Circulation of Crimean-Congo haemorrhagic fever virus in ticks in Upper Guinea-Republic of Guinea

Abstract

The aim of this study was to map the distribution of agents carrying the Crimean-Congo haemorrhagic fever virus (arbovirus-tica) in the natural region of Upper Guinea. The prefectures of Kankan, Dabola and Faranah were used as collection areas. Random sampling of different types of animals was used to collect the biomaterial. Two types of analysis methods (RT-PCR and ELISA) were used. Out of a total of 578 ticks collected and divided into 254 pools, the genus *Amblyomma* was the most frequently encountered with 83 tick pools. Molecular analysis (RT-PCR) for the detection of virus RNA revealed 2 positive cases (0.8%). Direct enzyme-linked immunosorbent assays (ELISA) for the detection of Ag yielded only one positive case (0.4%). We found that the species *Rhipicephalus geigyi* was the main vector and reservoir of the pathogen in Upper Guinea.

Key words: CCHF, ticks, RT-PCR, ELISA, Upper Guinea.

INTRODUCTION

CCHF was first identified in 1944, during an epidemic of haemorrhagic fevers among Russian servicemen in the Crimea. The aetiology of the epidemic was quickly suspected to be arboviral. This hypothesis was confirmed after the injection of filtrates of ticks of the genus Hyalomma marginatum in 1965 reproduced the same symptoms in volunteers, and the virus was thus named "Crimean virus" [1]. In 1969, it was discovered that the Crimean virus was antigenically identical to a virus isolated in the Democratic Republic of Congo in 1956, known as the Congo virus, which caused a disease similar to that caused by the Crimean virus. The virus was therefore renamed "Crimean-Congo haemorrhagic fever virus" [1]. Crimean-Congo haemorrhagic fever (CCHF) is an acute viral infection (arthropozoonosis) transmitted by ticks of the genera *Hyalomma, Amblyomma and Rhipicephalus* (reservoir and vector), endemic throughout Africa, Asia and the Middle East [2, 8].

According to the World Health Organization's Africa Bureau, in 2019, a new case of Crimean-Congo haemorrhagic fever (CCHF) occurred in Mauritania in Kithat, in the Wilaya of Guidimakha [3].

In Guinea, a study carried out by E.V Naidenova et al. in 2020 in rural areas showed that the prevalence of CCHFV was 1.3 ± 0.4%. Five of the eight tick species studied have been identified as carriers of CCHFV in Guinea. The aim of this study is to map the distribution of agents carrying the Crimean-Congo haemorrhagic fever virus (arbovirus-tica) in the natural region of Upper Guinea [4].

METHODOLOGY

The prefectures of Kankan, Faranah and Dabola in the Upper Guinea region were used as the study area. This prospective and descriptive study was carried out at the Institut de Recherche en Biologie Appliquée de Guinée (IRBAG) from June to December 2022. The biomaterial

consisted of pools of ticks. Enzyme-linked immunosorbent assays (ELISA) and molecular RT-PCR were used.

AIM OF THE STUDY

The aim of this study was to determine the prevalence of CHF Congo viruses in the species collected (ticks) in this region of Guinea; to inventory and identify tick species and to identify virus markers in ticks.

COLLECTION TECHNIQUE

The tick is removed from the animal using fine tweezers (tick forceps) placed as close as possible to the skin. Using a firm, constant movement, pull the tick so that the tick-pulling forceps are placed perpendicular to the skin, without rotating, jerking or moving too quickly. Then place the tick in the tube. Avoid crushing the tick during sampling. Then disinfect the site of the bite and wash your hands thoroughly after sampling to avoid contamination.

TRANSPORTING BIOLOGICAL MATERIAL

When transporting biological material, whether alive or dead, the tick must be kept dry, in a small, perforated and secure container. It must be sent to the laboratory and described using the appropriate information form.

IDENTIFICATION

Ticks were identified based on morphological criteria using a binocular magnifying glass. Direct ELISA for the detection of CHF Congo virus antigens in ticks and PCR (Molecular RT-PCR Analysis) for the detection of virus RNA.

MAP OF GUINEA

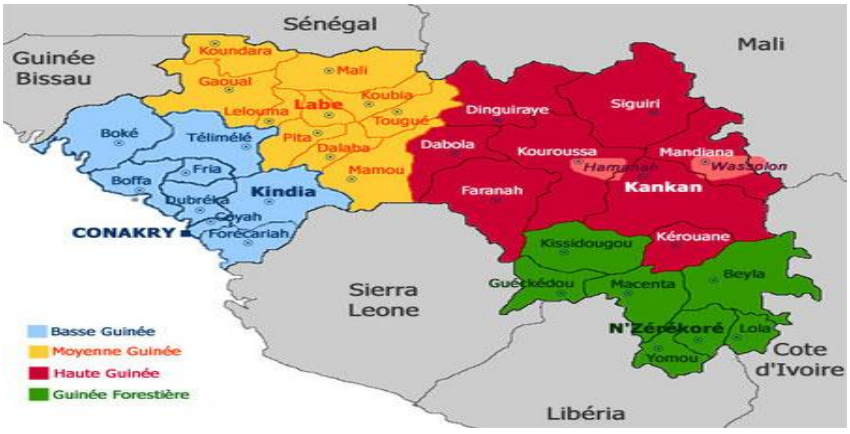

Fig 1. Study area

RESULTS and DISCUSSION

Table 1: Number of positive cases in Upper Guinea by prefecture and according to the two analysis methods.

