Original Article

Seroprevalence and factors associated with CCHF virus infection in cattle and sheep in Mopti region (Mali)

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ABSTRACT

Crimean-Congo hemorrhagic fever (CCHF) is a zoonotic arboviral disease caused by a virus of Bunyaviridae family, genus *Orthonairovirus* and transmitted by tick bite. The virus causes subclinical infection in animals and severe viral hemorrhagic disease, with a fatality rate of 10-40% in humans.Between January and February 2020, eighteen human cases of CCHF including nine deaths, were recorded in the health district of the Mopti region in Mali. The present study carried out to determine CCHF seroposivity in cattle and sheep and to identify the risk factors associated with the presence of antibodies in cattle and sheep in the Mopti region. A cross-sectional survey was conducted on a total of 200 cattle and sheep sampled in the localities of Konna and Mopti urban area. The double antigen sandwich ELISA technique allowed to establish a true overall seropositivity of 43.8% (95% CI: 36.9 - 50.6) including seropositivity of 40% (95 CI: 30.4-49.6) in Konna and 45.5% (95% CI: 35.2-54.8) in Sevare. According to species, seropositivity was 58.6% (95% CI: 48.3-67.7) in cattle and 27% (95% CI: 18.3-35.7) in sheep. Biostatistical analysis showed that cattle (OR=3.77; 95% CI: 2.07-6.87) were more likely to be seropositive compared than in sheep. This study demonstrates the circulation of virus in animals and the need to conduct joint actions according to the "One Health" approach for the control of this zoonosis.

Keywords: Seroepidemiological studies, Cattle, Sheep, Mali.

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1. Introduction

Mali, with its agropastoral tradition, is the largest livestock-producing country in the West African Economic and Monetary Union (UEMOA) and the second largest in the Economic Community of West African States (ECOWAS), after Nigeria [1]. The national livestock population is estimated as 12 848 696 cattle, 21 149 809 sheep, 29 201 079 goats, 607 786 horses, 1 190 567 donkeys, 1 291 233 camels, 88 262 pigs and 54 703 373 poultry [2]. Livestock farming contributes around 15.2% to Mali's Gross Domestic Product (GDP), behind agricultural products (16.2%) and ahead of gold (7.2%). Because of its importance, it is one of the country' s top développemental priorités. Thus, in areas where agricultural production is low or almost absent, the practice of livestock farming in these areas is becoming one of the economic means providing jobs and income. Livestock farming contributes around 80% of the income of populations in pastoral areas [1]. This sector provids most of the income needed to meet the food, healthcare and clothing expenses of the Malian population, whose activities are concentrated on pastoralism. Mali exports around 20% of its livestock products to several West African countries. Despite its importance to the country's economy, livestock development in Mali is hampered by endemic zoonoses and transboundary diseases such as contagious bovine pleuropneumonia (CBPP), peste des petits ruminants (PPR), pasteurellosis, Newcastle disease, foot-and-mouth disease, lumpy skin disease, Crimean-Congo hemorrhagic fever and Rift Valley fever. The context in Mali is characterized by wildlife cohabiting with livestock and humans particularly in rural areas that offer an ideal setting for the emergence and spread of zoonotic diseases [3]. Among the important zoonoses affecting animals and humans in Mali, Crimean-Congo Hemorrhagic Fever (CCHF) plays an important role. Crimean-Congo hemorrhagic fever is a zoonotic arbovirosis caused by a tick-borne virus of the Bunyaviridae family of the *Orthonairovirus* genus. Many ticks of different genus transmit the virus. The most effective and common vectors belong to the *Hyalomma* genus. Other tick genus, *Rhipicephalus* and *Dermacentor*, are also capable of transmitting the disease [4]. The geographical existence of the disease coincides with that of ticks of the Hyalomma genus [5]. It causes outbreaks of severe viral hemorrhagic fever, with a fatality rate of 10 to 40% in humans. The hosts of Crimean Congo hemorrhagic fever include a wide range of wild and domestic animals, including cattle, sheep and goats. Infection is asymptomatic in animals, and the CCHF virus is transmitted to humans either through tick bites or contact with infected body fluids. It is an emerging zoonosis, causing numerous epidemics in sub-Saharan Africa. In Mali, Crimean-Congo hemorrhagic fever is on the list of priority zoonotic diseases. Between January and February 2020, 18 human cases of Crimean-Congo hemorrhagic fever including 9 deaths (7 from the village of Kera, 1 in the Konna health area and 1 in the village of Senore in Douentza) were recorded in the Mopti health district [6]. Investigations have also identified the probable index case, a 39-year-old shepherd who was infected through contact with his cattle [7]. The first human cases of CCHF were identified in the Koulikoro region in 1991, with a seroprevalence of 4.5% out of a total of 228 people [8]. Between 2005 and 2014, out of 1,075 bovine sera collected in the country within the national epizootic surveillance program, an overall seroprevalence of 66% was noted [9]. This indicates the inapparent circulation of the CCHF virus in these animals. However, despite the gravity and zoonotic character of CCHF, available data on its epidemiological situation in domestic animals in Mali are scarce. The study was carried out to update data on the epidemiological situation of CCHF in cattle and sheep in Mali.

