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SHORT REPORT

Detection of possible spillover of a novel hantavirus in a Natal mastomys from Guinea

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Abstract

To date, only two rodent-borne hantaviruses have been detected in sub-Saharan Africa. Here, we report the detection of a yet unknown hantavirus in a Natal mastomys (*Mastomys natalensis*) in Méliandou, Guinea, in 2014. The phylogenetic placement of this virus suggests that it might represent a cross-order spillover event from an unknown bat or eulipotyphlan host.

Keywords Hantavirus · *Mastomys natalensis* · Guinea

Hantaviruses, family *Hantaviridae*, are emerging viruses causing two life-threatening zoonoses in humans: hemorrhagic fever with renal syndrome (HFRS) in Asia and Europe and hantavirus pulmonary syndrome (HPS) in the Americas (1). In Africa, human hantavirus infections were shown in epidemiological studies and in single patients using serodiagnostics (2–4).

The hantavirus genome consists of three segments of negative-stranded RNA; the large (L) segment encodes the viral RNA polymerase, the medium (M) segment the glycoprotein precursor which is co-translationally cleaved into the envelope glycoproteins Gc and Gn, and the small (S) segment the nucleocapsid protein (N) (5). Hantaviruses are known to be transmitted to humans via inhalation of excreta of infected rodents. Initially, rodents were considered to be the only hantavirus reservoirs. However, the discovery of several hantaviruses in moles, shrews and bats has extended the range of potential reservoirs, notably in Africa (6–8). In West Africa, most hantaviruses have actually been detected in bats and shrews, with the exception of Sangassou virus (SANGV) from Guinea whose reservoir is the African wood mouse (*Hylomyscus simus*) (9).

To further investigate the occurrence of hantaviruses in West African rodents, we collected samples in Côte d'Ivoire in 2013 and in Guinea in 2014 and 2015, specifically targeting small mammals living in close proximity to humans.

In Côte d'Ivoire, three ecologic zones were chosen for small mammal trapping: (a) dry bushland in northern Côte d'Ivoire (around Korhogo) (b) semi-arid bushland in central Côte d'Ivoire (around Bouaké) and (c) rainforest in southwestern Côte d'Ivoire (near Taï National Park). In Guinea, small mammals were captured in the course of an investigation of the origin of the Ebola virus disease epidemic, in and around Méliandou which is situated in a semi-arid area (near Guéckédou). In addition, small mammals were captured in the urban environment of Conakry. Animals were caught with Sherman traps, anesthetized, euthanized and dissected in the field. Species field identifications were confirmed by amplification and sequencing of an 800 bp fragment of the

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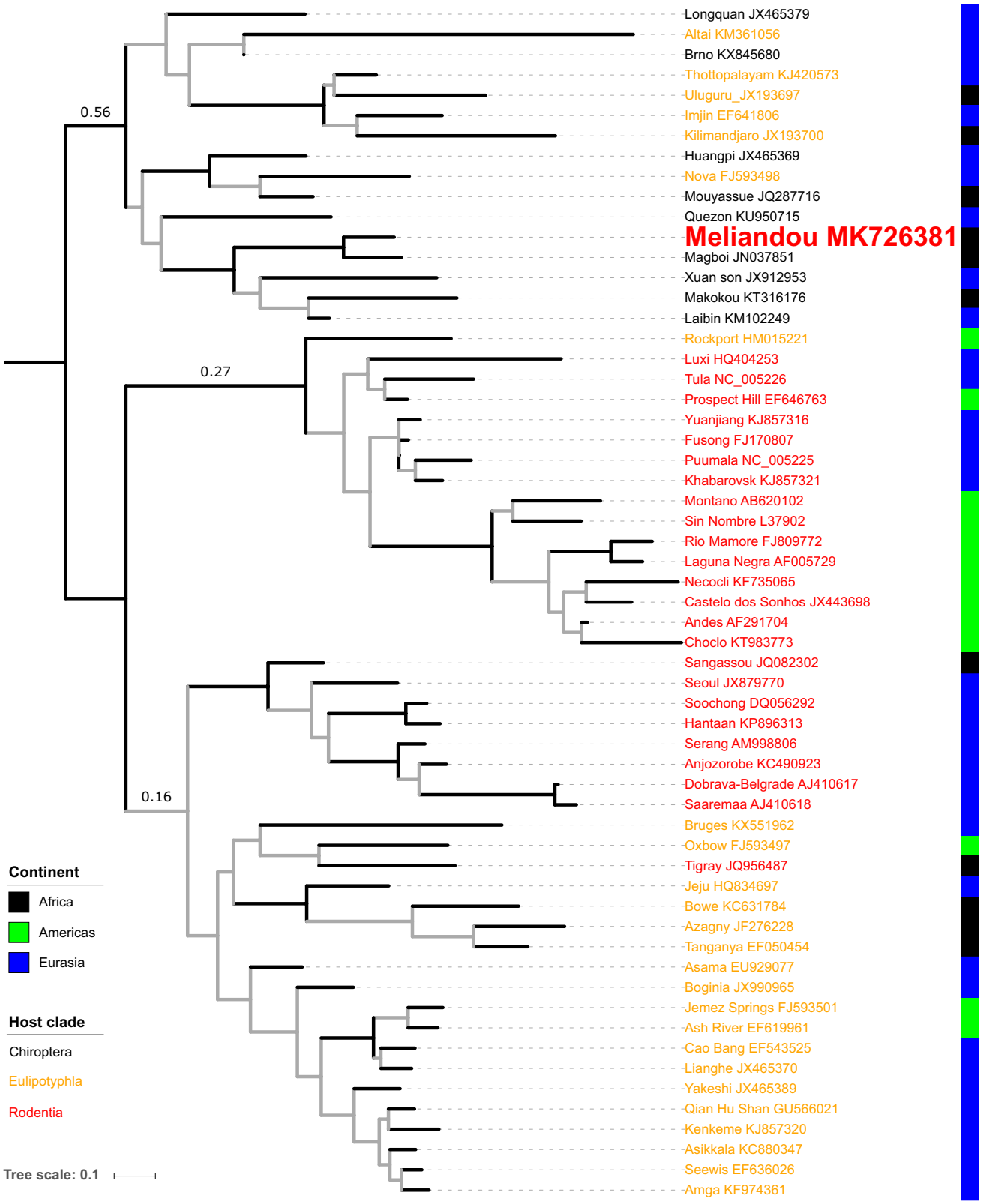

