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Persistence of Hantavirus circulation in *Neoromicia Nanus* (banana pipistrelle) in Aboisso, South East Côte d'Ivoire

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ABSTRACT

The hantavirus Mouyassué was first described in 2009 in banana pipistrelle (Neoromicia nanus) near Aboisso South East Côte d'Ivoire. In order to investigate the persistence of hantavirus in this region, eight years later we captured 163 banana pipistrelle in the same region. The use of nested RT -PCR allowed the detection of the virus within 30 banana pipistrelle that represents 18%. Phylogenetic analysis confirmed that the detected viruses all belonged to the hantavirus Mouyassué virus. These results demonstrated that banana pipistrelle constitute natural host of hantavirus in this area. Given that banana pipistrelles are commonly found in and around villages and frequently roost in the roofs of houses, further investigation is warranted to assess the potential risk of hantavirus transmission to humans.

Keywords : Hantavirus, Bat, *Neoromocia nanus*, Côte d'Ivoire

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Introduction

Bats constitute the second largest order of mammals representing more than 20% of all mammalian species (Wang et al., 2011). They help control insects population, reseed cut forests, and pollinate plants that provide food for humans and other species. Moreover, their guano can be used as a fertilizer and for manufacturing soaps, gasohol, and antibiotics (Barbosa Dos Santos et al., 2024; Calisher et al., 2006).

However bats are thought to be the source of several nastiest viruses that can jump to humans from animals during the past 40 years, including Ebola hemorrhagic fever and severe acute respiratory syndrome (SARS), an outbreak that killed more than 900 people (Gilbert, 2011).

Hantaviruses are emerging zoonotic viruses naturally carried by rodents from Europe, Asia, and Americas, although bats, moles, shrews, reptiles, and fish have also been identified as reservoirs (Fořtová et al., 2024; Weiss et al., 2022).

All known human pathogenic hantaviruses are rodent borne and belong to the genus Orthohantavirus; they can cause febrile illnesses with renal and/or cardiopulmonary impairment and even organ failure (Weiss et al., 2022).

Since the discovery of the first indigenous African hantavirus in 2006, several studies have been focused on Hantaviruses in Africa (Klempa et al., 2006; Sudi et al., 2018). In Côte d'Ivoire, serological investigations demonstrated evidence of shrew-borne hantavirus infections in humans but no hantavirus genetic material was amplified from any patients (Heinemann et al., 2016). However, novel hantavirus RNAs were detected in a West African pygmy shrew (Azagny virus), captured in Azagny National Park, Côte d'Ivoire, (Kang et al., 2011) and also in ethanol-fixed liver tissue from two banana pipistrelles (Mouyassué virus), captured near Mouyassué village in Côte d'Ivoire, (Sumibcay et al., 2012).

Hantaviruses persistence is problematic because they are enzootic viruses that maintain persistent infections in their hosts without apparent disease symptoms. The spillover of these viruses to humans can lead to one of two serious illnesses, hantavirus pulmonary syndrome and hemorrhagic fever with renal syndrome (Jonsson et al., 2010).

In Côte d'Ivoire since the detection of the presence of hantavirus in Aboisso, it is important to determine whether or not this virus is still persistent in this region. In this study, we investigated whether or not the virus detected earlier (Sumibcay et al., 2012) still permanently circulates in banana pipistrelles. The objective was 1) to detect the virus within lung tissues from banana pipistrelle captured from the same region where the virus was initially described and 2) identify the virus phylogetically

Materials and Methods

Samples collection

Field sampling was conducted in January 2019, during which bats were captured in four villages (Mouyassué, Anvo, Dougninikro and Ehia) in Aboisso region southeastern Côte d'Ivoire (Figure 1). Bats were captured at various sites using 12-m mist nets strategically placed between 30 cm and 2.5 m high arranged in parallel inside the villages during the 3 nights. In agreement with the local guides, houses harboring bats were identified and with the permission of the property owner nets were placed the evening from 06 to 10 pm (Figure 2). Animals were humanely euthanized under anesthesia with ketamine/xylazine followed by exsanguination through cardiac puncture and whole blood and Tissue samples of liver, spleen, kidney, lung and intestine were collected. Weight, forearm length, sex, and age were recorded, for each individual and samples stored immediately in liquid nitrogen and stored at -80°C when arrived at the laboratory.