2. Materials and Methods

2.1. Area, period and type of the study

This study was carried out in the Mopti region. It is the 5th administrative region of Mali. This region is located in the center of the country. It extends from 15°30' to 13°45' north latitude and 5°30' to 6°45' west longitude, and covers a total area of 79,017 km², or 6.34% of the national territory. Climatically, the region is located between the Sahelian zone and the northern Sudanian zone. The first zone is characterized by an arid to semi-arid regime, while the second, more humid, covers only a small part of the region [10]. Livestock plays a very 0important role, with 3,551,543 cattle, 3,742,058 sheep, 5,394,599 goats, 40,851 horses and 18,585 camels [2]. The Mopti region accounts for 22% of the national livestock population, and livestock farming is practiced in two (2) main modes: sedentary livestock farming, with limited radius of movement around towns and villages, and transhumant livestock farming, which is the most common. *Hyalomma spp*. tick, the vector of CCHF, lives in areas with a hot biotope [14]. The type of climate present in this region could be a favorable environment for this tick species. Also the seasonal movement of livestock farmers and their herds at the end of the rainy season towards areas that are more likely to fulfil their herd's nutritional needs presents an exchange oppotunity with tiks wich may infected by the CCHF virus. The study was carried out in two localities in the Mopti region, the communes of Konna and the urban commune of Mopti (Figure 1). This was a descriptive cross-sectional study from April to May 2021.

2.2. Sampling and data collection in the field

The animal population investigated included cattle and sheep in the study area. The study covered cattle and sheep without any discrimination as to breed, sex or age. Given the prevailing insecurity in the region, a rational sampling method was applied, targeting localities according to their accessibility. In the two localities selected for the study, 24 herds were sampled. A total of 200 samples were collected for the entire study. Blood sampling in the field was carried out in collaboration with the veterinary services of the

Mopti region. After contention, blood was collected from the jugular vein in Vacutainer vacuum tubes. Each sample was coded according to the site. Information on the species, animal age, sex and location were collected using a questionnaire. The age of the animals sampled was determined from their teeth. based on this, the animals were classified into two age groups: young (0-2 years) and the adult (3 years and over). The sera were then collected in 2ml cryotubes and stored at -20°C until analysis.

2.3. Serological analysis

In this study, samples were analyzed using the sandwich ELISA (Enzyme Linked Immuno-Sorbent Assay) method for antibodies to the CCHF virus nucleoprotein (NP). For this purpose, the ID Screen CCHF Double Antigen Multi-species Kit from the IDvet laboratory (ID.Vet, France) was used. This kit can detect both IgM and IgG simultaneously and without discrimination, with a sensitivity of 98.9% and a specificity of 100% [11]. The kit was used according to the manufacturer's instructions, and the optical densities obtained using an ELISA reader (MULTISKAN EX) at 450nm were recorded and exported to an Excel file for calculation and conversion into percentages of positivity (S/P). S/P % = OD sample/OD positive control x 100. Samples with S/P % less than or equal to 30% were considered as negative samples, and those with S/P % greater than 30% as positive samples.

