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Research Article

Circulation of the Crimean-Congo Hemorrhagic Fever Virus in Cattle Farms in the Municipality of Abobo (Abidjan, Côte d'Ivoire) Following the First Human Case of Crimean-Congo Hemorrhagic Fever

Adjogoua Edgard Valery[1]*, Diaha-Kouamé Claude Aimée[2], Diobo N'Guessan Fidèle[3], Kouao Diané Maxime[4] and Dosso Mireille[1]

[1]Département des Virus Epidémiques, Institut Pasteur de Côte d'Ivoire, Abidjan, Côte d'Ivoire

[2]Département Environnement et Santé, Institut Pasteur de Côte d'Ivoire, Abidjan, Côte d'Ivoire

[3]Laboratoire de Biologie et Santé, Unité de Formation et de Recherche Biosciences, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire

[4]Centre de Ressources Biologiques, Institut Pasteur de Côte d'Ivoire, Abidjan, Côte d'Ivoire

***Corresponding Author:** Adjogoua Edgard Valery, Département des Virus Epidémiques, Institut Pasteur de Côte d'Ivoire, Abidjan, Côte d'Ivoire.

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Abstract

Crimean-Congo Hemorrhagic Fever (CCHF) is a major viral zoonosis that is often fatal in humans. Controlling this disease requires knowledge of its epidemiological situation in reservoir hosts, particularly cattle. This study was conducted in a locality of the Abobo municipality following a human infection with the Crimean-Congo Hemorrhagic Fever Virus (CCHFV) from a bovine carcass, with the aim of contributing to a better understanding of CCHF epidemiology in cattle in Côte d'Ivoire. To this end, 167 bovine serum samples were collected and tested using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The study showed the presence of CCHFV in the serum of cattle in southern Côte d'Ivoire, with six (06) animals testing positive. It also revealed that among these positive samples, 83% were from female cattle compared to 17% from males. In conclusion, this investigation highlighted, for the first time, the circulation of CCHFV in cattle serum in southern Côte d'Ivoire, indicating that precautionary measures should be taken to prevent the emergence of this disease in humans.

Keywords: *CCHFV; Cattle; Farms; Abobo; Côte d'Ivoire*

Introduction

Livestock remains a highly important sub-sector in the economy of many African countries, including Côte d'Ivoire (Soffo). The low level of animal production in developing countries, particularly in Côte d'Ivoire, is attributed to multiple constraints. These include high-impact animal diseases as well as emerging and re-emerging zoonoses. Among these diseases is Crimean-Congo Hemorrhagic Fever (CCHF), a tick-borne zoonosis present in several countries, which could pose a significant threat [1]. It is caused by the CCHF virus, an *Orthonairovirus* belonging to the *Nairoviridae* family. Humans become infected through tick bites or contact with biological fluids from infected animals or humans. The disease has already been reported in cattle in some countries of the sub-region that supply livestock to Côte d'Ivoire, notably Burkina Faso (prevalence of 72.2%) [1], Mali (prevalence of 66%) [2], Niger (prevalence of 9.1%) [2], and Ghana (prevalence of 5.7%) [3]. Moreover, a recent CCHF outbreak caused human deaths in Mali [4]. In Côte d'Ivoire, livestock farming practices are characterized by the

movement of cattle through cross-border (Burkina Faso, Mali, Guinea, and Ghana) and regional transhumance. This movement led to the infection of a 39-year-old man with the CCHF virus. He was a cattle breeder living in the Akeïkoi neighborhood, located within the urban commune of Abobo, Abidjan, Côte d'Ivoire [5]. The infection resulted from handling the carcasses of two cattle imported from Burkina Faso, which, after a short stay in local enclosures, developed severe morbidity and subsequently died. It is important to note that these two cattle had been kept in three adjacent pens with other asymptomatic animals. In this context, the present study was conducted to assess the epidemiological status of the other animals that had contact with the two infected cattle. It is also worth emphasizing that this study aimed to strengthen epidemiological data on CCHF in cattle in Côte d'Ivoire, which remains insufficient to date. Therefore, this study was carried out to contribute to a better understanding of the epidemiology of CCHF in cattle in Côte d'Ivoire.

Materials and Methods

Study area and setting

This study was conducted in southern Côte d'Ivoire in August 2022, following the report of the first human case of CCHF. Samples were collected from three (3) cattle enclosures in the village of AkeiKoi. This village is located in Abobo, one of the ten municipalities of the city of Abidjan (Figure 1). These three urban farms are thus located in the city of Abidjan, the economic capital of Côte d'Ivoire, situated in the southern part of the country, which is a large and rapidly expanding urban area.