Hantavirus detection using RT-PCR

Total nucleic acids were extracted from lung samples using the NucleoSpin kit (Macherey-Nagel, Düren, Germany). Reverse-transcription was performed using the SuperScript II Reverse transcription kit (Invitrogen, Carlsbad, CA, USA) with random hexamer primers (Bioline, London, UK). Five microliter of complementary DNA (cDNA) were tested for the presence of hantavirus genetic material by using a nested PCR assay, targeting the large (L) genomic segment as described previously (Klempa et al., 2006) by using degenerated primers (Table 1).

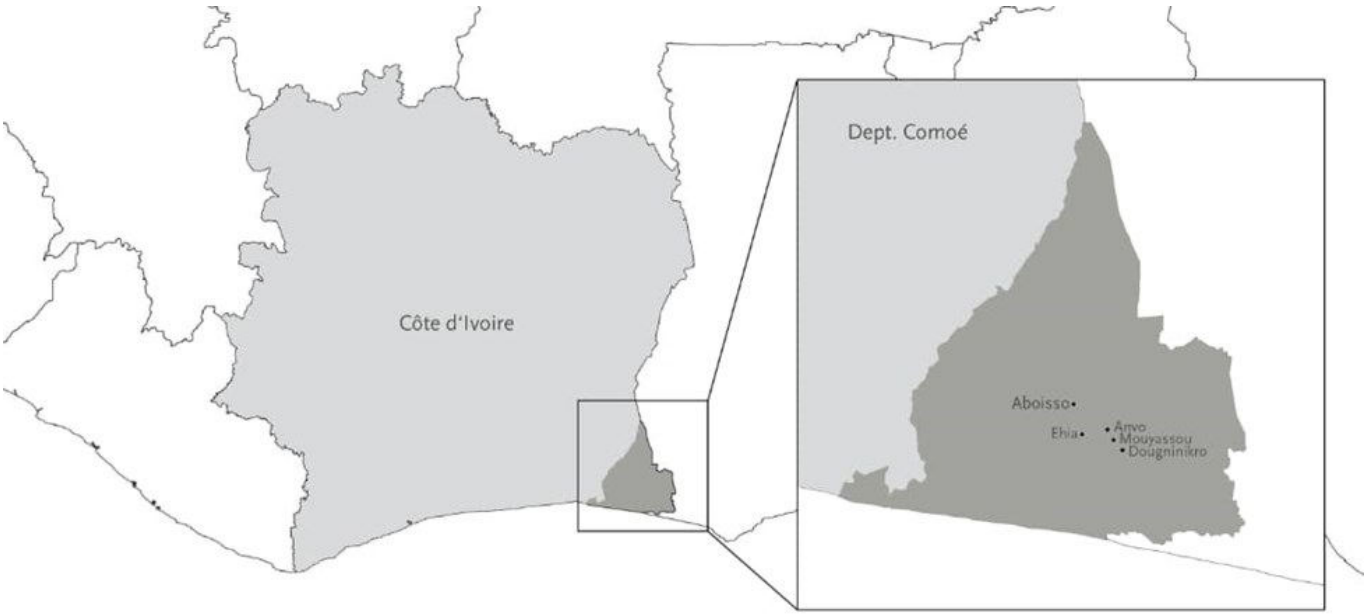

Figure 1. Map of the collection site, Aboisso region is located in the southeastern Côte d'Ivoire. The figure was generated using QGIS v.3.10 software

Figure 2: Net setting for bat capture inside the village

Table 1:Primer sequences for pre-nested and nested amplification of a conserved region in the RdRp gene on the hantavirus L segment (Klempa et al., 2006).