2.4. Statistical analysis of data

Field survey data and serological test results were collected and saved in Microsoft Office 2016 Excel spreadsheets. For each seropositivity rate, the 95% confidence interval (CI) was calculated using the following formula: $CI= p\pm1,96\times\sqrt{p(1-p)/N}$. The true seropositivity was calculated using the following formula: $Pr = [Pa + (Sp-1)] / [Sp + (Se-1)]$. Statistical analyses were performed using R software version 4.1. Odds ratios were calculated using STATA 15 software. The animal's serological status was considered as a dependent variable, and the variables collected in the field as independent variables. The association between dependent and independent variables has been checked using the chi-square test or Fisher's exact test. To identify factors associated with infection, univariate logistic regression was used to determine unadjusted odds ratios. At the end of this analysis, the variables for which the univariate logistic regression provided a *p-value* less than or equal to 0.20 are included in a multivariate analysis using a logistic regression model. Factors associated with CCHF virus infection are identified by analyzing odds ratios and their confidence intervals, as well as the p-values associated with each analysis. For all these analyses, the significance level was set at 0.05.

3. Results

3.1. Seroprevalence

After analysis, 85 animals out of 199 were positive giving an apparent overall seropositivity of 42.7% (95% CI : 36.2% - 49.8%) (Table 1). The overall seropositivity was 43.8% (95% CI : 36.9 - 50.6).

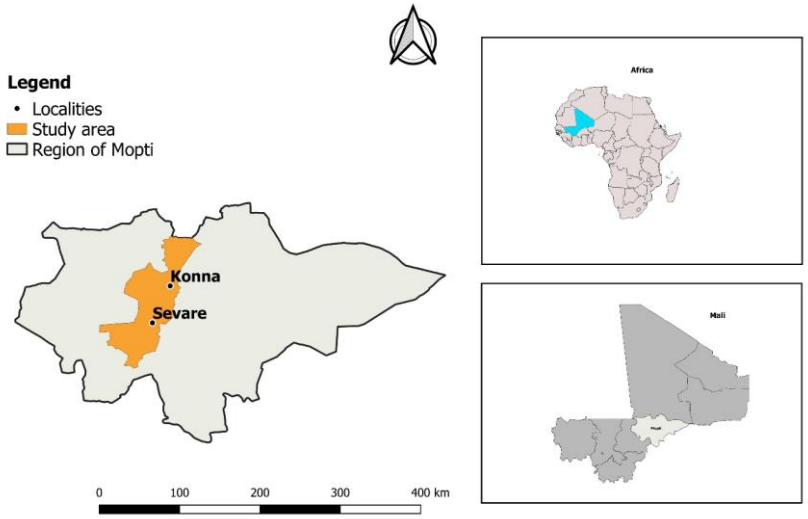

Figure 1. geographical schematic of research area

Table 1 : CCHF seropositivity in the localities of Konna and Sevare

Area	Number of animals	Positives	Prevalence (%)	95% CI
Konna	100	40	40	(30,4-49,6)
Sevare	99	45	45,5	(35,2-54,8)
Total	199	85	42,7	(36,2-49,8)

3.2. Variation of seropositivity by location, age, sex and species

Table 2 shows the variation of seropositivity of CCHF according to locality, age, sex and species. According to age, seropositivity is significantly higher in adults than in young animals ($p<0.05$). A variation in seropositivity was also observed between the two species, with a significantly higher prevalence in cattle ($p<0.05$).

3.3. Identification of factors associated with CCHFV infection

3.3.1. Univariate logistic analysis

In univariate analysis, cattle were more likely to have antibodies against CCHF virus compared to sheep (OR= 5.41; 95% CI: 2.17-13.48).

3.3.2. Multivariate logistic analysis

The results of multivariate logistic regression showed that cattle were 3.77 times (OR=3.77 ; 95% CI : 2.07 6.87) more likely to have antibodies against CCHF virus compared to sheep (Table 3).

