



## **Serosurvey of Crimean-Congo Haemorrhagic Fever Virus (CCHFV) in Cattle in Livestock Areas of Côte d'Ivoire, West Africa**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author AEV designed the study and had full access to the data. Authors AEV, DKCA and DKM undertook full responsibility for the data, accuracy of analysis and final decision-making for submission. Author AEV designed the study. Authors DKCA and KKARMC collected the data. Authors DKM, AEV and DKCA conducted data management. Author DKCA contributed to the study concept and design. Author DKM conducted the statistical analysis and authors AEV and DKCA contributed to statistical analysis. Authors AEV, DKM and DKCA drafted the manuscript. All authors contributed to the acquisition, analysis, or interpretation of data. Authors DM, AEV and DKM critically revised the manuscript. Author AEV supervised the study. All authors have read and approved the manuscript.*

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### **ABSTRACT**

**Background:** Crimean-Congo haemorrhagic fever virus (CCHFV) is an arbovirus transmitted by Ixodid ticks and causes a highly pathogenic disease called Crimean-Congo haemorrhagic fever with a mortality rate of up to 50% in humans.

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**Methods:** From January to December 2019, tick and blood samples were taken from seven cattle farms located in the towns of Korhogo, Bondoukou, Man and Bouaflé. In each of the farms, samples were taken from twenty cattle. These tick and blood samples were sent to the national reference laboratory of the Department of Epidemic Viruses of the Pasteur Institute of Côte d'Ivoire for diagnosis of CCHFV as part of the surveillance of haemorrhagic fever viruses in Côte d'Ivoire. Molecular detection of the Crimean-Congo haemorrhagic fever virus in ticks was performed by real-time PCR. For the serological analysis, the ELISA was used for the detection of antibodies to CCHFV (IgM and IgG).

**Results:** The study allowed the identification of 411 ticks divided into three genera *Amblyomma*, *Hyalomma* and *Rhipicephalus*. The predominant species were *Rhipicephalus (Boophilus) microplus* with (47.4%) and *Amblyomma variegatum* with (42.8%). No ticks gave positive results for the research of the CCHFV. Serological analysis showed that all 140 blood samples were IgM negative and 53.57% were IgG positive. Cattle from Bouaflé (90%) and Korhogo (70%) showed a higher seroprevalence than cattle from other regions. There was no significant difference in seropositivity between female bovines (54.1%) and male bovines (52.5%) ( $P>0.05$ ). There is an association between age and anti-CCHF IgG positivity ( $P\leq 0.05$ ), as cattle less than 3 years old (59.3%) showed a higher seroprevalence.

**Conclusion:** CCHFV serological tests have been detected in cattle, one of the livestock known to play a role in the amplification of CCHFV. Our results demonstrate that the prevalence of CCHFV is high in livestock areas of Côte d'Ivoire and suggest that surveillance for CCHFV should be established.

**Keywords:** Ticks; Crimean-Congo haemorrhagic fever virus; seroprevalence; cattle breeding; Côte d'Ivoire.

## 1. INTRODUCTION

Crimean-Congo haemorrhagic fever (CCHF) is an Anthro-zoonosis caused by a virus (Nairovirus) of the Bunyaviridae family. It is endemic in Africa, the Balkans, the Middle East and Asia [1]. Crimean-Congo haemorrhagic fever (CCHF) is a potentially fatal viral disease transmitted by ticks [2]. Outbreaks of viral haemorrhagic fever have a case-fatality rate of 10-40% in humans. The Crimean-Congo haemorrhagic fever virus (CCHFV) is transmitted to humans either by tick bites or by contact with the blood or tissues of infected animals during or immediately after slaughter. Human cases are most often described in people working in the livestock sector: farmers, slaughterhouse employees, veterinarians [1]. Several studies show that CCHF is widespread in West Africa with documented cases of human deaths in Senegal and Mauritania [3,4]. CCHF hosts include a large number of wild and domestic animals, including cattle, sheep and goats. However, the virus causes limited or no disease in their zoonotic hosts [5,6]. Because it causes an unapparent infection or mild fever in cattle, sheep and goats with a viremia of sufficient intensity to infect adult ticks [7]. Given the important involvement of ruminants in the life cycle of the CCHFV, these animals constitute a practical sentinel species group that is often used to measure the epizootic activity of the CCHFV in

