



EVIDENCE OF RIFT VALLEY FEVER VIRUS CIRCULATION IN CATTLE PARKS IN SLAUGHTERED OF MAN DISTRICT (CÔTE D'IVOIRE).

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ABSTRACT

Background: Rift Valley Fever Virus is a viral zoonosis caused by an arbovirus transmitted by Culicidae. This arbovirus can seriously affect different species of domestic ruminants, causing severe outbreaks, economic losses and humans' mortality. This study was designed to investigate RVFV infection in cattle and mosquito in the slaughterhouse of Man city, western Côte d'Ivoire. **Methods:** Blood samples were collected from two cattle parks during dry seasons. Mosquito were collected during rainy and dry seasons. Mosquitos were trapped using six light traps, installed in general at 6:00 pm and visited the following morning at 6:00 am during four consecutive days. Molecular diagnosis of RVFV was carried out by real-time RT-PCR with specific primers; Forward: 3'-TGAAAATTCCTGAGACACATGG-5', Reverse: 3'-ACTTCCTTGCATCATCTGATG-5' and Probe: FAM - CAATGTAAGGGGCTGTGTGGACTTGTG-TAMRA for L segment. Blood samples were analysed for RVF IgM and IgG detection using Enzyme-Linked immunosorbent assay. **Results:** Forty blood samples from animals and 634 adults mosquitoes belonging to five species: *Anopheles gambiae* (1.7%), *Culex cinereus* (6.6%), *Culex quinquefasciatus* (91%) *Culex nebulosis* (0.5%) and *Mansonia* sp. (0.2%) were collected. No IgG and IgM antibodies against RVFV were found in any of 40 sera. More than 70% of *Culex quinquefasciatus* regardless of site and season was observed. RVFV was successfully detected in 50% of *Culex cinereus*' pools and 6% of *Culex quinquefasciatus*' pools. **Conclusion:** This study diagnosed for the first time RVFV from mosquito in western country. It was found in *Culex* (*Culex quinquefasciatus* and *Culex cinereus*) which represents the most abundant genera in study site.

KEYWORDS: Rift Valley Fever Virus, domestic ruminants, Mosquitos vectors, Relative abundance, Côte d'Ivoire.

INTRODUCTION

Rift Valley fever (RVF), an acute arthropod-borne viral disease, is due to a member of *Phlebovirus* genus belonging to Bunyaviridae family. Rift Valley fever Virus (RVFV) causes an acute viral zoonotic disease of varying severity including haemorrhagic fever, encephalitis and mortalities. RVFV is transmitted to ruminants by Arthropod vector or by direct contact with viremic fluids such as blood, foetal membranes or amniotic fluids.^[1,2] Transmission is also possible by contact with infectious animal tissues and inhalation of aerosols generated by slaughtering and necropsy.^[3] RVFV has been isolated from many vectors in the field^[4], such as ticks and sand flies which are able to transmit the virus in experimental conditions.^[5-7]

However, mosquitoes are the main insects involved in the spread of RVFV during outbreaks. RVFV has been isolated from *Aedes*, *Culex*, *Anopheles*^[7,8] and *Mansonia*.^[9]

Rift Valley Fever mainly affects domestic ruminants such as sheeps, goats and cattle.^[10,11] In livestock, RVFV causes abortion storms and high mortality rates have been observed among younger animals.^[12,13] Abortion rate in pregnant ewes is around 95 to 100%.^[1] More than 90 % of lambs infected with RVFV die.^[14] RVFV has been responsible for loss of more than 100,000 pets in the last 15 years.^[15] The significant impact of RVF on livestock health, production, food security and trade, induce significant economic losses.^[16,17] The risk of

introducing RVF into disease-free countries by importation of an infected animal or mosquito is real, and the consequent restriction of access to export markets may induce dramatic economic consequences for national and local economies.^[16]

Several outbreaks of RVF have been described in sub-Saharan Africa, notably in Kenya, where the disease was first described^[10], Senegal^[18] and recently in Niger, where it caused 363 cases of abortion and 370 cases of animal deaths in 2016.^[19] It has also been reported in Burkina Faso and Mali^[20] and Guinea^[12], neighboring countries of Côte d'Ivoire. Ivorian livestock, particularly those in areas close to the borders with countries that have experienced rift valley fever epidemics, are highly exposed. The city of Man, in addition to being close to the border with Guinea, has a high concentration of rice-growing activity that is conducive to the development of mosquitoes. This study was therefore designed to study RVF virus infection in cattle and mosquitoes in two

packs in slaughterhouses in Man district, Côte d'Ivoire. in slaughterhouses in Man district, Côte d'Ivoire.

