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

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Abstract

Introduction: The last few decades have seen the emergence of numerous human and animal infectious diseases worldwide. Among emerging and re-emerging diseases, viral haemorrhagic fevers are a major public health problem.

Objective: The aim of this study was to map the distribution of agents carrying Crimean-Congo haemorrhagic fever virus (arbovirus-tica) in the natural region of Middle Guinea.

Method: Two types of analysis methods (RT-PCR and ELISA) were used. The prefectures of Mamou, Dalaba and Pita were used as collection areas. Random sampling of different types of animals was used to collect the biomaterial.

Results: Out of a total of 789 ticks collected and divided into 229 pools, the genera *Amblyomma* and *Rhipicephalus* were encountered. Molecular analysis (RT-PCR) for the detection of virus RNA revealed 4 positive cases (1.8%). Direct enzyme-linked immunosorbent assays (ELISA) for the detection of Ag were positive in only 2 cases (0.8%).

Conclusion: We found that the *Rhipicephalus* and *Amblyomma* species were the main vectors and reservoirs of the pathogen in Middle Guinea.

Keywords: Ticks; CHF-Congo; RNA; Middle Guinea.

1. Introduction

CCHF was first reported in the Crimean Peninsula between 1944 and 1945, when there was a large outbreak of severe haemorrhagic fever with a mortality rate of 10%. The disease became known as Crimean haemorrhagic fever. Subsequently, cases were reported in all the European and Central Asian republics of the former Soviet Union and in other countries. The virus was later shown to be antigenically identical to the Congo virus that had been isolated from a fever patient in the Democratic Republic of Congo in 1956, and was subsequently named CCHFV [1, 2, 3].

Of all the tick-borne viruses that infect humans, CCHFV has the widest geographical distribution [12]. CCHFV infection has been reported from more than 30 countries, with major outbreaks reported in south-eastern Europe, Asia, the Middle East and Africa. [4,5]

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South Eastern Europe: outbreaks reported in Crimea (1944-1945), Astrakhan (1953-1963), Rostov (1963-1969), Bulgaria (1953-1974, 1975-1996 and 1997-2003), Albania (2001), Kosovo (2001) and Turkey (2002-2005 and 2007-2008) [4,6].

Africa: outbreaks reported in Zaire (1956), Uganda (1958-1977), Mauritania (1983 and 2004), Burkina Faso (1983), South Africa (1981-1986), Tanzania (1986), South West Africa (1986), Kenya (2000) and Sudan (2008) [7,8].

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 Crimean-Congo haemorrhagic fever virus (arbovirus-tic) in the natural region of Upper Guinea [8].

2. Methodology

The prefectures of Mamou, Dalaba and Pita in the Middle 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.

2.1. Objective 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.

2.2. 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. Disinfect the site of the bite and wash your hands thoroughly after sampling to avoid contamination.

2.3. Transporting biological material

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

2.4. Identification

Ticks were identified on the basis of 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.

2.5. Map of guinea

Figure 1 Map of Guinea

3. Results

Table 1 Number of positive cases in Middle Guinea by prefecture and by enzyme-linked immune sorbent assay (ELISA) and molecular RT-PCR methods.

Prefectures	**Number of pools**	**Positive cases**					
		RT-PCR	(%)	IC95%	ELISA	(%)	IC95%
Mamou	112	2	0.9	0.97-0.83	1	0.4	0.47-0.33
Dalaba	66	2	0.9	0.97-0.83	1	0.4	0.47-0.33
Pita	51	0	0	-	0	0	-
Total	229	4	1.8	1.87-1.73	2	0.8	0.87-0.73

In the natural region of Middle Guinea we were able to collect a total of 229 tick pools. Over the entire collection period, we obtained many more tick pools in Mamou than in the rest of the prefectures, i.e. 112 out of the 229 collected, and among these pools only 2 cases were positive. In Dalaba prefecture, 66 pools/229 were obtained, with 2 positive cases. In Pita, 51 pools were harvested and no positive cases were observed. Analysis using the RT-PCR method resulted in 4 positive cases, giving a percentage of 1.8. The prefectures of Mamou and Dalaba each had 2 positive cases, while Pita recorded no cases using the molecular method.

. Using the enzyme immunoassay method, we obtained 2 positive cases, i.e. 0.8% out of a total of 229 pools collected in central Guinea: 1 case in Mamou, 1 case in Dalaba and no cases in the prefecture of Pita. These results are similar to those obtained using the RT-PCR method, the only difference being that the positivity rate is 2 times lower than those obtained using the enzyme-linked immunosorbent assay.

