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Demonstration of the DNA carriage rate of Coxiella burnetii (Q fever agent) in ticks in the prefectures of Kindia, Beyla, Lola and N'Zérékoré in the Republic of Guinea

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Abstract*: Q fever or Query fever or Queensland fever is a cosmopolitan zoonosis mainly affecting people working with animals. The infection is caused by an intracellular bacterium Coxiella burnetii. The reservoir of this bacterium is ubiquitous, consisting of many mammals, but also ticks. The present research which took place from October 2017 to October 2020 focused on the analysis of C. burnetii DNA carriage in ticks, on an analyzed sample of 980 tick collections, there were 735 cases positive, i.e. a prevalence of 75%. ELISA and real-time PCR methods were used for data analysis. Species like Amblyomma, Rhipicephalus (boophilus), Rhipicephalus and Hyalomma are found in Kindia (Lower Guinea). In the prefectures of Beyla, Lola and N'Zérékoré (Guinée Forestière), Rh decoloratus, Am variegatum and Rh (boophilus) geigyi are found. The presence of this pathogen constitutes a threat to human and animal health in these investigated areas, and must be taken into account in the biological diagnosis of Q fever.*

Keywords: Q fever, Coxiella burnetii DNA, ticks, biological diagnosis

1. INTRODUCTION

Coxiella burnetii is a global zoonotic pathogen responsible for Q fever in various animal and human species. It is frequently detected in ticks which are ectoparasites of livestock, some tick species are competent vectors [1, 2]. Ticks that infest cattle and small ruminants in particular have a major economic impact on the development of rural populations [3]. Several species of ticks naturally infected with Coxiella burnetii have been identified in Africa, America, Asia, Europe and Oceania. Ticks would have allowed the circulation of C. burnetii between wild and domestic animals, and would have helped to maintain this bacterium in animal populations [4, 5]. In Guinea, studies on Q fever date back to 1986 [6].

Ticks play an important role in transmission between wild vertebrate mammals and birds. They infect vertebrates either by biting or through their droppings. In ticks, there is transovarian transmission [7]. The study area of this research was the prefecture of Kindia in Lower Guinea and the prefectures of Beyla, Lola and N'Zérékoré in Forest Guinea. These prefectures are part of the largest agro-pastoral areas of Guinea.

2. MATERIALS AND METHODS

2.1 Presentation of the study area

The investigation sites of this work are: the prefecture of Kindia in lower Guinea and the prefectures of Beyla, Lola and N'Zérékoré in Forest Guinea. The prefecture of Kindia has an area of 9115 km^2. It has a population of 438,315 inhabitants, including 226,300 women. The main activities are: trade, agriculture and animal husbandry. The density is 52 inhabitants per km2 unequally distributed between ten (10) decentralized communities and one (01) urban municipality which includes thirty-three (33) districts. With a growth rate of 34%. Its climate is of the humid tropical type, characterized by the alternation of two seasons of variable duration, a dry season from November to April and a rainy season with abundant rainfall from May to October with an average rainfall of 2500 mm of water per year with temperatures ranging from 25°C to 39°C [8].

The prefectures of Beyla, Lola and N'Zérékoré are the three largest agro-pastoral prefectures in the Forest region. This region is located in the south-east of Guinea and covers an area of 49,500 km², or 20% of the national territory. Its population is 1.1 million. Its density is estimated at 22 inhabitants per km². Its relief is on a plateau. It has seven (7) prefectures: Beyla, Guéckédou, Kissidougou, Lola, Macenta, N'Zérékoré and Yomou. Its climate is of the humid subequatorial type characterized by the alternation of two seasons, a rainy season from March to November and a dry season from December to February. In August and September, the monthly rainfall can reach 300 to 400 mm³ with relatively high intensities. Average temperatures are lowered by altitude and vary between 17°C and 22°C [9].

The Institute for Research in Applied Biology of Guinea (IRBAG) and the laboratory of the Center for Research in Epidemiology, Microbiology and Care (CREMS) served as a framework for carrying out this study.

2.2 Materials and equipment

The work material consists of: biological material (tick pools, serum); protective equipment (gloves, overalls, goggles, boots, etc.); sampling equipment (tubes, alcohol syringes, cotton, ice cream, dry ice and swabs); identification material (identification key, magnifying glass, etc.).

2.3 Method

2.3.1 Collection and identification of ticks

This research, which focuses on the evaluation of the DNA carriage rate of Coxiella burnetii in ticks in the prefectures of Kindia, Beyla, Lola and N'Zérékoré in the Republic of Guinea, took place from October 2019 to October 2020. This study focused on a population of 980 ticks, namely Kindia (554), Lola (203), Beyla (189) and N'Zérékoré (34).

2.3.3 DNA extraction and PCR amplification

Total DNA was extracted from ticks using the DNeasy Blood and Tissue Kit. Adult ticks were analyzed individually; foraging nymphs were grouped by species, collection date, and sampling site into groups of five individuals. In each round DNA extraction, a template-free negative control sample was included to exclude any contamination in the extraction process. Five (5) µl of tick DNA was used in all PCR reactions. Samples were tested for the presence of C. burnetii by PCR assays.