MATERIAL AND METHODS

Study area and sampling sites

This study was carried out at two cattle parks of slaughterhouse of Man district (7° 24'45" N, 7° 33'13" W), in western Côte d'Ivoire (600 km from Abidjan) (Figure 1). Man has only one rainy season (RS) (April to October) and dry season (DS) (November to March).^[21] Rainfall reaches more than 2000 mm per year with peak in August (302 mm) and September (262 mm). Except for January, the climate is generally warm and humid. The average annual temperature varies little around 25°C. Relative humidity is very high, with an annual average of 90%. This study was conducted in two cattle parks, each containing at least 30 oxen. Man slaughterhouse is located in the city, surrounded by housing.

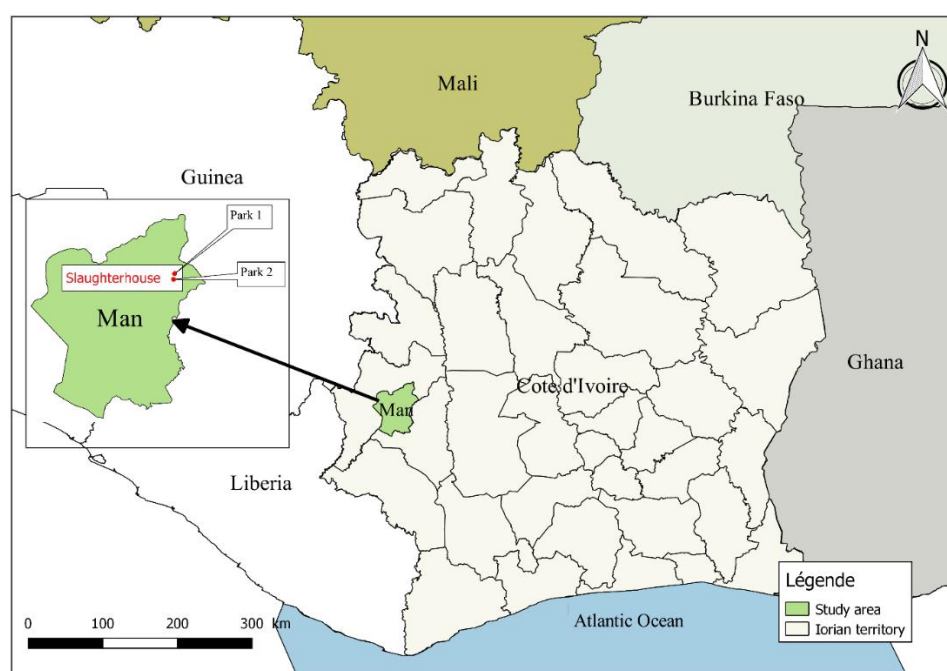


Figure: Study area location.

Study design

This study was designed as a cross-sectional study. Data collection was done in two cattle parks selected in the slaughterhouse in Man city. The distance between these two cattle parks was about 100 meters. In each cattle park, blood samples were collected from 20 randomly chosen cattle. The choice of number of animals sampled per park was based on the total number of livestock of park, which was maximum 30. To avoid colostral immunity, cattle were selected were at least one year of age. Animals were sampled at March 2019 during the dry season; during which animal restraint is easier and less tiring for the herdsman. Adult mosquitoes sampling was done twice in August 2018 and March 2019

corresponding to rainy season (RS) and dry season (DS) respectively. Three sampling points were choosing in each park.

