collected in Ethiopia and Ambryomma hebraeum, from Zimbabwe (Lagier *et al*., 2009). *R. africae* was subsequently identified by the technique of Polymerase Chain Reaction (PCR) in several countries in sub-Saharan Africa. This technique has been used in particular for the identification of pathogens Rickettsia in ticks ixodid of Keur Momar Sarr zone (Louga) in Senegal (Parola *et al*, 2003, 2005b;. Sambou, 2012), Chad and Ethiopia (Lagier *et al*., 2009) and in many countries of Equatorial Africa and South (Sekeyová *et al*., 2012). Overall, these results would show a varied diversification of etiologic agents of rickettsial diseases in the world. They also show that investigations based on PCR for detection of C. burnetii and other rickettsial agents in ticks, using the methods currently available, should be interpreted with caution without sequencing of the amplified products. Methods known improved molecular diagnostics for the detection of C. burnetii must consider the possibility of cross-reactions with Coxiella kind of bacteria.

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Conclusion

This study shows the movement of germs responsible for rickettsial diseases in the investigation zone. Coxiella burnetii Identifying, Bartonella spp. Borrelia burgdorferi and from ticks indicates the presence of several etiological agents of rickettsial diseases in Guinea. Research should be conducted in order to continue to assess the epidemiological and clinical importance of these infections in endemic areas like in other regions of the country. In the end, these results should allow the establishment of a real program against rickettsial diseases, a program must include epidemiological surveillance and entomological aspects as part of the "One Health".

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