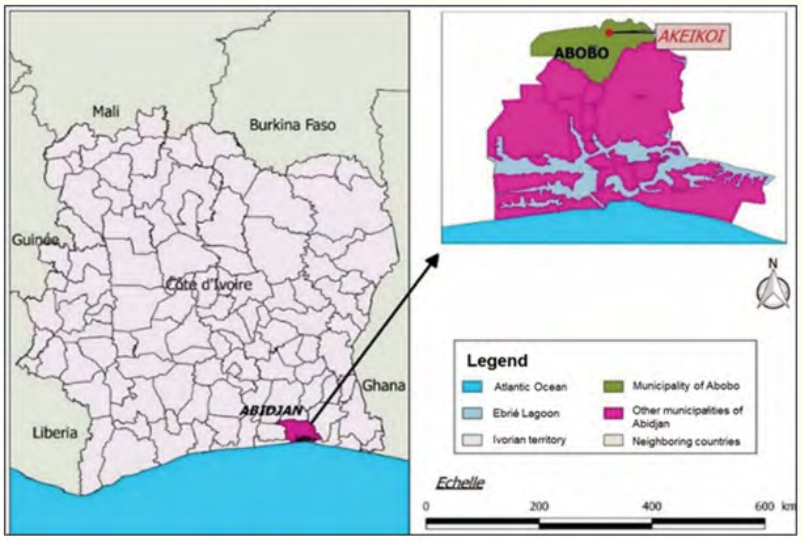

Figure 1: *Location of the village of Akeikoi (Abobo, Abidjan).*

For the investigation, the team was composed of researchers, veterinarians, and technicians from the Pasteur Institute of Côte d'Ivoire and the Directorate of Veterinary Services.

Sampling

During this investigation, blood samples were collected from three cattle enclosures in the village of Akeikoi, located in the Abobo municipality (Abidjan). Blood samples (serum and plasma) were taken from all the cattle present in these enclosures. The distribution of animals in the three enclosures was as follows: 32 animals in enclosure 1, 70 animals in enclosure 2, and 65 animals in enclosure 3.

After restraining the animal, blood was drawn using a sterile 5 cc syringe and collected into dry tubes for serum and EDTA tubes for plasma. The blood samples (serum and plasma) were stored in a cooler with ice packs at approximately +4°C.

Sample biobanking

The cattle blood samples were transferred to the laboratory of the Pasteur Institute for the detection of the Crimean-Congo Hemorrhagic Fever Virus (CCHFV). For each sample, one aliquot was used for nucleic acid extraction, and the other was stored at -80°C in the Pasteur Institute's biobank for medium- or long-term preservation, with the aim of future research on other tick-borne pathogens.

Sample analysis

Viral RNA extraction

For the analysis of bovine blood serum, viral RNA was extracted using the QIAGEN extraction kit (QIAamp Viral RNA) according to the manufacturer's protocol. This kit includes all the necessary tubes and reagents for the extraction process. After centrifugation, the supernatants of the bovine serum were collected in sterile 1.5 ml Eppendorf tubes. To inactivate the virus, 140 µl of bovine serum supernatant was added to 560 µl of AVL buffer in a microcentrifuge tube and mixed using a vortex mixer for 15 seconds to obtain a homogeneous solution. The tubes were then incubated for 10 minutes at room temperature (15°C to 25°C).

Molecular characterization by RT-PCR

The detection of Crimean-Congo Hemorrhagic Fever Virus (CCHFV) RNA was performed using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) with the AMBION amplification kit. A specific pair of primers and probe for virus detection were used following the manufacturer's protocol. The forward primer (designated CCHF S1) was 5′-TCT CAA AGA AAC ACG TGC C-3′, the reverse primer (designated CCHF S122) was 5′-CCT TTT TGA ACT CTT CAA ACC-3′, and the probe (designated CCHF probe) was FAM-ACT CAA GGKAAC ACT GTG GGC GTA AG-BHQ1, all specific for the virus. These were used to amplify the S segment of the virus.

Data analysis

The collected data were entered and processed using the Excel spreadsheet. The proportions according to the sex of the cattle suspected of infection with the Crimean-Congo Hemorrhagic Fever virus were calculated using the following formula:

Results

Proportion of cattle suspected of cchfv infection according to sex

Blood samples were collected from a total of 167 cattle during this study. Table 1 shows that females were sampled twice as much as males, representing 67.67% versus 32.33%, resulting in a sex ratio of 0.48.

Gender	Number of suspected CCHFV cases	Proportion %	Male/Female ratio
Mâle	54	32,33	0,48
Female	113	67,67	
Total	167	100	

Table 1*: Proportion of cattle suspected of CCHFV infection according to sex.*

Molecular diagnostic results of CCHFV in bovine serum samples

Total number of positive bovine serum samples

Molecular analyses revealed that out of the 167 bovine serum samples tested, six (06) were positive for CCHFV. Among these six positive cattle, five (05) came from Enclosure 3 and one (01) from Enclosure 2 (Table 2). It was noted during blood sampling that these two enclosures are adjacent.

