Case Report

Molecular analyses of human rabies virus associated with encephalitis in two children in Gabon

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A B S T R A C T

Rabies is a zoonotic neurological life-threatening neglected tropical disease present worldwide, and Gabon is listed as an endemic country. However, despite strong clinical suspicion in humans and molecular confirmation of rabies virus (RABV) infections in dogs for several decades, no molecularly confirmed case in Gabon has ever been reported. In this study, we describe two cases of human rabies and provide the first molecular diagnostic report on suspected human rabies cases in Gabon. Our results showed that the RABVs isolated from the patients are closely related to other RABV strains belonging to the African 1A subclade in the Cosmopolitan lineage isolated more than 20 years ago from Gabonese dog brains, suggesting that only this species circulates in the country. Because both patients had a history of dog bites a few weeks before symptom onset and the main causative agent of human rabies worldwide is dog-associated RABV, we conclude that dogs are likely to be the source of human infection in this study.

1. Introduction

One of the oldest and most terrifying human diseases, Rabies is an acute fatal zoonotic neglected neurological disease. It is endemic in more than 150 countries worldwide and continues to cause approximately 59,000 deaths in humans annually, mainly in children under 15 years old (Scott and Nel, 2021). Rabies is caused by RNA viruses belonging to the Lyssavirus genus in the Rhabdoviridae family. The rabies virus (RABV) genome consists of a single-stranded, negative-sense RNA virus with a genome size of approximately 12 kb that encodes five proteins: the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the glycoprotein (G) and the large protein or polymerase (L).

According to an epidemiological report from the World Health Organization (WHO), rabies is present in Gabon (Briggs et al., 2018). However, there are no molecular diagnostic reports of human or dog rabies encephalitis cases in the country (Mbilu et al., 2020). Similarly, there...
are no data on the lineages circulating in Gabonese human populations. Previous cases reported to the WHO were diagnosed based on clinical symptoms alone. The only Gabonese study on rabies dates back to 1988 and regarded vaccinated dogs (Bourhy et al., 1988). However, in 2003, a 3-year-old child with a history of travel in Gabon was diagnosed with furious rabies in France (Floret et al., 2005), and some scarce data report RABV sequences from domestic dogs in Gabon (Bourhy et al., 2016; Trupin et al., 2016), confirming the presence of the virus in the country. In this study, we report the first molecularly confirmed cases of human rabies encephalitis in Gabon.

2. The study

The first case of human rabies was a 7-year-old boy admitted on 13 May 2019 to the intensive care unit of the Centre Hospitalier Universitaire de Libreville. The patient had a history of agitated state with fever and uncontrollable vomiting for 3 days. The clinical examination found a marked psychomotor agitation during aerophobia and hydrophobia stimulation without hydrosponic spasms. The patient had several wounds, especially on the inside of the left knee and on the forehead, in addition to the meningeal and infectious syndromes. He had been bitten by a dog 8 weeks before but did not receive any post-exposure prophylaxis. Given the circumstances, a RABV infection was suspected. The evolution was marked by confusion, hypersialorrhrea and cutaneous dyschromia. The patient died on 30 May 2019 with fever, loss of consciousness and hydroelectric disorder.

The second case of human rabies was a 9-year-old boy admitted on 27 July 2020 to the intensive care unit of the Centre Hospitalier Universitaire d’Owendo (CHUO). The patient first consulted at the Nzeng-Ayong Health Center for febrile psychomotor agitation, which had been evolving for 2 days. He had been bitten by a dog approximately 2 months before admission and had not received adequate treatment. The examination during the consultation found the following symptoms: psychomotor agitation with cries, hypersialorrhrea, an infectious syndrome and a scar on the left hand. Faced with a suspicion of rabies, and due to their lack of capacity, the doctors referred the patient to the CHUO for better care. After his admission to the intensive care unit of the CHUO, the medical team found hydrophobia and aerophobia, in addition to the previously mentioned symptoms, both reinforcing the suspicion of rabies. The evolution of the disease was marked by the worsening of the initial symptoms and the patient died on 29 July 2020.

Figure 1. (A) Phylogenetic tree based on 250 bp nucleotide sequences of the polymerase gene of the lyssavirus responsible for human rabies. GenBank accession number is followed by the name of the isolate. Sequences from this study are indicated in red. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model [18]. The tree with the highest log likelihood (-2506.26) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 43 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 252 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [19].