specific areas and thus define potential risk areas [8–10], seroepidemiological studies in livestock are important because they can determine the prevalence of CCHFV circulation in an area and help to define potential risk areas [11]. CCHF virus has been isolated from ticks, domestic and wild vertebrates and humans in some sub-Saharan West African countries such as Senegal [4], Mauritania [3] Burkina Faso [12] and Mali [13]. It should also be noted that the cattle encountered in the livestock farms and livestock market come mainly from the various sub-Saharan countries bordering Côte d'Ivoire. To date, very few studies have been carried out on the prevalence rates of this virus in Côte d'Ivoire. The importance of the involvement of ticks and cattle in the transmission of CCHFV requires knowledge of the geographical distribution of ticks and the epidemiology of CCHFV in cattle present in the major breeding areas of Côte d'Ivoire. The objective of this study is to identify the different species of ticks that parasitize cattle in four geographical areas of Côte d'Ivoire and to determine the prevalence of CCHF virus in ticks and cattle in these different areas.

## 2. MATERIALS AND METHODS

### 2.1 Study Site and Type of Study

This cross-sectional study was conducted in four regions of Côte d'Ivoire belonging to different

geographical areas. These regions include the towns of Bondoukou in the north-east, Bouaflé in the centre, Korhogo in the north and Man in the west. A total of seven farms were visited for tick and blood sampling of cattle. It involved the analysis of tick and bovine blood samples sent to the national reference laboratory of the Epidemic Virus Department of the Pasteur Institute of Côte d'Ivoire between January and December 2019 for diagnosis of CCHFV as part of the surveillance of haemorrhagic fever viruses in Côte d'Ivoire.

## 2.2 Sampling of Ticks and Blood from Cattle

Tick and blood samples were taken from seven cattle farms located in the towns of Korhogo, Bondoukou, Man and Bouaflé. In each of the farms, twenty (20) oxen were sampled for tick and blood sampling. Ticks were collected from animals of all ages randomly selected from farms with at least twenty (20) animals. Three (3) ticks were collected from each bovine animal. For sampling, ticks were searched all over the body, starting with areas of tick preference, i.e. udders, testicles, ears and in the vicinity of mucous membranes (anogenital region).

The collected ticks were stored individually in tubes of nunc previously labelled with a park number. Blood samples were taken by puncture of the jugular vein, on red plan tubes for blood collection. After centrifugation 3500 RPM, the serum was refrigerated at 4°C, then sent to the laboratory of the Pasteur Institute of Côte d'Ivoire and stored in a -80°C freezer.

## 2.3 Ticks Identification

Identification was carried out using OPTIKA binocular magnifiers at 10X and 20X magnification and dichotomous identification keys [14–17]. After identification, the batches of ticks for each farm were placed in a liquid nitrogen canister for storage prior to transport. Long-term storage of the ticks was done in a freezer at -80°C.

## 2.4 Detection of CCHFV in Ticks

The ticks were crushed with 500 µl of phosphate buffered saline buffer (PBS) by an automatic grinder. The FastPrep-24TM5G. After centrifugation, the supernatants were recovered in a 1.5 ml microcentrifuge tubes. 140 µl of supernatant was used for RNA extraction. 140 µl

of bovine serum was used for RNA extraction. RNA extractions were performed using the QIAamp Viral RNA Extraction Kit (QIAGEN, Valencia, CA, USA), according to the manufacturer's protocol. The RNAs eluted after extraction were stored at a temperature of -80°C in ultra low temperature.

Molecular detection of the virus was performed by RT-PCR (One-step Applied Biosystems) in real time using Ambion's AgPath™ One-step RT PCR kit. Sense primers (designated CCHF S1): 5-TCT CAA AGA AAC ACG TGC C-3, anti sense primers (designated CCHF S122): 5-CCT TTT TGA ACT CTT CAA ACC-3 and the probe (designated CCHF probe): FAM-ACT CAA GGKAAC ACT GTG GGC GTA AG-BHQ1 specific to the FHCV virus were used for the amplification of the S segment of the CCHFV as previously described in the work of Atkinson and collaborator [18]. RT-PCR was performed in a reaction volume of 25 µl containing 1 µl of extracted RNA, 2xQuantitect Probe, nuclease-free water, primer and probe. The following amplification programme was used: 50°C for 15 minutes for the reverse transcription phase, 95°C for 15 minutes for the denaturation phase followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute.