Primer Name	5' → 3' Primer Sequences
Han-L-F1	ATG TAY GTB AGT GCW GAT GC
Han-L-R1	AAC CAD TCW GTY CCR TCA TC
Han-L-F2	TGC WGA TGC HAC IAA RTG GTC
Han-L-R2	GCR TCR TCW GAR TGR TGD GCA A

The first round reaction volume was 25µl with 2.5µl cDNA added to a reaction mix containing 12.5µl of 2× Maxima® Hot-start Mix, 2.5µl of 1µM Han-L-F1 and Han-L-R1, 0,5µl of MasterAmp® PCR enhancer with betaine (Epicenter Biotechnologies, USA) and 4.5µl of nuclease-free water. The reaction was run under the following cycling conditions on the PE GeneAmp® 9700 thermocycler: 95°C for 15 minutes, 40 cycles at 95°C for 30 seconds, 53°C for 45 seconds and 72°C for 45 seconds, and final extension at 72°C for 6 minutes. The nested amplification was performed with 2µl of pre-nested PCR product added to a reaction mix containing 12.5µl of Maxima Hot-start Mix, 2.5µl of 1µM primers Han-L-F2 and Han-L-R2, 0.5µl of MasterAmp® PCR enhancer with betaine and 5µl of nuclease-free water. Amplification was performed using the following cycling profile: 95°C for 15 minutes, 25 cycles at 95°C for 30 seconds, 53°C for 45 seconds and 72°C for 45 seconds, final extension at 72°C for 6 minutes. All positive PCR products were sequenced on both strands according to Sanger's method. Sequence identity was confirmed with BLAST (National Center for Biotechnology Information, Bethesda, MD, USA) (Altschul et al., 1990).

Phylogenetic analysis

To determine the phylogenetic identification of our sequences, we assembled a data set comprising CIV sequences as well as 39 partial and complete L sequences from other hantaviruses extracted from publicly availably genomes (retrieved from NCBI). Data sets were aligned at amino acide level using MAFFT, as implemented in Geneious Prime® 2024.0.3 (available at https://www.geneious.com). Conserved alignment blocks were selected using Gblocks (Castresana, 2000) as implemented in SeaView version 4 (Gouy et al., 2010). Phylogenetic inferences were conducted using the Maximum Likelihood (ML) method, implemented in the IQ-TREE v.2 (Nguyen et al., 2015) , employing the Q.yeast+I+G4 substitution model using the ultra-fast bootstrap procedure with 1000 pseudo-replicates bootstrap replicates for statistical support (Nguyen et al., 2015). The visualization and editing of the resulting trees were conducted using FigTree (available at http://tree.bio.ed.ac.uk/software/figtree /) and Itol (https://itol.embl.de) respectively.

Results and Discussion

In total, 168 bats were captured and assigned at genus level in the field based on morphology with 164 *Neoromicia Nanus* and four *Chaerephan sp*. Among them 74 female (44.05%) and 94 male (55.95%). The sex ratio was 0.79 female for one male. Using the PAN-Hanta-PCR, hantavirus RNA was detected in lung samples of 30 insectivorous *Neoromicia nanus* captured in all villages (**Table 2**) among them six were females and 24 males.

