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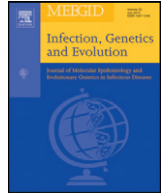
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Short communication

Identification of lymphocytic choriomeningitis mammarenavirus in house mouse (*Mus musculus*, Rodentia) in French Guiana



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ABSTRACT

Thirty-seven house mice (*Mus musculus*, Rodentia) caught in different localities in French Guiana were screened to investigate the presence of lymphocytic choriomeningitis mammarenavirus (LCMV). Two animals trapped in an urban area were found positive, hosting a new strain of LCMV, that we tentatively named LCMV “Comou”. The complete sequence was determined using a metagenomic approach. Phylogenetic analyses revealed that this strain is related to genetic lineage I composed of strains inducing severe disease in humans. These results emphasize the need for active surveillance in humans as well as in house mouse populations, which is a rather common rodent in French Guianese cities and settlements.

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The family Arenaviridae is made up of single-stranded RNA viruses and includes two genera, *Mammarenavirus* and *Reptarenavirus* (Radoshitzky et al., 2015). The *Mammarenavirus* genus is divided into two main groups according to phylogenetic and serological criteria: arenaviruses from the Old World with viruses such as Lassa and LCMV, and New World arenaviruses such as Tacaribe, Junin and Machupo viruses, among others (Salvato et al., 2011). All these viruses are hosted by rodents with the exception of Tacaribe virus, which has been described in a bat species (*Artibeus jamaicensis*, Phyllostomidae) (Downs et al., 1963). The geographical distribution and phylogenetic relationships of arenaviruses have been associated with the distribution of their reservoirs. Indeed, arenaviruses have been suggested to co-evolve with their rodent hosts (Emonet et al., 2009). Arenaviruses from the Old World are associated with rodents from the family Muridae, subfamily Murinae, whereas those from the New World are related to a different subfamily of Muridae, namely the Sigmodontinae. Each arenavirus species seems to be hosted by a unique reservoir species, or by closely related species within a given genus, and is distributed in patches (Charrel

et al., 2008). Among arenaviruses, LCMV is the only one distributed worldwide (America, Africa, Asia and Europe). This wide distribution is explained by the geographic distribution of its main reservoir, *Mus musculus*. In the Old World, other Murinae species can be infected by LCMV strains such as *Apodemus sylvaticus*, *Mus spretus* and *Rattus norvegicus*, and even a distantly related rodent, the squirrel *Sciurus vulgaris*, which was detected positive through serological and molecular approaches, although no viral sequence was obtained (Blasdell et al., 2008; Ledesma et al., 2009). Serological studies performed on *M. musculus* captured in urban areas as well as in undisturbed environments revealed that 8 to nearly 13% of tested individuals can be infected with LCMV (Ledesma et al., 2009; Lledó et al., 2003; Riera et al., 2005). Human beings can be infected with LCMV through direct contacts with infected rodents or through inhalation of contaminated rodent feces and/or urine. LCMV infection is usually asymptomatic in humans. It can nevertheless induce febrile illness and even sometimes severe cases of aseptic meningitis and encephalitis (Barton and Hyndman, 2000; De Ory et al., 2009; Martos Fernández et al., 1996). In the Americas, numerous arenaviruses have been described as causing severe disease (hemorrhagic fevers) and the LCMV presence has been investigated in rodents and human populations in several areas (Riera et al., 2005). In French Guiana, the recent identification of a new arenavirus (Lavergne et al., 2015) belonging to the New World clade A (Charrel and de Lamballerie, 2010), which includes viruses that are known to be poorly pathogenic, led us to investigate the circulation of

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Table 1
Nucleotide and amino acid identities between LCMV Comou and other representative LCMV strains based on the polymerase (L), small zinc finger-like protein (Z), nucleocapsid (NP) and glycoprotein precursor (GP) sequences.