(B) Maximum Likelihood phylogenetic tree of the 475 bp nucleoprotein gene depicting the phylogenetic relationship of the two Gabonese RABV strains with other RABV strains from Africa. GenBank accession numbers are followed by the name of the isolate and isolate date for some strains. Sequences from this study are indicated in red. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model [18]. The tree with the highest log likelihood (-2239.81) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 35 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 475 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [19].
For both patients, the search for malaria parasites in the blood and the cerebrospinal fluid bacterial culture were negative. Six samples of saliva swabs were made every 3 hours (2 swabs per hour). These samples and the cerebrospinal fluid (CSF) were sent to the Centre Interdisciplinaire de Recherches Médicales de Franceville (CIRMF) on 20 May 2019 for the first patient and on 29 July 2020 for the second patient for rabies diagnostic.

All saliva swabs tested for RABV and lyssavirus by real-time and conventional polymerase chain reaction (PCR), respectively, were found positive by the two systems, while testing of CSF samples was negative. In addition, CSF PCR testing for other viruses (alphaviruses, flaviviruses, Herpesviridae, Arenaviridae, and Paramyxoviridae) by conventional PCR was negative. All protocols are available in the supplementary files.

All positive saliva swabs were confirmed by the molecular analysis of the partial P and N genes. The obtained sequences named IICV (first patient) and ENND (second patient) were accessioned in the NCBI GenBank. Sequence accession numbers of polymerase and nucleoprotein genes of IICN are, respectively, MW553267 and MW553269; and for ENND, MW553268 and MW553270.

The phylogenetic tree was constructed with the MW553267 and MW553268 partial P gene sequences from this study and other lyssavirus (RABV, Mokola virus, Duvenhage virus, Lagos Bat lyssavirus, European Bat lyssavirus 1 and 2, and Australian Bat lyssavirus) sequences. Our sequences clustered with RABV strains (Figure 1A), confirming that this virus was the causative agent for both cases of encephalitis.

In order to gain more information on our isolated RABV strains, we conducted additional phylogenetic analysis with the MW553269 and MW553270 partial N gene sequences from this study and other RABV strains (Figure 1B). This analysis placed our sequences with all the other Gabonese sequences isolated from the brains of dogs more than 20 years ago. All the strains clustered with others belonging to the African 1A subclade of the Cosmopolitan clade of RABV strains. These data are consistent with previous studies indicating the circulation of the Cosmopolitan African 1A subclade in Gabon and neighboring central African countries (Bourhy et al., 2008; Kissi et al., 1995) and accord with the RABV strain grouping according to their geographical origin (Bourhy et al., 2008).

Together, these data suggest that after 20 years, only one lineage, the Cosmopolitan African 1A lineage, circulates in Gabon despite the presence of the African 2 clade in neighboring countries such as Cameroon and the Central African Republic (Sadeuh-Mba et al., 2017; Talbi et al., 2009), the presence of the African 3 clade in southern Africa, or the recently detected African subclade 4 in Egypt (Brunker et al., 2015; Troupin et al., 2016)(Bourhy et al., 2008). However, these data come from only two patients; thus, data from more patients and dogs with rabies encephalitis are needed to gain further information about the rabies lineages circulating in Gabon. A nationwide rabies surveillance program should be implemented.

Based on previous studies demonstrating the existence of two major groups among RABV strains, bat- and dog-associated RABV groups (Bourhy et al., 2008; Floret et al., 2005; Troupin et al., 2016), we suggest that the source of these two human rabies encephalitis cases is likely to be dogs. Indeed, among the RABV animal reservoirs, dogs are responsible for >99% of human rabies cases around the world (Hampson et al., 2015), and they are probably the primary vector for inter-species transmission of dog-related RABV (Troupin et al., 2016). In addition, both patients had a history of dog bites in the weeks before the onset of symptoms with no use of post-exposure anti-rabies vaccination. Furthermore, the rabies virus sequences clustered with those of viruses isolated from dogs in Gabon. All these data strongly support the hypothesis that dogs are the likely source of infection for these two human rabies cases. However, looking for RABV strains in domestic and wild animal reservoirs in Gabon would be necessary to deepen our knowledge of the animals carrying the virus in the country and their capacity to transmit to humans.

**Authors’ contributions**

NN, GDM conceived and designed the study. VS, RMM, AN, JFN, FCO, TPP, JK, LB, HMM, AMZ, GEN collected the samples of patients and data. DKM, LBK, JBMP performed the laboratory analyses.
SELD performed the sequencing. NN analyzed the data and wrote the manuscript. All authors approved the final version of the manuscript.

**Declaration of Competing Interest**

The authors report no conflicts of interest.

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**Ethical approval**

At the request of the Gabonese Ministry of Health, samples were taken in order to diagnose rabies infection. Oral and consent form was obtained from the children's parents.

**Institutional Review Board Statement**

At the request of the Gabonese Ministry of Health, samples were taken in order to diagnose rabies infection. The study was conducted according to the guidelines of the Declaration of Helsinki.

**Informed Consent Statement**

Informed consent was obtained for all subjects involved in the study.

**Supplementary materials**

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijirreg.2022.01.006.

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