4. Discussion

Laboratory analysis in the present study gave an overall apparent seropositivity of 42.71%. The true overall seropositivity found was 43.8%, showing that the CCHF virus is circulating in these 2 location of the Mopti region. These results reveal an apparent seropositivity of 58.6% in cattle and 27% in sheep, which are lower than those obtained in cattle in other previous study in the same region (69%) [9] and in Mauritania [12, 13]. These differences in seropositivity may be linked to the sampling method used in these studies. Indeed, These studies were carried out on 394, 495 and 201 cattle respectively, a larger sample size than in the present study. Nevertheless, the prevalences found in this study were higher than those obtained in Niger (9.01% in cattle), in Nigeria (24.0% in cattle and 0% in sheep), in Burkina Faso (14.8% in sheep) [14, 15, 16]. In Senegal, a study conducted in the regions of Saint-Louis, Louga, Matam and Tambacounda reports a serological prevalence of 6.52% in sheep [17]. This difference may be explained by the lower exposure of animals in these areas to the CCHF virus. The study showed that seropositivity varies according to locality but this variation was not significant. Seropositivity also varied according to the age of the animals. It was significantly higher in adults than in young animals and the same observations were made in others african countries [13, 17]. This result could probably be due to the cumulative exposure of animals to CCHF virus. In another study conducted in Kenya, the authors report that this could also be attributable to the presence of maternal immunity against CCHF virus, which may have had an effect on the low seroprevalence in young animals [18] . The seropositivity did not vary significantly according to the sex of the animals. This result differs from those found in other studies where females are more infected than males [14, 16, 17]. Our results also showed a variation in seropositivity by species, with seropositivity significantly higher in cattle than in sheep. The same observation was made in neighboring Mauritania and in Nigeria [13, 15]. This may be attributed to the breeding mode of these two species. In Mali, cattle breeding generally differs from that of small ruminants. Cattle are bred extensively. They spend more time in the wild and more in contact with wildlife. This species is therefore more exposed to the CCHF virus. Small ruminants, on the other hand, are bred semi-extensively. They spend less time on pasture and are therefore less exposed to the CCHF virus.

Table 2. Variation of CCHF seropositivity

Variables	Modalitys	Nb analyzed	Nb positives	Seropositivity (%) and 95% CI	*P-value*
Location	Konna	100	40	40 (30,4-49,6)	0,525
	Sevare	99	45	45,5 (35,2-54,8)	
Age	Young	89	31	34,83 (25,1-44,9)	0,045
	Adult	110	54	49,09 (39,7-58,3)	
Sex	Male	84	42	50 (39-61)	0,083
	Female	115	43	37,39 (28,2-45,8)	
Species	Cattle	99	58	58,6 (48,3-67,7)	$1{,}298.\ 10^{-5}$
	Sheep	100	27	27 (18,3-35,7)	

Table 3 : Univariate and multivariate logistic regression of CCHF virus infection

Variables	Modalitys	Univariate regression		Multivariate regression	
		Odds ratio (CI*)	*P value*	Odds ratio (CI*)	*P value*
Location	Konna	Ref		Ref	-
	Sevare	1,25 (0,71-2,19)	0,43	-	-
Age range	Young	Ref		Ref	-
	Adult	1,73 (0,73-4,13)	0,21	-	-
Sex	Female	Ref		Ref	Ref
	Male	1,67 (0,94-2,96)	0,077	1,61 (0,88 – 2,94)	0,11
Species	Sheep	Ref		Ref	Ref
	Cattle	5,41 (2,17-13,48)	0,000*	3,77 (2,07 – 6,87)	0,000*

*CI : 95% confidence interval

Other studies have also reported that infection with CCHF virus seems to occur most frequently in large mammals such as cattle, which are the preferred hosts of adult *Hyalomma* ticks [19]. After univariate and multivariate logistic regression analyses, only bovine species was identified as a factor associated with CCHF virus infection. These results show that sheep are less likely to have antibodies against CCHF virus than cattle, suggesting that cattle may play a major role in the epidemiology of this disease in Mali. The same observation is made by other authors [13, 15]. The results of the present study showed that the sex of the animal was not associated with infection by the CCHF virus. Schulz and al. (2021) [13], also reported in their study that sex was not a factor associated with infection by the CCHF virus. Contrary to the studies carried out in other neighboring countries such as Niger [14] and Burkina-Faso [16], these results also showed that the age range of the animals was not a factor associated with CCHF virus infection.

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Authors' Contribution
Study concept and design : M A D, L D D and R B-A.
Acquisition of data : J B T and C O F
Analysis and interpretation of data : M A D, L D D
Drafting of the manuscript : MA D
Critical revision of the manuscript for important intellectual content : YH, N M
Administrative, technical, and material support : A D, M A D, F D, C D, S S.

Ethics
Data collection and blood sampling are carried out with the informed consent of herd owners. Samples are taken by qualified personnel after appropriate restraint of the animals to ensure the safety of both personnel and animals.

Conflict of Interest
The authors declare that they have no conflict of interest.