	(% Identity in nucleotides with LCMV-Comou)				(% Identity in amino acids with LCMV-Comou)			
	S		L		S		L	
	NP	GP	L	Z	NP	GP	L	Z
Traub_USA_1936	91.5	90.4	84.8	80.1	96.4	96.8	91.4	89.9
200312154_810,366_California_2003	90.3	90	76.4	74.5	95.7	95.1	83.7	85.4
OQ28_Osaka_Japan_1990	89.8	90.4	87.7	85	95.9	96.2	93.6	92.1
Pasteur_France_2006	86.2	83	81.6	83.5	94.3	94.1	89	91
201102714_USA_2011	86	84.4	NA	NA	96.2	96.4	NA	NA
IN_2012_M435_USA_2012	85.7	84.4	NA	NA	94.8	95.5	NA	NA
EEB_7_Spain_2008	85.5	85	80.8	82	95.3	95.3	88.6	91
Douglas_4707_810938_NY_N938_1947	85.5	85.6	83.2	80.1	95.5	95.1	90.4	89.9
WHI_8107_810906_NY_H909_1949	85.5	83.8	NA	NA	94.4	94.3	NA	NA
811316_Massachusetts_2008	85.4	85.4	84.4	81.6	94.8	95.5	91.7	92.1
200501927_810850_Rhode_island_2005	85.4	83.5	81.4	78.7	95	93.1	88.5	88.8
200504219_810885_Michigan_2005	85.3	84.4	81.5	80.9	95.3	96	89.2	88.8
200504261_810896_Ohio_2005	85.3	83.6	81.4	78.7	94.6	93.3	88.7	88.8
WE_UBC_57135_810940_NY_H940_1935	84.9	84.1	82.1	82.4	95.3	94.5	89.4	92.1
200312181_810362_Wisconsin_2003	84.9	85.1	81.6	80.9	93.9	94.9	89.5	93.3
WE_UBC_A337_810909_NY_H909_1935	84.9	84.2	NA	NA	95.5	94.7	NA	NA
WE_Nagasaki_Japan_2011	84.8	84.7	82.7	81.6	94.4	94.9	89.6	91
Armstrong_53b_Missouri_USA_1933	84.8	84.7	81.5	77.5	94.4	95.3	88.8	88.8
Makokou_Gabon_2012	84.7	85.1	81.6	NA	94.3	94.7	89.2	NA
HP65_2009_France	84.4	83.2	80.3	83.5	90.1	91.1	87.1	91
Marseille_France_2004	84.3	84	81.2	77.3	94.3	95.5	88.5	83
CH_5871_Germany_2000	84.3	83.2	NA	NA	94.6	93.5	NA	NA
CH_5692_Germany_1999	84.3	82.6	80.5	81.3	94.6	93.9	87.5	92.1
Bulgaria_1956	81.1	77.6	72.6	NA	92.8	89.9	80.1	NA
M2_Austria_2005	80.9	78.3	NA	NA	93	89.7	NA	NA
M1_Austria_2005	80.9	78.3	NA	NA	93	89.7	NA	NA
BRC_Nagasaki_2005	80.5	78.5	73	76.9	92.5	90.3	79.5	81.8
Dandenong_0710_2678	79.6	78.6	72.9	78.4	93.4	91.1	80.6	85.2
GR01_Spain_2004	79.6	76.4	NA	NA	89.4	83	NA	NA
SN05_Spain_2004	79.3	72.8	NA	NA	89.6	79.6	NA	NA
CABN_Spain_2004	79	72.6	NA	NA	89.8	80.6	NA	NA
810935_Lyles_Georgia_1984	78.9	78	72.5	70.4	92.1	88.5	79.8	82
Lunk_virus_NKS_1	72.6	70.7	NA	58.1	82.2	79.6	NA	55.6

the ubiquitous LCMV. We report here the first genetic characterization of LCMV in *M. musculus* in French Guiana.

As part of an investigation program on the diversity of viruses hosted by rodents, captures were implemented in various localities of northern French Guiana during a 10-year period. Among *c.a.* 500 rodents, 37 *M. musculus* were collected in two different types of environments. Most of them ($n = 20$) came from a small Amerindian village located along the Oyapock River (Saint-Georges municipality) at the Brazilian border, while the others ($n = 17$) came from the urban and periurban areas of Cayenne (the largest city in French Guiana). Rodents were caught alive, brought to the laboratory, anesthetized and euthanized to collect their organs (kidney, lungs, intestine and spleen) and sera. All *M. musculus* individuals were identified morphologically and molecularly confirmed by sequencing a fragment from the mitochondrial Cytochrome Oxidase I gene (Borisenko et al., 2008). For all *M. musculus*, total RNA was extracted from the lungs and kidneys and cDNA synthesized as previously described (Lavergne et al., 2015). All individuals were screened for the presence of LCMV using a hemi-nested PCR targeting a 650-bp fragment of the S segment. Primers were designed to fit with LCMV strains. First-round PCR was performed using primer LCMV-1638F: 5'-TGGAGAGTCAGGAGGCC-3' (designed in this study) and modified primer 1010C: 5'-TCNGGNGARGGNTGCC-3' (Bowen et al., 1996). The second-round PCR was performed using the modified primer 1010C and primer 1696: 5'-ATNATGCARTCCATRAGWGCACA-3' (Bowen et al., 1996).