Adult mosquito collection and identification

Mosquitos were collected using light traps technique.^[22] In brief, a light trap is a devise powered by power bank (10400mAh, Input: 5V/2A, Output/ 5V/1A&5V/2A) and which use UV technology to attract mosquitoes and other flying insects. The light traps were hanged on the parks fence approximately 1.5 m above the ground. They were installed at 6:00 pm and collected the following morning at 6:00 am. The trapping occurred once a month during four consecutive days. All mosquitoes collected were

observed under microscope (OPTIKA LAB-10 Binocular Stereo Microscope 20x & 40x Magnification) and identified to species level using taxonomic keys as previously describe by Edwards (1941) and Mattingly (1971).^[23,24] They were grouped into monospecific pools of 10 mosquitoes in 1.5 ml cryotube labelled. The pools were stored in liquid nitrogen (-180°C) and transported to the laboratory, where they were stored at -80 °C until virus RNA extraction and molecular biology analysis.

Blood sampling in cattle

Five ml of whole blood was collected from the jugular vein of cattle in Vacutainer tubes (Becton Dickinson, USA). All the work was done taking care to secure each of the actors involved, including the animals. The collected blood samples were subsequently properly stored and transported in a cool box at 4°C to the virology department of Institute Pasteur, where sera was separated from whole blood by centrifugation; samples stored at -80°C until tested.

Laboratory analysis

Serological analysis using capture IgM and IgG by Elisa test

All sera samples were tested for evidence of IgG and IgM by using an ELISA technique. The plate reading was done by spectrophotometry (450-620 nm).

RNA extraction and diagnosis of RVF viruses by RT-PCR

RNA was extracted from female mosquitoes and cattle sera. The mosquitoes grouping in batches of 10 were ground with 500 µl of phosphate buffered saline buffer (PBS) using with a disposable pestle. After homogenization, the supernatants recovered in 1.5 ml Eppendorf tube. As for the cattle serum, they were also collected in cryotubes (1.5 ml) from whole blood after centrifugation. For real-time PCR 140µl of supernatants were used for RNA extraction using the QIAamp Viral RNA Extraction Kit (QIAGEN, Valencia, CA, USA), according to the manufacturers protocol. RNA eluted after extraction are stored at a temperature of -80 °C until the amplification tests specific for the RVFV RNA sequences. The molecular detection of the virus was carried out by real-time RT-PCR (One step Applied

Biosystems) using the Ambion kit with Forward primer: 3'-TGAAAATTCCTGAGACACATGG-5', Reverse primer: 3'-ACTTCCTTGCATCATCTGATG-5' and FAM - CAATGTAAGGGGCCTGTGTGGACTTGTG-TAMRA specific probe for L segment of RVFV as previously used by Jackel and al. (2013). The RT-PCR was performed in a 25 µl reaction volume containing 1 µl of extracted RNA, 2xQuantitect Probe, nuclease free water, primer and Sonde Probe. The following amplification program was used: 50 °C for 10 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.

Data analysis

Data were entered into a spreadsheet, cross-checked and transferred into R version 3.5.1. The Person's Chi-square test (χ^2) was used to compare, the relative abundance (RA) of mosquito species, and proportion by season which expressed as the percentage of the total number of mosquitoes collected. However, when the number of mosquitoes was below 5, the Fisher's exact test was applied. All differences were considered significant at $P < 0.05$.

RESULTS

Serology of animal tested

Sera samples were obtained and tested from 40 cattles; no IgG and IgM antibodies anti-RVFV was found in any of the 40 sera.

Diversity and relative abundance of mosquito species

A total of 634 adult's mosquitoes belonging to at least five mosquito species were collected. They were composed of three genera: *Anopheles* (1 species), *Culex* (3), and *Mansonia* (1). *Culex* and *Anopheles* were captured in both parks. *Mansonia* was collected only in park 2. The genus *Culex* was the most represented with three species (*Culex cinereus*, *Culex quinquefasciatus*, *Culex nebulosis*). The other two genera had one species each: *Anopheles gambiae* and *Mansonia sp.* *Cx. quinquefasciatus* was the most abundant. Park 1 and DS recorded the highest number of mosquitoes collected (Table 1).

Table 1: Spatial and seasonal variation of the relative abundance of mosquito species collected in two cattle parks of Man district.