Data Availability
All detailed Data and informations of this study are available upon request from the corresponding author

References

1. Mali. Programme for the development of meat exports from Mali (PRODEVIM), Project to support the improvement of livestock health and production. Working document. 2018.
2. Mali. National Direction of Animal Production and Industries (DNPIA). Annual report. 2021. 151p.
3. Centers for Disease Control and Prevention. Résumé de l'atelier: Priorisation des maladies zoonotiques pour l'engagement multisectoriel au Mali. 2017.19p.
4. Zientara S, 2013. La fièvre hémorragique de Crimée-Congo est en recrudescence en Europe orientale. Bulletin Epidémiologique., n 33, pp.13.
5. Whitehouse CA. Fièvre hémorragique de Crimée-Congo. *Antiviral Res*. 2004 ;64(3) :145-60.
6. Mali. Mopti Regional Direction of Veterinary Services (DRSV Mopti). Report on the evolving situation of Crimean-Congo hemorrhagic fever in the Mopti region. 2020. 08p.
7. WAHO. Epidémiological-newsletter 2020. Bamako: WAHO. 2020;10p.
8. Traoré AK, Dao S, Jouanelle JC, Bougoudogo F, Toure YT, Maiga Kl. A propos des premières observations sérologiques de la fièvre hémorragique de Crimée Congo au Mali. *Mali Médical*. 2005 ;20(4) : pp 52-53.
9. Maiga O., Andrada Sas M., Rosenke K., Kamissoko B., Mertens M., Sogoba N., et al,. Serosurvey of Crimean–Congo Hemorrhagic Fever Virus in Cattle, Mali, West Africa. *American Journal of Tropical Medicine and Hygiene*. 2017 ;96(6):pp. 1341–1345.
10. Mali. Direction nationale de la conservation de la nature., Evaluation intégrée des écosystèmes: cas de la région de Mopti au Mali. Rapport provisoire, 2009 ;114p.
11. Sas, M.A., Comtet, L., Donnet, F., Mertens, M., Vatansever, Z., Tordo, N., Pourquier, P., Groschup, M.H., "A novel double-antigen sandwich ELISA for the species-independent detection of Crimean-Congo hemorrhagic fever virus-specific antibodies". *Antiviral Research*. 2018 ;151 :24-26.
12. Sas MA., Mertens M., Isselmou E., Reimer N., Mamy B., Doumbia B., Groschup MH., Crimean-Congo hemorrhagic fever virus specific antibody detection in cattle in Mauritania. *Vector Borne Zoonotic Dis*. 2017 ;17 (8) :582-587.
13. Schulz A, Barry Y, Stoek F, Ba A, Schulz J, Haki M.L, et al, Crimean-Congo hemorrhagic fever virus antibody prevalence in Mauritanian livestock (cattle, goats, sheep and camels) is stratified by the animal's age. PLoS *Neglected Tropical Diseases.* 2021 ;15(4): 12p.
14. Maïna A., Ibrahim A. I., Abdou Alassane A., Adakal H., Epidémiologie de la fièvre Hémorragique de Crimée-Congo (FHCC) chez les bovins dans le département de Boboye au Niger. *International Journal of Biological and Chemical Sciences*. 2020;14(3): 698-705.

15. Oluwayelu D., Afrough B., Adebiyi A., Varghese A., Eun-Sil P., Fukushi S. et al., Prevalence of Antibodies to Crimean-Congo Hemorrhagic Fever in Ruminants, Nigeria. *Emerging Infectious Diseases*. 2015;26(4) :744-747.
16. Dahourou L. D., Akio S., Savadogo, M., Yougbaré B., Ouoba L. B., Tapsoba A. S. R.,et al. Serological evidence and factors associated with Crimean-Congo haemorrhagic fever in sheep in Burkina Faso. *Veterinary Medicine and Science*. 2024 ;10(1322) :10p.
17. Dieye A., Enquête sérologique sur la fièvre hémorragique Crimée Congo et la fièvre de la vallée du Rift chez les ruminants dans les régions de Saint-Louis, Louga, Matam et Tambacounda. Mémoire de Master SPV–EGRS/EISMV: Dakar. 2019 ;30p.
18. Blanco-Penedo, I.; Obanda. V.; Kingori, E.; Agwanda, B.; Ahlm, C.; Lwande, O.W., Seroepidemiology of Crimean-Congo Hemorrhagic Fever Virus (CCHFV) in Cattle across Three Livestock Pastoral Regions in Kenya. *Dairy*. 2021 ;2 :425–434.
19. Shepherd A. J., Swanepoel R., Leman PA., Antibody response in Crimean-Congo hemorrhagic fever. Rev *Infectious Disease*. 1989; 11(4): S801–6.