## 2.5 Serological Analysis of Samples

The ELISA (Enzyme-Linked Immunosorbent Assay) for the detection of antibodies to CCHFV was chosen as the serological test.

IgM detection consisted of sensitizing a Microplate ELISA 96 well Maxisorp plate with bovine IgM antibodies diluted 1/1000 in PBS 1X at 100µl per well. After an overnight incubation at 4°C, the plate wells were washed 3 times with 350 µl of wash buffer (PBS 1X at 0.05% Tween20) and then the serum of the test sample (diluted 1/100 in wash buffer with 1% skimmed milk) was deposited in two wells (100 µl each): one for the specific antigen of the virus for which antibodies were being tested and the other for the negative control. After incubation at 37°C for 1 hour, the viral antigen and the control antigen were each deposited in one well for each sample tested. The presence in the serum of antibodies directed against the virus of interest causes the formation of an immune complex with the viral antigen during the new incubation phase (37°C, 1 hour) while no specific reaction occurs with the control antigen, allowing the background of the aspecific binding to be evaluated. The next step

was to add the immuno acid specific to the viral antigen of interest prior to the deposition of a peroxidase-labelled bovine IgM conjugate (each of these two steps requiring a 1 hour incubation phase at 37°C). IgG detection is performed on the same type of 96-well plate with sensitisation by the specific immuno acid of the virus to be tested diluted 1/1000 in PBS 1X with two wells per sample. After overnight incubation at 4°C, either control antigen or virus antigen (diluted 1/100 in wash buffer with 1% skim milk) is added to each well to capture the virus. The addition of the test serum (diluted 1/100 in wash buffer plus 1% skim milk) allows the capture of antibodies to the specific viral antigen in the case of a sample from a positive subject. Revelation is done during a phase of addition of antibodies against bovine IgG antibodies coupled with peroxidase. The plates were read by spectrophotometry dual wave length (450-620 nm). The analysis results were interpreted according to the optical density (OD). Each OD was divided by the positivity threshold, so a sample was considered positive when the ratio obtained was greater than 1 with a positivity threshold of 0.492.

## 2.6 Statistical Analysis

Descriptive statistics on the characteristics of the study population and laboratory results were carried out using EPI-Info 6 version 3.3.2. The seroprevalence of CCHFV was determined for our entire sample. Qualitative variables were

characterised by numbers and percentages. Chi-square was performed between the qualitative variables to establish a relationship, P values below 0.05 were considered statistically significant.

## 3. RESULTS

### 3.1 Tick Genera and Species

In the four regions studied, 7 farms were visited. A total of 411 ticks were sampled from 140 cattle. Of the ticks sampled 95.86% (394) were adults and 4.14% (17) were nymphs. The identification of these ticks revealed the presence of 3 distinct genera. These are the genera *Amblyomma*, *Hyalomma* and *Rhipicephalus*. The genus *Rhipicephalus* composed of 5 species included 218 (53.04%) of the collected ticks. The genus *Amblyomma* with 176 (42.82%) was represented by only one species. The genus *Hyalomma* was only composed of nymphs. The predominant species were *Rhipicephalus (Boophilus) microplus* (Canestrini, 1888) with 195 (47.4%) and *Amblyomma variegatum* (Fabricius, 1794) 176 (42.8%) (Table 1).

### 3.2 Prevalence of CCHFV in Ticks

No positive results were obtained from any of the 411 ticks collected for the CCHFV's research using the real-time PCR method.