Among the 37 animals, two were found positive, in lungs and kidneys, for LCMV after sequencing the PCR products. These two animals were male, adult, with tissue kept in the JAGUARS collection (CITES agreement FR-973A) at the Institut Pasteur de la Guyane with the reference numbers M1504_JAG and M1812_JAG. The two animals were

captured at the same trapping site, 2 months apart in 2013, in a small residential house, Rémire-Monjoly municipality, in the suburbs of Cayenne. We determined the complete sequence of LCMV using a viral metagenomic approach (detailed sample preparation protocol is available upon request). Briefly, analyses were done on lung, kidney, intestine and spleen tissues as well as on serum of the two positive specimens. Samples were pooled by organ. They were crushed in Hank's buffered saline solution (Gibco BRL) and aliquots were centrifuged to eliminate the particles and filtrated. Samples were then extracted using the automated nucleic acid extractor NucliSENS® EasyMAG® from BioMérieux. cDNA was generated using SuperScript III reverse transcriptase and was submitted to random amplification using the WTA kit (Invitrogen, Life Technologies, Paisley, UK). The libraries were prepared using the NEBNext Ultra DNA Library Prep Kit from Illumina (New England BioLabs) and were then sequenced on a MiSeq machine in 250-base paired-end reads (Illumina, San Diego, CA, USA). Sequence files were generated using Illumina Analysis Pipeline version 1.8 (CASAVA). Raw sequence files were submitted to removal of adapter sequences using Trimmomatic (Bolger et al., 2014) and subsequently quality filtered with a quality threshold of 20 using the fastx-toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Reads were then assembled into contigs using SPAdes (Bankevich et al., 2012) and velvet (Zerbino and Birney, 2008) using different k-mer sizes. Samples showing high levels of read duplications were deduplicated using fastqMcf from the ea-utils package (Aronesty, 2011). Contigs were submitted to a blast search (version 2.2.28+) (Altschul et al., 1990) on nr and viral databases and 380 contigs gave a positive hit on LCMV. Reads were then mapped against the complete sequence of the S and L segments of LCMV strain OQ28 (accession numbers AB627952 and AB627955). Of 5 million reads nearly obtained for the two individuals, 6168 (0.08%)