Species	Park 1	Park 2	χ^2	Pvalue	RS	DS	χ^2	Pvalue
	n (%)	n (%)			n (%)	n (%)		
<i>Anopheles gambiae</i>	8 (2.2)	3 (1.1)	0.920	0.539*	10 (3.7)	1 (0.3)	10.584	0.001*
<i>Culex cinereus</i>	18 (4.9)	24 (9.1)	3.871	0.049	0 (0)	42 (11.4)	29.054	0.011*
<i>Culex quinquefasciatus</i>	343 (92.7)	234 (88.6)	0.147	0.701	256 (96.2)	321 (87.2)	0.716	0.397
<i>Culex nebulosis</i>	1 (0.3)	2 (0.8)	0.769	0.574*	0	3 (0.8)	2.161	0.269*
<i>Mansonia sp.</i>	0	1 (0.4)	1.398	0.417*	0	1 (0.3)	0.722	1*
Total	370	264			266	368		

χ^2 : Chi-square; RS: Rainy Season; DS: Dry Season; *: The exact Fisher test was applied

The statistical tests on Relative Abundance (RA) of species had shown that no significant difference between the proportions of *An. gambiae*, *Cx. quinquefasciatus*, *Cx. nebulosis* and *Mansonia sp.* caught in both parks. However *Cx. cinereus* was more abundant in park 2 ($\chi^2 = 3.871$; $p = 0.039$). The proportion of *An. gambiae* was significantly higher during RS than DS ($\chi^2 = 10.584$; $p = 0.001$), contrary at *Cx. cinereus* which was higher during DS than RS ($\chi^2 = 29.054$; $p = 0.011$). There was no significant difference between seasons at *Cx. quinquefasciatus* ($\chi^2 = 0.716$; $p = 0.397$) and *Cx. nebulosis* ($\chi^2 = 2.161$; $p = 0.269$). Nevertheless *Cx. quinquefasciatus* was the most abundant among the mosquitoes captured in each park. *An. gambiae*, *Cx. nebulosis* and *Mansonia sp.* captured were in very small proportion.

RVFV detection by RT-PCR amplification

A total of 628 females mosquitoes were sorted in 68 monospecific pools at the rate of 10 mosquitoes per pool. Pooling has made possible to obtain 38 and 30 pools for the park 1 and 2 respectively. The pools of park 1 are divided into 1 pool of *An. gambiae* 2 of *Cx. cinereus*, 34 of *Cx. quinquefasciatus* and 1 of *Cx. nebulosis*. In park 2, the catches made it possible to constitute 1 pool of *An. gambiae*, 3 of *Cx. cinereus*, 24 of *Cx. quinquefasciatus*, 1 of *Mansonia sp.* and 1 of *Cx. nebulosis*. No RVFV was detected in all mosquitoes pools obtained in P2. RVFV was successfully detected in mosquitos' females at P1. Virus was detected in 1 pool (50%) of *Cx. cinereus* on 2 pools obtained and in 2 pools (6%) of *Cx. quinquefasciatus* on 34 pools. Infection rate for all mosquitoes caught in both parks was estimated as 4.4% (3/68). No virus was detected in *An. gambiae*, *Cx. nebulosis*, and *Mansonia sp.* (Table 2) and cattle samples.

Table 2: Summary of Rift Valley Fever Virus diagnosed from mosquitoes captured in park 1 of the slaughterhouses in Man city (western Côte d'Ivoire).

Species	Total	RS	Number of pools	Number of positive PCR pools	DS	Number of pools	Number of positive PCR pools
<i>Anopheles gambiae</i>	8	8	1	0	0	0	0
<i>Culex cinereus</i>	18	0	0	0	18	2	1
<i>Culex quinquefasciatus</i>	343	129	13	2	214	21	0
<i>Culex nebulosis</i>	1	0	0	0	1	1	0
Total	370	137	14	2	233	24	1

RS: Rainy Season; DS: Dry Season

DISCUSSION

This was the first time that the animals in the west of Côte d'Ivoire (Man district) have been involved in RVF antibodies detection, and to our knowledge no reports of RVFV circulation from this region have been available. Non-detection of anti-RVF IgG and IgM in domestic animals is surprising considering the presence of virus in Mosquitos. This observation could be ascribed to different factors such as the small number of cattle analysed. It is the first time RVFV was detected in these localities, perhaps a recent introduction of the virus in these localities, or again the renewal of animal populations.^[25] The latter hypothesis is supported by the fact that animals in slaughterhouse parks were intended for slaughter and therefore frequently renewed.