Préfectures	The number of samples	Results of tests			
		PCR	**(%)**	**ELISA**	**(%)**
Faranah	90	1	0.4	0	0
Dabola	58	0	0	0	0
Kankan	106	1	0.4	1	0.4
Total	254	2	0.8	1	0.4

In Upper Guinea, tick collection yielded a sample of 254 pools distributed as follows: 106 in Kankan, 90 in Faranah and 58 in the prefecture of Dabola; analysis of the results revealed 2 positive cases. One case was found in Kankan and the other in Faranah, with a rate of 0.8%. No positive cases were found in Dabola. As regards the ELISA enzyme-linked immune sorbent assay, we found only 1 positive case in the prefecture of Kankan, which proves the sensitivity of the molecular method compared with the ELISA enzyme-linked immune sorbent assay.

Table 2: Positivity rate of the different tick species collected in the Faranah prefecture using the RT-PCR method.

Tick species	The number of samples	**Positive cases**		
		PCR	**(%)**	**$IC_{95\%}$**
Am varigeatum	29	0	0	-
Hy trucatum	5	0	0	-
Rh annulatus	7	0	0	-
Rh decoloratus	33	0	0	-
Rh geigyi	15	1	0.4	0.5-0.3
Rh senegalensis	1	0	0	-
Total	90	1	0.4	0.5-0.3

This table showing the distribution of tick species in the Faranah prefecture shows that out of a total of 90 tick pools collected, only one positive case was found using the RT-PCR analysis method, i.e. 0.4%. No cases were found using the enzyme-linked immune sorbent assay. The species concerned was *Rhicipicephalis geigyi*.

Table 3: Positivity rate of the different tick species collected in the prefecture of Kankan by the analysis methods.

Tick species	The number of samples	Positive cases			
		PCR	(%)	ELISA	(%)
Am varigeatum	35	0	0	0	0
Hy trucatum	8	0	0	0	0
Rh annulatus	7	0	0	0	0
Rh decoloratus	36	0	0	0	0
Rh geigyi	18	1	0.9	1	0.9
Rh senegalensis	2	0	0	0	0
Total	106	1	0.9	1	0.9

Observation of the results of the two analysis methods (RT-PCR and ELISA) shows a similarity in terms of the species concerned (*Rhicipicephalis geigyi*) and the positivity rate (0.9%).

Table 4: Positivity rate of the different tick species collected in the prefecture of Dabola by the analysis methods.

Tick species	The number of samples	Positive cases		
		ELISA	(%)	IC
Am varigeatum	19	0	0	-
Hy trucatum	5	0	0	-
Rh annulatus	6	0	0	-
Rh decoloratus	20	0	0	-
Rh. geigyi	8	0	0	-
Rh. senegalensis	0	0	0	-
Total	58	0	0	-

From this table, we can see that of the 58 tick pools collected in the prefecture of Dabola, no positive case was detected by the two RT-PCR and ELISA analysis methods, but it should be noted that all the species encountered in the other two prefectures are represented in Dabola.

Molecular analysis (RT-PCR) revealed 2 positive cases (0.8%). Enzyme-linked immunosorbent assays revealed only one positive case (0.4%). We found that the species Rhipicephalus geigyi was the main vector and reservoir of the pathogen in Upper Guinea.

A study carried out in Guinea by a team of Russian-Guinean researchers led by E.V Naidenova, in 2020, on the prevalence of Crimean-Congo haemorrhagic fever virus in rural areas of Guinea, showed that among the ticks studied, the estimated prevalence of CCHFV was 1.3 ± 0.4%. Five of the eight tick species studied were identified as carriers of CCHFV in Guinea [4].

These results are higher than ours (0.4% by enzyme-linked immunosorbent assay and 2 positive cases (0.8%) by molecular method (RT-PCR).

The results found by F. Farhadpour et al. in 2016 in southern Iran show a detection rate of 4.5% for 9 samples examined, and the species concerned were *Hyaloma marginatum, Hyaloma anatolicum* and *Rhicicephalus sanguinus*; these results are higher than those found in Upper Guinea by both analytical methods. In our case, we had not encountered the genus *Hyaloma* as a carrier of the CHFCongo virus [5].

The results obtained by a team of researchers (Aillen E. et al., 2016) working on the serosurveillance of pathogenic viruses circulating in West Africa indicate a higher result than

ours, using the molecular RT-PCR method and the enzyme-linked immunosorbent assay, with a frequency of Crimean-Congo haemorrhagic fever virus of 2%. [6].

Our results can be compared with studies carried out by Sanidad M. in 2017 in Spain, which show a similar geographical distribution to Guinea; the results obtained mention 3.2% as the prevalence obtained. This result is higher than that found in our study [7].

CONCLUSION

Following the investigations carried out and the results obtained, the CCHF virus antigen was detected in 1 tick pool and the virus RNA in 2 tick pools. All tick pools in which viral antigen was detected contained CCHF RNA. Positive results were obtained in two prefectures (Kankan and Faranah). We found that the species *Rhipicephalus geigyi* was the main vector and reservoir of the pathogen in Upper Guinea.

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