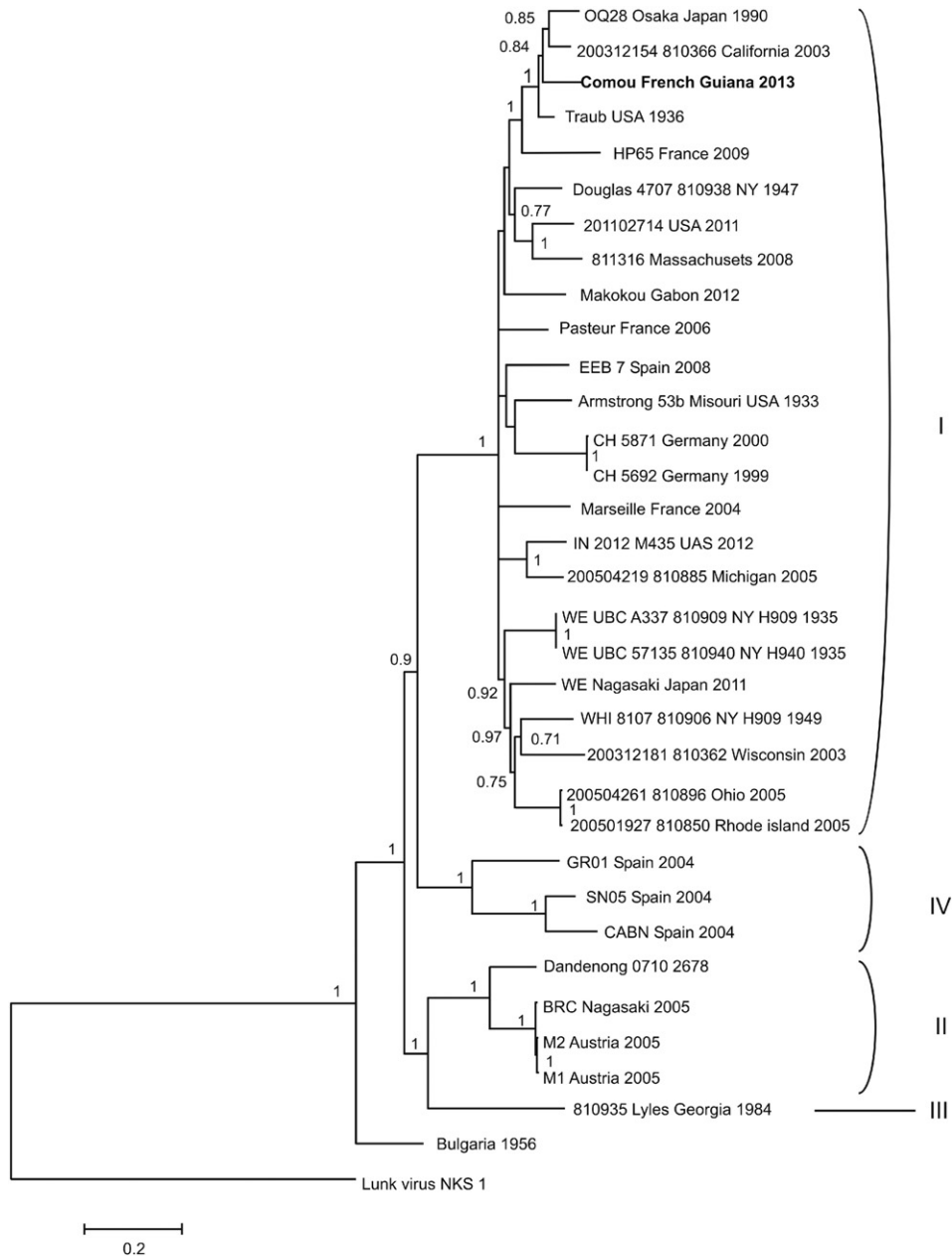


Fig. 1. Phylogenetic tree based on analysis of the complete nucleotide sequence of the NP gene of LCMV Comou and representative strains of LCMV. The tree is based on the GTR+I+G model of amino acid evolution. Virus names are associated with their locality of origin, the year of identification and their accession numbers. Support for nodes is provided by the posterior probabilities of the corresponding clades. All resolved nodes have posterior probability greater than 0.7. Scale bar indicates nucleotide sequence divergence among sequences.

corresponded to the LCMV virus with an average depth of coverage of 99.94 and 59.49 for the S and L segments, respectively. The obtained sequences of LCMV identified from M1504_JAG and M1812_JAG showed a very high percentage of identity (99.5% on 3196 bp of the S segment and 99.92% for 6958 bp of the L segment).

The sequences of the S and L segments were nearly complete except for the 5' and 3' end for the two segments. Only one sample (M1504_JAG) was subsequently analyzed to obtain the full sequences of the S and L segments. Primers were designed to amplify the two extremities based on the conserved terminal nucleotide sequences with specific primers. Amplification products were sequenced and completed the constructed genomes. Nucleotide sequences of the LCMV strain identified in French Guiana with other available sequences were aligned using MEGA5 (Tamura et al., 2011) software and sequence identities were determined in nucleotide and amino acid.

The complete sequences of the S and L segments are 3320 and 7205 nucleotides in length, respectively. The S segment encodes the nucleocapsid (NP), which is 1677 bp long, and the glycoprotein precursor (GP), 1497 bp long. The L segment encodes the viral RNA-dependent RNA polymerase (L), which is 6624 bp long, and the small zinc finger-like protein (Z) measuring 273 nucleotides. We named this new LCMV strain LCMV Comou ("Comou" is an Amerindian name for the common and widespread palm-tree *Oenocarpus bacaba*).