**Table 1. Distribution of tick genera and species by region**

Ticks	Bondoukou n (%)	Bouafle n (%)	Korhogo n (%)	Man n (%)	Total n (%)
<i>Rhipicephalus (Boophilus) annulatus</i>	6 (5.7)	0.0	3 (2.5)	0.0	9 (2.2)
<i>Rhipicephalus (Boophilus) decoloratus</i>	11 (10.5)	0.0	1 (0.8)	0.0	12 (2.9)
<i>Rhipicephalus (Boophilus) geigy</i>	0.0	0.0	0.00	1 (0.8)	1 (0.2)
<i>Rhipicephalus (Boophilus) microplus</i>	20 (19.0)	61 (92.4)	7 (5.8)	107 (89.2)	195 (47.4)
Nymphe	15 (14.3)	0.0	1 (0.8)	1 (0.8)	17 (4.1)
<i>Rhipicephalus sanguineus</i>	0.0	0.0	1 (0.8)	0.0	1 (0.2)
<i>Amblyomma variegatum</i>	53 (50.5)	5 (7.6)	107 (89.2)	11 (9.2)	176 (42.8)
Total	105 (100.0)	66 (100.0)	120 (100.0)	120 (100.0)	411 (100.0)

*The species Rhipicephalus (Boophilus) annulatus, Rhipicephalus (Boophilus) decoloratus (Koch, 1844), Rhipicephalus (Boophilus) geigy (Aeschlimann & Morel, 1965), Rhipicephalus sanguineus (Latreille, 1806) had small proportions*

**Table 2. Breakdown of the 140 results of the Congo crime serological survey by region, sex and age of cattle**

	Negative n (%)	Positive n (%)	Total n (%)	P-value
<b>Region</b>				
Bondoukou	25 (62.5)	15 (37.5)	40 (28.6)	0.0001
Bouaflé	2 (10.0)	18(90.0)	20 (14.2)	S
Korhogo	12 (30.0)	28 (70.0)	40 (28.6)	
Man	26 (65.0)	14 (35.0)	40 (28.6)	
<b>Sex</b>				
Female	39 (45.9)	46 (54.1)	85 (60.7)	0.87
Male	26 (47.3)	29 (52.7)	55 (39.3)	NS
<b>Ages (in year)</b>				
[1- 3]	37(40.7)	54 (59.3)	91 (65)	0.05
[3- 6]	18(51.4)	17(48.6)	35 (25)	S
[6-10]	10 (71.4)	4 (28.6)	14 (10)	

NS: Not significant / S: Significatant

### 3.3 Seroprevalence of CCHFV

For the sero-epidemiological study, 140 bovine serum samples were tested for IgG and IgM CCHFV antibodies. Serological analysis showed that all 140 samples were IgM negative. Serological analysis for the detection of IgG showed that 53.57% (75/140) were positive. Bouaflé was predominantly 18/20 (90%) positive for anti-CCHF IgG followed by Korhogo with 28 /40 (70%). Female bovines 46/85 (54.1%) were positive compared to 29/55 (52.5%) for males. There were no significant associations ( $P>0.05$ ).

Cattle under 3 years old 54/91 (59.3%) were positive compared to 17/35 (48%) for those aged 3-6 and 4/14 (28.6%) for those over 6. There is a link between age and anti-CCHF IgG positivity. ( $P\leq 0.05$ ) (Table 2).

## 4. DISCUSSION

Crimean Congo Haemorrhagic Fever (CCHF) is a major viral zoonosis that is often fatal in humans. Clinical signs are observed following infection in humans while in animals it is asymptomatic. Ticks act both as a vector and a reservoir for Crimean Congo Haemorrhagic Fever virus (CCHFV) [19]. The virus can therefore be transmitted by many species of ticks belonging to different genera. In this study five (5) tick species belonging to three genera were identified. These three genera have also been shown in several studies in Côte d'Ivoire [20–22] Among the species identified, *Rhipicephalus* (*Boophilus*) *microplus* (47.4%) and *Amblyomma variegatum* (42.8%) were the predominant species. Our results are in line with

those obtained by Toure et al. [21] and Diaha-Kouame et al. [23] indicating the predominance of these two tick species in cattle breeding in these same regions [21,23]. The individuals of the genus *Hyalomma* obtained in this study were all nymphs. However, the same authors identified adult ticks belonging to the genus *Hyalomma*.

In this study the ticks tested for the detection of CCHFV were negative. And according to some authors, ticks such as *Hyalomma marginatum* (Koch, 1844) and *Amblyomma variegatum* are known to be involved in the distribution of the Crimean-Congo haemorrhagic fever virus [24,25]. Moreover other studies carried out in Ghana [26], in Mali [27] in Mauritania [28] and in Senegal [29] showed prevalences of CCHFV in the three types of ticks identified in this study.