Table 2: Detection of hantavirus RNA in tissues of insectivorous bats by RT-PCR

Genus species	Mouyassué	Anvo	Dougninikro	Ehia	Total
N nanus	8/36	4/34	11/52	7/41	30/163
Chaerephan sp	0/3	-	-	0/2	0/5
Total	8/39	4/34	11/52	7/43	30/168

The prevalence of hantavirus in *Neoromicia nanus* was 18%. Hantaviruses have been detected and characterized in bat species from Africa (e.g. Mouyassue virus in Cote d'Ivoire; Magboi virus in Sierra Leone; Makokou virus in Gabon, Asia (e.g. Xuan Son virus in Vietnam; Huangpi virus, Longquan loanvirus; Laibin mobatvirus in China; Quezon mobatvirus in the Philippines; Đakrông virus in Vietnam and Brno loanvirus in Europe. But for most of these viruses, the host specificity is not well documented (Dafalla et al., 2023). Most of African bat-borne hantaviruses were limited to single findings suggesting the possibility of an accidental spillover events. Recently, a novel hantavirus, named Kiwira virus, was molecularly detected in six Angolan free-tailed bats captured in Tanzania and in one free-tailed bat in the Democratic Republic of Congo (Weiss et al., 2022). Eight years later after the first detection of Mouyassué virus (Sumibcay et al., 2012), we investigated the same village and the surroundings. We demonstrated the persistent circulation of MOUV in Mouyassué and surrounding villages. Our findings show for the first time a high prevalence of hantavirus infection in *Neoromicia nanus*. The detection of

Mouyassué virus in several bats of the same species from different locations indicates a host specificity to *Neoromicia nanus* and points to this species as a natural host. Insectivorous bats of the genus Neoromicia (family Vespertilionidae) referred to commonly as either Serotine or Pipistrelle bats, are distributed in the Afro-Malagasy regions. Neoromicia bats habitually roost in small colony numbers (up to 10 individuals) in naturally occurring crevices, though some species may also be found in anthropogenic structures such as the roofs of houses (Geldenhuys et al., 2018).

Phylogenetic analysis comprising sequences from 27 out of 30 positive displaying strong gel band showed that CIV virus were related to Mouyassué virus (MOUV) detected in banana pipistrelle eight years earlier in Mouyassué village (**Figure 3**). The amino acid pairwise comparison of the sequences analysed revealed an identity varying between 96.05 to 100% and that between these sequences and MOUV varying between 96.08 and 99.24%. The closest relative of this group comprising our newfound Mouyassué-like and MOUV was Nova virus (NVAV), previously reported in the European common mole (*Talpa europaea*) (Kang et al., 2009). These analyzes confirm they phylogenetic placement within the *Mobatvirus* genus. Additionally to Mouyassué virus detected in Cote d'Ivoire, a novel paramyxovirus sequences were identified from the same host species and coronaviruses from both the genera alphacoronavirus and betacoronavirus have been reported from the sister species *Neoromicia capensis* in South Africa (Geldenhuys et al., 2013; Mortlock et al., 2015). There is no evidence of bat-borne hantaviruses causing disease in humans, but the continually discovery of hantaviruses in non-rodent hosts raises the question whether these viruses can infect humans and induce disease as rodent-borne hantaviruses do. Hantavirus disease often manifests as a febrile illness with non-specific symptoms. These clinical symptoms are quite common for several pathologies like malaria, particularly in Africa, and might be easily undiagnosed. The adaptation of these bat species to human dwelling greatly increases the chance of contacts between humans and infected animals. Further studies should be performed to analyze the zoonotic potential of this bat-borne hantavirus and evaluate its transmission within bat populations.

Conclusion

The present study reports the persistent circulation of a hantavirus related to bats in the Aboisso region. Although Neoromicia nanus is not consumed as food, its frequent presence in house roofs increases the risk of human exposure among residents in the area.

Figure3: Maximum likelihood analysis of hantavirus L (66 amino acid) segments, visualized by the iTol online server. The newfound Mouyassué-like reported in this study are highlighted with in red and deposited in GenBank under the accession numbers of PQ008937-63. Only bootstrap supports of >70%, are shown.

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Data availability: The consensus sequence of this research have been submitted to the National Center for Biotechnology Information under accessions PQ008937-PQ008963 and available at July 20/2024.

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