Table 2 Positivity rate of the different tick species collected in the Mamou prefecture using the RT-PCR and ELISA methods

Tick species	**Number of pools**	**Positive cases**					
		RT-PCR	(%)	$IC_{95\%}$	ELISA	(%)	IC95%
Am. variegatum	49	1	0.9	1.62-1.38	1	0.9	1-0.8
Rh geigyi	31	1	0.9	1.62-1.38	0	0	-
Rh. decoloratus	20	0	0	-	0	0	-
Rh. sanguineus	1	0	0	-	0	0	-
Rh. senegalensis	1	0	0	-	0	0	-
Total	112	2	1.8	1.9-1.7	1	0.9	1-0.8

In this table, out of a total of 112 tick pools sampled in the Mamou prefecture, we found 2 cases positive by the molecular RT-PCR method, i.e. 1.8%. The species concerned were : *Am* variegeatum and *Rh geigyi*, each of which recorded one positive case. The ELISA enzyme-linked immunosorbent assay revealed 1 positive case in the Mamou prefecture, i.e. 0.9% of a total of 112 tick pools.

Table 3 Positivity rate of the different tick species collected in Dalaba prefecture using the RT-PCR and ELISA methods

Tick species	**Number of pools**	**Positive cases**					
		ELISA	(%)	IC95%	RT -PCR	(%)	IC95%
Am. Variegatum	32	0	0	-	1	1.5	1.62-1.38
Rh. Geigyi	20	0	0	-	0	0	-
Rh. decoloratus	14	1	1.5	1.62-1.38	1	1.5	1.62-1.38

Rh. sanguineus	0	0	0	-	0	0	-
Rh. senegalensis	0	0	0	-	0	0	-
Total	66	1	1.5	1.62-1.38	2	3	-

This table shows that of the 66 pools collected in the Dalaba prefecture, only 2 were positive after analysis by the molecular method, i.e. 3%, 1 case each of *Amblyomma variegatum* and *Rh decoloratus* in the Dalaba prefecture. The results of the enzyme-linked immunosorbent assay (ELISA) showed only one positive case, i.e. a percentage of 1.5 for the prefecture of Dalaba.

Table 4 Positivity rate of the different tick species collected in the prefecture of Pita by the two analysis methods

Tick species	**Number of pools**	**Positive cases**			
		PCR	(%)	ELISA	(%)
Am. variegeatum	26	0	0	0	0
Rh. geigyi	15	0	0	0	0
Rh .decoloratus	10	0	0	0	0
Rh. sanguineus	0	0	0	0	0
Rh. senegalensis	0	0	0	0	0
Total	51	0	0	0	0

This table shows that no positive results were found in the prefecture of Pita out of a total of 51 tick pools collected, using the RT-PCR and ELISA analysis methods. The results in this table indicate that the herds from which tick samples were collected show no traces of infection or presence of the CHF- Congo virus particle. It should be noted that the animals from which the samples were taken were penned and grazed in protected pastures.

4. Discussion

Enzyme-linked immunosorbent assays yielded only 02 positive cases (0.8%). Molecular analysis (RT-PCR) revealed 04 positive cases (1.8%). 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 [8].

These results are slightly lower than ours (1.8%) by molecular method (RT-PCR) and higher than our results by Enzyme-Linked Immunosorbent Assay with a frequency of 0.8%.

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 Middle Guinea by the two analytical methods. In our case, we had not encountered the genus *Hyaloma* as a carrier of the CHFCongo virus [9].

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%. [10].

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 by our research team [11].

5. Conclusion

Following the investigations carried out and the results obtained, CCHF virus antigen was detected in 2 tick pools and virus RNA in 4 tick pools. All tick pools in which viral antigen was detected contained CCHF RNA. Positive results were obtained in two prefectures (Mamou and Dalaba). We found that *Rhipicephalus* and *Amblyomma* species were the main vectors and reservoirs of the pathogen in Middle Guinea.

Compliance with ethical standards

Disclosure of conflict of interest

There are no conflicts of interest between the authors with respect to this article.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

Authors' contributions

Mamadou Gando DIALLO was the principal author and contributed to all stages of the work. The participation of the other co-authors Aissatou BOIRO, Thierno Amadou Labé BALDE, Ekaterina NAYDENOVA, Boubacar Sidy Sily BAH, Sanaba BOUMBALY, Bonaventure KOLIE, Souleymane DIALLO, Mohamed DIALLO and Abdoulaye Djibril DIALLO. is not negligible.

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