Comparison of sequence identity of the complete NP gene of the S segment of LCMV Comou with other LCMV strains revealed that it shares the highest percentage of identity in nucleotides (91.5%) with a strain identified in North America called Traub 1936 and with two other strains identified in California (USA) in 2003 and in Japan (OQ28) in 1990 with 90.3% and 89.8% of nucleotide identity, respectively (Table 1). Similarly, it shows a high percentage of identity at the

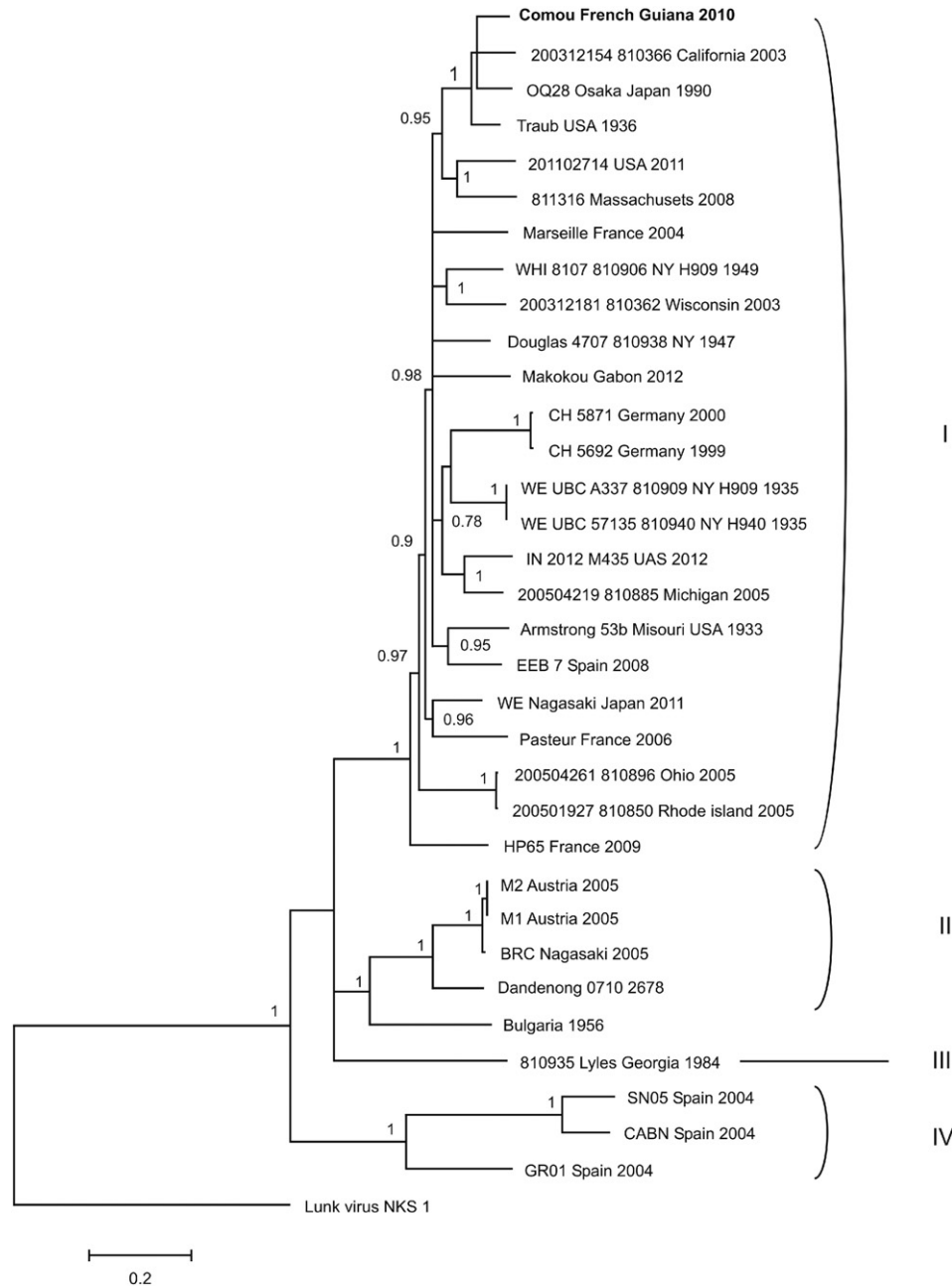


Fig. 2. Phylogenetic tree based on analysis of the complete nucleotide sequence of the GP gene of LCMV Comou and representative strains of LCMV. The tree is based on the GTR+I+G model of amino acid evolution. Virus names are associated with their locality of origin, their year and their accession numbers. Support for nodes is provided by the posterior probabilities of the corresponding clades. All resolved nodes have posterior probability greater than 0.7. Scale bar indicates nucleotide sequence divergence among sequences.