	Enclosure 1		Enclosure 2		Enclosure 3		Total
	Male	Female	Male	Female	Male	Female	
Positive	0	0	0	1	1	4	6
Negative	9	23	27	42	17	43	161
Total	32		70		65		167

Table 2: *RT-PCR results of blood samples collected by cattle enclosure.*

Proportions of cattle infected with CCHFV according to sex

Figure 2 shows the proportions of CCHFV-positive serum samples according to sex. Indeed, among these positive samples, 83% came from female cattle compared to 17% from male cattle.

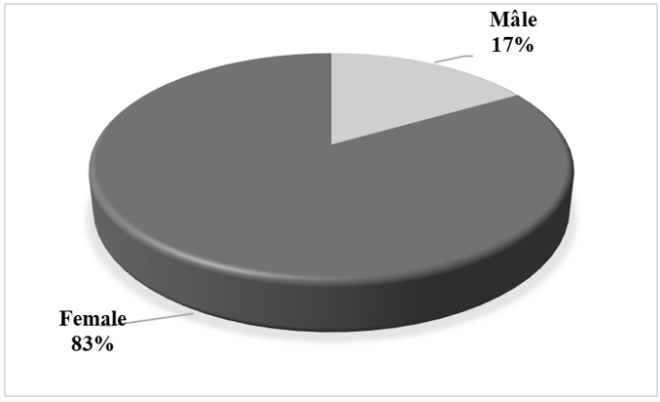

Figure 2: *Proportions of blood samples positive for CCHFV according to the sex of the cattle.*

Discussion

This study was conducted in the Abobo municipality, which is part of the city of Abidjan. Abidjan hosts the country's largest slaughterhouses and livestock markets, thus accommodating the majority of cattle from neighboring cross-border countries. The choice of this study area was based on epidemiological considerations, notably following a human infection with the Crimean-Congo Hemorrhagic Fever Virus (CCHFV) in a locality within the Abobo municipality linked to a bovine carcass. Indeed, this study was carried out to contribute to a better understanding of the epidemiology of CCHF in cattle in Côte d'Ivoire.

To this end, blood sampling was performed in the index enclosure as well as in two other nearby enclosures. The results demonstrated the presence of the Crimean-Congo Hemorrhagic Fever Virus by RT-PCR in bovine serum. This study therefore highlights for the first time

the circulation of the Crimean-Congo virus in cattle in southern Côte d'Ivoire. However, serological studies conducted by Valery., *et al*. [6] have reported the circulation of CCHFV in bovine serum in four regions of Côte d'Ivoire, namely Korhogo, Bondoukou, Man, and Bouaflé. These authors also revealed the presence of this virus in vectors (ticks) in the same locations.

The overall molecular prevalence observed was 3.59%. Indeed, out of 167 bovine serum samples analyzed, six (06) tested positive. This overall molecular prevalence is significantly lower than that reported in cattle from Niger (9%) according to Mangombi., *et al*. [7], Mali (66%), and Mauritania (67%) [2,8]. Additionally, Grech-Angelini., *et al*. [9] reported a prevalence of 13% in Corsica and Mourya., *et al*. [10] found a prevalence of 12.09% in India. These differences may be explained by the sample sizes used in the analyses, as those studies included much larger samples than ours. Moreover, those authors investigated multiple localities, unlike our study which was limited to a single study area.

The results revealed a predominance of infection in female cattle compared to males, 83% versus 17%. This difference could be explained by the sample sizes for each sex. Indeed, during our investigation, it was observed that females were more abundant in the enclosures than males, which naturally resulted in an imbalance in sample size since females were sampled more than males. Furthermore, since the virus is transmitted by ticks, this result could be explained by a higher tick infestation in females at the time of the study. Beyond this study, several other authors have also shown a higher prevalence of the virus in females compared to males-for example, [7] in Senegal and [1] in Burkina Faso. According to the latter, regarding herd management, females are preferred by breeders for reproduction and are therefore kept longer than males, which are sold for slaughter. As a result, female cattle remain in herds longer and are thus more exposed to tick bites, increasing the risk of contamination [1,2].

Finally, it was found that among the positive cattle, the majority originated from Enclosure 3, specifically five (05), compared to one (01) from Enclosure 2. This could be explained by the fact that the index bovine—that is, the one that died—came from Enclosure 3. The results of this study indicated horizontal transmission among cattle. However, the study area is not an ecological zone conducive to the development of the tick vectors primarily responsible for CCHFV, particularly species of the genus *Hyalomma*. This observation suggests that transmission of the virus may occur via other types of vectors, which remain to be identified.

Conclusion

The present study aimed to contribute to a better understanding of the epidemiology of Crimean-Congo Hemorrhagic Fever (CCHF) in cattle in the Abobo municipality. This epidemiological investigation in the three cattle farms surrounding the first human case of CCHF revealed bovine serum samples positive for the Crimean-Congo Hemorrhagic Fever Virus. This study thus confirms, for the first time, the circulation of the Crimean-Congo Hemorrhagic Fever Virus in cattle farms in southern Côte d'Ivoire. The first human case of CCHF in Côte d'Ivoire is therefore closely linked to the handling of animal tissue from cattle farms in Akeikoi (Abobo, Abidjan).

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