Fig. 1 Phylogenetic placement of the new hantavirus detected in a Natal mastomys, Guinea. This maximum likelihood phylogenetic tree is based on partial L amino acid sequences (alignment of 112 amino acid positions). Gray branches are only supported by low Shimodaira–Hasegawa approximate likelihood ratio test values (< 0.90). Bayesian analyses supported a similar topology. The values above three branches correspond to root posterior probabilities for the three best supported locations; the root of the tree was placed on the best supported branch. Scale bar in amino acid substitutions per site

mitochondrial cytochrome *b* gene. A total of 38 eulipotyphlans and 503 rodents were captured in Côte d'Ivoire and Guinea representing 11 genera and 19 species (Supplemental Material Table 1). We extracted total nucleic acid from all lung samples using the NucleoSpin kit (Macherey–Nagel, Düren, Germany). A nested RT-PCR amplifying a 387 bp fragment of the highly conserved region of the RNA polymerase (L) gene of hantaviruses was then performed using degenerate primers as described before (8).

Hantavirus RNA was detected in the lungs of 1 Natal mastomys (*Mastomys natalensis*) (Gui_126), captured in the village Méliandou (8.62243° N, 10.06462° W), Guinea. Sanger sequencing of the PCR product generated a 345 nt-long sequence (GenBank MK726381). Further attempts to detect hantavirus RNA in blood and liver samples from the same individual and attempts to generate a full genome sequence using in-solution hybridization capture coupled with high-throughput sequencing were unsuccessful, most likely due to low copy numbers.

We performed phylogenetic analyses of an amino acid sequence alignment of the new sequence and a set of 58 representative hantavirus sequences in maximum likelihood and Bayesian frameworks (Supplemental Material Table 2). The Gui_126 sequence did not nest within the diversity of rodent-infecting hantaviruses but rather clustered with hantaviruses infecting bats and eulipotyphlans from Africa and Eurasia; its closest relative was the Magboi virus (MGBV) a bat-borne hantavirus discovered in hairy slit-faced bats (*Nycteris hispida*) in Sierra Leone (8) (Fig. 1).

Hantavirus species are demarcated when the pairwise evolutionary distance of the complete coding sequences of their nucleocapsid protein and glycoproteins exceeds 10% (10). While we cannot apply this criterion here, we note that the patristic distance between the Gui_126 sequence and MGBV exceeds patristic distances observed in the same tree between some recognized and putative new species approved and ratified by the International Committee on Taxonomy of Viruses (ICTV), e.g. Andes and Choclo hantavirus. This preliminary observation suggests that the virus identified in Gui_126 might represent a new species.