Assessment of abundance of RVF vectors and diagnosis of RVFV provides important data of entomological risk for RVF occurrence. These information can provide guidance in the design of appropriate prevention and control measures, as there currently there is no commercially available vaccine for RVFV, and vector control is likely to be a major component of outbreak response efforts.

Mosquito catching by the light trap technique in the two cattle parks of the slaughterhouse in the city of Man showed a low diversity of mosquito species. This result

may be related to the capture technique. Indeed, the light trap technique less effective in *Aedes* genus catching.^[26] Alternatively, this could be explained by the short collection period which was 4 days in one month per season. *Cx. quinquefasciatus* represents more than 91% of captured mosquito. Presence and abundance of *Cx. quinquefasciatus* in our study may be due to its close link with urban habitat.^[27] Its proliferation in urban areas results from the acceleration of urbanization process in West Africa without sufficient infrastructures for water collection and elimination. Thus creating breeding sites (open canals, sewage drainage pit, temporary pond on the periphery etc.) conducive to Culicidae proliferation.^[28] This makes is sometimes considered an ecological benchmark for urbanization.^[29] This phenomenon has also been observed everywhere in Africa: Bouaké in Côte d'Ivoire^[30], Dakar in Senegal^[28] and Yaoundé in Cameroon.^[31]

Except of *Mansonia sp.* occasionally found in urban areas, all the mosquito species found were observed during the two seasons and in the two parks. Their small proportion could be linked to the effect of using insecticides unlike *Cx. quinquefasciatus* which shows more resistance.^[32] In general, mosquitoes are more abundant in the rainy season, contrary to the results of this study. The rain on the traps during catches in the rainy season could explain these results. In addition, the

high humidity of the study area (mountainous area) during the year, and the presence of artificial breeding sites (livestock waterers, stagnant wastewater, etc.) on the study site (slaughterhouse) would have favoured the presence of mosquitoes during the dry season.

This study is part of the arthropod-borne viruses' vector surveys and reports the first identification of RVFV mosquito in Côte d'Ivoire. Four out of the five species of mosquitoes (*Cx. quinquefasciatus*, *Cx. nebulosis*, *An. gambiae*, *Mansonia. sp.*) collected during this study had already been identified as vector of RVFV during major epizootics in some African countries. They have been identified in Kenya^[33] and in Senegal.^[4] An entomological study carried out during the last outbreak of RVF in Kenya in 2006/2007 also revealed the presence of RVFV in these mosquitoes.^[33]

RVFV has been identified in *Cx. quinquefasciatus* and *Cx. cinereus*. *Cx. quinquefasciatus* has been several times involved in RVFV transmission^[34, 35], unlike *Cx. cinereus*, which is described for the first time as a vector for this virus in this study. Absence of RVFV in other species of mosquitoes (*Cx. nebulosis*, *An. gambiae*, and *Mansonia sp.*) in our study can be explained by their small number, unlike the more abundant of *Cx. quinquefasciatus* and *Cx. cinereus*, thus offering more opportunities for RVFV discovery within them. Our results are similar to a studies conducted in Sudan by Seufi and Galal in 2010, which showed the presence of RVFV in the most abundant species.^[35]

CONCLUSION

This study demonstrated for the first time the presence of RVFV in mosquitoes collected in western part of Côte d'Ivoire. The virus was found in *Culex* (*Cx. quinquefasciatus* and *Cx. cinereus*) which represents the most abundant genus of the study site. *Cx. cinereus* is described for the first time as a vector of RVFV. These data contribute to the knowledge of RVFV circulation and its vectors in the Culicidae population. These data are important for the epidemiological surveillance of vector-borne diseases in the management of animal health in Côte d'Ivoire. As the Culicidae species identified in this study are present in other geographical areas of Côte d'Ivoire, a more extensive study would allow a better characterization of the RVFV vector population in the country.

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CONFLICT OF INTEREST

All authors declared that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

KKARMC, AEV, KY and FH designed the study; KKARMC, G-CN, AEV implemented the study and collected the data; KKARMC, G-CN and KKY managed the data; KKARMC and KKY analysed and interpreted the data; KKARMC wrote the first draft of the paper. All authors revised and approved the final manuscript before submission.

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