Real-time PCR amplifications were performed in a StepOne PlusTM System and end-point PCR assays were performed in a thermal cycler. Positive (corresponding pathogen DNA tested) and negative controls were included in each assay. All PCR products of the expected size were purified using NZYGelpure for Sanger sequencing with the corresponding forward and reverse PCR primers [5].

2.3.4 Statistical analyzes

IBM SPSS Statistics version 20 software was used for statistical analysis. Logistic regression was used to compare the prevalence of a given pathogen regarding tick species and their origin (vegetation or wild animal). The differences were considered statistically significant at $p \leq 0.05$ [10].

3. RESULTS AND DISCUSSIONS

4.1 Results

The results obtained during this experimental study on the identification and distribution of cases of C. burnetii in ticks in four prefectures of Guinea and according to the species are shown in Table 1.

Table 1: Distribution of cases of C. burnetii in ticks by prefecture and according to species

Area	Species	Collared ticks	Positive cases	Frequency (%)
Kindia	*Amblyomma*	301	237	24,18
	Haemaphysalis	86	36	3,67
	Hyalomma	27	17	1,73
	Rhipicephalus	39	31	3,16
	Rhipicephalus (boophilus)	101	89	9,08
Total Kindia		**554**	**410**	**41,82**
Beyla	*Am variegatum*	69	47	4,79
	Rh decoloratus	98	**77**	7,85
	Rh (boophilus) geigyi	22	19	1,93
Total Beyla		**189**	**143**	**14,57**
Lola	*Rh decoloratus*	175	135	13,77
	Rh (boophilus) geigyi	28	20	2,04
Total Lola		**203**	**155**	**15,81**
N'Zérékoré	*Am variegatum*	5	3	0, 30
	Rh decoloratus	29	24	2,44
Total N'Zérékoré		**34**	**27**	**2,44**
Totaux		**980**	**735**	**75**

For all the areas investigated, the number of cases of C. burnetii DNA detected in ticks is 735 corresponding to a prevalence of 75%. This shows that ticks could well be carriers of the bacteria from animals to humans.

4.2 Talks

The results in Table 1 show that in all the places investigated there was detection of C. burnetii DNA in the ticks collected and subjected to the analyses. Out of a total of 980 ticks, the number of cases of C. burnetii DNA detected in ticks is 735 corresponding to a prevalence of 75%. With 410 positive cases out of 554 samples in Kindia, i.e. 41.82% frequency; in Beyla, out of 189 ticks, there were 143 positive cases, i.e. 14.57% frequency; in Lola out of 203 ticks there were 143 positive

cases i.e. 14.57% frequency and in N'Zérékoré out of 34 ticks there were 27 positive cases i.e. 2.44% frequency.

The Amblyomma species was the most frequent in Kindia with 237 positive cases corresponding to 24.18%, the Hyalomma species was the least frequent with 17 positive cases corresponding to 1.73%.
In the Lola and N'Zérékoré prefectures, the Rh decoloratus species was the most frequent, with varying proportions ranging from 24 positive cases in N'Zérékoré, i.e. 2.44%, and 135 positive cases in Lola, i.e. 13.77%. of frequency. The Rh species (boophilus) geigyi is the least frequent in this study with 19 positive cases in Beyla, a frequency of 1.93%.

On the other hand, in the study by David N, et al. (2017) carried out in Kenya, only the genus Rhipicephalus was found to be positive for C. burnetii DNA with a prevalence of 2.96% [6]. This prevalence is much lower than that found by the present study. The work of Abdoulaye Ahmat Nassour et al. (2020) carried out on the prevalence of Coxiella burnetii DNA in ticks collected in the prefecture of Kindia, produced a prevalence of 42.2%. This rate is lower than that produced by the present study.

The work of Fatoumata Dramé et al. (2021) carried out on the carriage of Coxiella burnetii DNA in ticks collected in the Forest Region, in particular in N'Zérékoré, Lola and Beyla, resulted in a prevalence of 76.29%. This rate is higher than that of the present study (75%). The carriage rate of C. burnetii DNA in ticks, achieved by the present study, is significantly higher than 2.4% presented in the study in Slovenia by Natacha K et al., [11].

5. CONCLUSION

Ticks transmit Coxiella burnetii from animal to animal and maintain the infection in animal populations that are the reservoir of Q fever. The disease often presents as an occupational disease. Infected domestic animals show no signs of infection, but the disease, although asymptomatic, can cause abortions in infected females. The clinic of this disease in humans includes an incubation period of about 20 days. The onset is marked by a flu-like syndrome with fever, chills, sweating, asthenia, anorexia, headache, myalgia which lasts 1 to 3 weeks. This work contributes to the knowledge of the DNA carriage rate of Coxiella burnetii by ticks in the areas of investigation of this thesis, the objective of which is to improve the biological diagnosis of Q fever in the structures of health of Kindia, of Guinée Forestière in particular and of Guinea in general. C. burnetii DNA was detected in ticks collected and analyzed.

This study also revealed some novel tick-host associations and tick-borne pathogens. Finally, wildlife could play an important role not only as reservoirs, but also in maintaining and spreading tick populations and tick-borne pathogens.

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