With regard to serological analysis, the overall seroprevalence observed for IgG was 53.57%, which is much higher than that obtained for cattle in Niger (9%) [30] and Egypt (3.83%) [31] and significantly lower than those obtained from cattle in Mali (66%) [11] and in Mauritania (67%) [32]. It should be noted that in Mali, a country bordering Côte d'Ivoire, serious cases of CCHF have been reported in humans [33] and detection of the virus in ticks [27] has also proved a circulation of CCHFV in this country.

Serological results varied according to the regions visited. Indeed, the cattle from Bouaflé (90%) and Korhogo (70%) showed a higher seroprevalence than cattle from other regions at  $p<0.05$ . This difference could be explained by the geographical positions of these two towns. And according to Hoek et al. (2012) climatic factors such as temperature, humidity and rainfall have

an important influence on the distribution of CCHF vector ticks in the regions [34]. Vorou, Pierroutsakos, and Maltezou (2007) also reported that climate and environmental change can affect CCHF epidemiology and trigger community outbreaks [35]. In addition, disparities in prevalences between locations may be due to the density of animals in the area as evidenced by the results obtained by Maiga et al. [11]. The search for the viral genome can truly establish with certainty a relationship between the presence of tick vectors of CCHF and seroprevalence. But no tick has tested positive. Given that antibodies to CCHFV remain for life in cattle, it is also possible that infection may have occurred months or years before our study in these two communities. The presence of IgG shows that the contact of animals with the virus is not recent. This could explain the fact that there are no positive ticks at CCHFV. The detection of CCHFV in bovine sera in these regions of Côte d'Ivoire shows a high risk of exposure to the virus, especially for humans, especially when handling animals during slaughter or consuming unpasteurised milk. Crimean-Congo haemorrhagic fever is a major public health threat that can have a considerable effect on livestock owners and their families living near livestock farms, slaughterhouse workers and health personnel, particularly in resource-poor countries. CCHF is a zoonotic disease that affects people who come into contact with livestock and ticks [36]. Currently, there is no CCHF monitoring programme in Côte d'Ivoire involving humans. No human cases of CCHF have been recorded in Côte d'Ivoire in the past. However, suspected cases can be confused with other human cases of haemorrhage. The CCHF is able to cause nosocomial outbreaks and the limited options available for treatment and care of infected people underline the need for surveillance [6].

In these farms the females are kept longer for breeding, and only a few males are kept for breeding performance and genetic improvement. There are therefore more females than males in the herds. These results are consistent with those of Maïna et al. [30] which showed that females were more infested than males.

On most farms the animals on the farms are for sale. Cattle are therefore sold by the owners, and consequently there are older animals on these farms. Serology has been positive in younger cattle than in adults, suggesting that age may influence the rate of infection in ruminants. A

study carried out in Egypt showed that the number of positive cases of CCHFV-specific IgG in cattle in age group B ( $\geq 2$  years) was significantly higher ( $p < 0.001$ ) than in age group A ( $< 2$  years) (5.7% versus 1.6%) [31]. This study confirms the circulation of CCHFV in these cattle farms in Côte d'Ivoire. It is therefore imperative to take effective control measures against tick infestations in these regions in order to prevent CCHFV infections in humans.

## 5. CONCLUSION

This study carried out for the serological surveillance of Crimean-Congo Haemorrhagic Fever (CCHF) revealed the presence of Crimean-Congo Haemorrhagic Fever Virus (CCHFV) in livestock farms in Côte d'Ivoire. Cattle from Bouaflé and Korhogo showed a higher seroprevalence than cattle from other regions. The results obtained show the circulation of the virus in these regions of Côte d'Ivoire. Our results indicate that the prevalence of CCHFV is high in these regions of Côte d'Ivoire and suggest that surveillance for CCHFV should be established to assess the presence and distribution of this virus. Appropriate surveillance measures must be implemented while taking into account those at risk such as herdsmen, slaughterhouse workers and others. It is also necessary to take effective measures to control ticks that are potential vectors by controlling tick infestations in livestock in order to prevent outbreaks of CCHF among human populations.

## ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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