This new hantavirus is the fourth putative hantavirus discovered in Guinea, after the Sangassou, Tanganya and Bowé hantaviruses (the latter two were discovered in shrews). We propose the name Méliandou virus (MELV) according to the place of finding. We only detected this hantavirus RNA in lungs from a single individual (out of 168 Natal mastomys tested). Previous studies that used the same screening approach did not detect MELV in even larger samples of Natal mastomys from West (N = 325; 11) and East Africa (N = 483; 12). In addition, we show here that MELV belongs to a large clade of hantaviruses primarily infecting bats and eulipotyphlans. Therefore, it is possible that *M. natalensis* is not the natural host of MELV. Instead, the infection observed may be the result of a spillover event from another host species. Natal mastomys closely track human habitats where they might be exposed to hantavirus-containing excreta from a number of human-adapted bats and eulipotyphlans. The lack of dilution effect in these low-diversity mammal communities might be particularly favorable to more intense (cross-species) transmission (13) and might have facilitated the transmission of MELV to this Natal mastomys individual. While resolving the uncertainty regarding the natural host of MELV will require additional research, its detection in an animal captured in a village already suggests local populations might be exposed to this virus.

In combination with our recent findings of human exposure to hantaviruses in West and Central Africa (2–4), the discovery of MELV in a human dwelling-associated rodent underlines again the importance to include hantaviruses in diagnostic panels for clinical investigations in these regions.

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Author contributions DHK, FHL, ECH, ACK and PTW conceived and designed the experiments.LK, KN, LK, KB, BKA, FZ and AD collected the samples. LK and SK performed the experiments. LK, SCS and FHL analyzed the data. LK and AD wrote the initial draft of the manuscript, SCS, FHL and DHK revised the manuscript.

Compliance with ethical standards

Conflict of interest No competing financial interests exist.

Ethical approval All applicable international, national, and institutional guidelines for the use and care of animals were followed.

References

1. Kruger DH, Figueiredo LT, Song JW, Klempa B (2015) Hantaviruses—globally emerging pathogens. J Clin Virol 64:128–136
2. Heinemann P, Tia M, Alabi A, Anon JC, Auste B, Essbauer S, Gnionsahe A, Kigninlman H, Klempa B, Kraef C, Kruger N, Leendertz FH, Ndhatz-Sanogo M, Schaumburg F, Witkowski PT, Akoua-Koffi CG, Kruger DH (2016) Human infections by non-rodent-associated hantaviruses in Africa. J Infect Dis 214:1507–1511

3. Klempa B, Koivogui L, Sylla O, Koulemou K, Auste B, Kruger DH, ter Meulen J (2010) Serological evidence of human hantavirus infections in Guinea, West Africa. J Infect Dis 201:1031–1034
4. Witkowski PT, Leendertz SA, Auste B, Akoua-Koffi C, Schubert G, Klempa B, Muyembe-Tamfum JJ, Karhemere S, Leendertz FH, Kruger DH (2015) Human seroprevalence indicating hantavirus infections in tropical rainforests of Côte d'Ivoire and Democratic Republic of Congo. Front Microbiol 6:518
5. Vaheri A, Strandin T, Hepojoki J, Sironen T, Henttonen H, Makela S, Mustonen J (2013) Uncovering the mysteries of hantavirus infections. Nat Rev Microbiol 11:539–550
6. Klempa B, Fichet-Calvet E, Lecompte E, Auste B, Aniskin V, Meisel H, Barriere P, Koivogui L, ter Meulen J, Kruger DH (2007) Novel hantavirus sequences in shrew, Guinea. Emerg Infect Dis 13:520–522
7. Sumibcay L, Kadjo B, Gu SH, Kang HJ, Lim BK, Cook JA, Song JW, Yanagihara R (2012) Divergent lineage of a novel hantavirus in the banana pipistrelle (*Neoromicia nanus*) in Côte d'Ivoire. Virol J 9:4
8. Weiss S, Witkowski PT, Auste B, Nowak K, Weber N, Fahr J, Mombouli JV, Wolfe ND, Drexler JF, Drosten C, Klempa B, Leendertz FH, Kruger DH (2012) Hantavirus in bat, Sierra Leone. Emerg Infect Dis 18:159–161
9. Witkowski PT, Klempa B, Ithete NL, Auste B, Mfune JK, Hoveka J, Matthee S, Preiser W, Kruger DH (2014) Hantaviruses in Africa. Virus Res 187:34–42
10. Maes P, Klempa B, Clement J, Matthijnssens J, Gajdusek DC, Kruger DH, Van Ranst M (2009) A proposal for new criteria for the classification of hantaviruses, based on S and M segment protein sequences. Infect Genet Evol 9:813–820
11. Klempa B, Fichet-Calvet E, Lecompte E, Auste B, Aniskin V, Meisel H, Denys C, Koivogui L, ter Meulen J, Kruger DH (2006) Hantavirus in African wood mouse, Guinea. Emerg Infect Dis 12:838–840
12. Tesikova J, Bryjova A, Bryja J, Lavrenchenko LA, Gouy de Bellocq J (2017) Hantavirus strains in east Africa related to western African hantaviruses. Vector Borne Zoonotic Dis 17:278–280
13. Dizney LJ, Ruedas LA (2009) Increased host species diversity and decreased prevalence of Sin Nombre virus. Emerg Infect Dis 15:1012–1018