amino acid level with these three strains (96.4% with Traub 1936) (Table 1). The complete GP sequence also shows the highest percentage of nucleotide (90.4%) and amino acid (96.8%) identities with the Traub strain. For the L segment, the highest percentage of identity is observed with the OQ28 strain (87.7% and 93.6% in nucleotide and amino acid, respectively) as well as for the complete sequence of the Z gene in the nucleotide (85% identity).

Phylogenetic relationships were inferred from the alignment of nucleotide sequences for the NP, GP and L genes using a Bayesian approach performed with Mr. Bayes 3.2.2 (Ronquist et al., 2012) after selection of the optimum model using MrModeltest v2 (Nylander, 2004). Phylogenetic analyses based on the NP and GP genes showed that LCMV Comou is closely related to the three above-cited strains (OQ28, Traub and 810366) with a high posterior probability (Figs. 1 and 2). This group of sequences is also associated with strains of different

geographical origins (mainly the US, France and Germany). This major clade, which is also supported by a high posterior probability, has been described as “lineage I” and includes strains that are associated with severe human cases (Albariño et al., 2010). Analysis of the L gene sequence shows that LCMV Comou clusters with a high posterior probability with strains OQ28, Traub and 810366 (supplemental data).

This study identifies for the first time a LCMV strain circulating in French Guiana in *M. musculus*. LCMV Comou is associated with numerous strains belonging to lineage I, which is composed of strains inducing severe disease in humans. Numerous strains of the lineage I have been identified worldwide and have *M. musculus* as the main reservoir (Albariño et al., 2010). The introduction of *M. musculus* in northern South America can be correlated with the arrival of human populations during colonization (Husson, 1978). In French Guiana, this commensal species is found in numerous localities, mainly in urban areas, but can



Fig. 3. Current distribution of *Mus musculus* in French Guiana. Records correspond to *M. musculus* caught during our field surveys and/or known by vouchered materials in museum collections and are indicated in transparent red. Dots show distribution of main human settlements and urban areas. (Data sources: “urban areas” derived from the human footprint index [de Thoisy et al. 2010] updated in 2012, and map of land occupancy of the national park, available on http://www.geoguyane.fr/PRRA/panierDownloadFrontal_parametrage.php?LAYERIDTS=90567.)

also be encountered in small remote human settlements, even those located in a forest environment and not permanently inhabited (Fig. 3). Its current distribution is supposedly rising progressively in relation to the increased movement of human populations (tourism, demographic expansion, gold mining, urbanization) in the area (Catzefflis F. and de Thoisy B., unpublished data). The circulation of LCMV could accordingly increase in forest areas where human populations live in close contact with wild, commensal and domestic species. In some remote places, native rodents and especially Sigmodontinae mouse-like species can live and/or visit traditional wood houses, as reported in the Wayampi Amerindian settlement of Trois-Sauts (Catzefflis, 2012). These close contacts between native rodents and *M. musculus* could lead to the transmission of LCMV to wild Sigmodontinae rodent species, or even Echimyidae, and further increase the risk of contamination to humans. With around 30 cases of lethal viral encephalitis reported every year in French Guiana, for which no defined etiological agent has yet been identified, the monitoring of LCMV in the human population using a serological approach could be of interest to better reveal the public health relevance of this virus in French Guiana. The present characterization of LCMV Comou offers the possibility to investigate its presence in the humans and in Murinae and shows the importance of monitoring the distribution of

M. musculus in French Guiana as a risk factor in this French overseas department whose demography is in rapid expansion.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.meegid.2015.11.023>.

Sequence accession numbers.

Sequences of LCMV Comou are deposited in the GenBank database with the following accession numbers: LCMV Comou complete S sequence KT731538, LCMV Comou complete L sequence KT731537.

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