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Short communication

Molecular epidemiology of rabies virus isolates in Uganda

Shinji Hirano[a], Takuya Itou[a,*], Hisashi Shibuya[b], Yoshito Kashiwazaki[c], Takeo Sakai[a]

[a] Nihon University Veterinary Research Center, 1866 Kameino, Fujisawa 252-8510, Kanagawa, Japan
[b] Laboratory of Veterinary Pathology, Nihon University School of Veterinary Medicine, 1866 Kameino, Fujisawa 252-8510, Kanagawa, Japan
[c] Japan International Cooperation Agency, 5-25 Niban-cho, Chiyoda-ku 102-8012, Tokyo, Japan

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ABSTRACT

African field rabies virus isolates from domestic animals can be phylogenetically divided into three geographic lineages: Africa 1a (North and West Africa); Africa 1b (East and South Africa); Africa 2 (West Africa). Partial nucleotide sequences of the rabies virus nucleoprotein gene (203-nt) were obtained from five dogs and three goats in Uganda. The analyzed Ugandan field rabies viruses were categorized into the Africa 1a and Africa 1b lineages. The present study thus demonstrates that two African lineage-derived rabies viruses coexist in Uganda, which is located in the geographical region between the known Africa 1a and Africa 1b lineage distributions.

The etiological agent classical rabies virus (RABV) is classified into the family *Rhabdoviridae*, genus *Lyssavirus* genotype 1. In Africa, domestic dogs are the most important source of infection to humans, while other carnivora, such as jackals, wolves and mongooses, also act as reservoirs for the disease (Nel et al., 1997). Carnivora has been reported to transmit RABV to livestock, resulting in economic losses (Bloch and Diallo, 1995; Courtin et al., 2000). Phylogenetic analyses of RABVs in Africa have shown that the RABVs isolated from domestic dogs and livestock can be divided into three geographical lineages: Africa 1a, which comprises isolates from northern and western Africa; Africa 1b, which is made up of isolates from eastern and southern Africa; Africa 2, which is composed of isolates from western Africa (Lembo et al., 2007; Johnson et al., 2004a,b; Kissi et al., 1995; Talbi et al., 2009).

Isolates of the Africa 1a and Africa 1b lineages are referred to as the Cosmopolitan lineage due to their similarities to isolates from European lineages (Nel et al., 2005). On the other hand, RABVs maintained among mongooses are made up of a monophyletic lineage, the Viverrid lineage, which is different from other known lineages (Kissi et al., 1995; Davis et al., 2007). Moreover, other rabies-related lyssaviruses that are also distributed in Africa include Lagos bat virus, Mokola virus and Duvenhage virus, which are classified as genotypes 2, 3 and 4, respectively (WHO, 2005).

Rabies is a fatal zoonosis that almost always kills infected mammals after onset. In Africa, rabies causes an estimated 24,000 human deaths annually (Knobel et al., 2005). In rabies-endemic Uganda, based on clinical diagnosis, the disease has killed more than 10 people every year since 2000 (WHO, Rabnet. www.who.int/globalatlas/default.asp, accessed 23 June 2009). Another report has suggested that 592 human deaths per year in Uganda can be attributed to dog, cat and other wildlife bite injuries (Fèvre et al., 2005). However, there have been no reports on the genetic analysis of field RABV isolates in Uganda. Therefore, this study investigated the epidemiological characters of field RABVs in Uganda.

Twelve RABV samples were obtained from six dogs, five goats and one cow with rabies infection, as diagnosed by a fluorescence antibody test of brain tissues in 2003 (Table 1). Due to prolonged storage at ambient temperature, brain samples in 50% glycerol saline had decomposed by 2008. Normal extraction of viral RNA using ISOGEN (Nippon Gene, Japan) and QIAamp Viral RNA kit (Qiagen, USA) was attempted, but only one sample was detected as positive using RT-PCR from the extracted viral RNA. Therefore, viral RNA was extracted using ISOGEN according to the manufacturer's instructions with some modifications. Briefly, each brain sample was homogenized in 1 mL ISOGEN at room temperature for extraction. Chloroform (200 μL) was added to the homogenate, followed by vigorous mixing for 15 s. The sample was kept for 5 min at room temperature, and centrifuged at 12,000 × *g* for 15 min at 4 °C. After centrifugation, the aqueous phase was collected, and the organic phase was used for re-extraction. For re-extraction, 200 μL TE buffer (pH 8.0) and 40 μL chloroform were added. The aqueous phase was again collected and combined with that of the first extraction; re-extraction was carried out twice. RNA was precipitated from the final aqueous solution by incubating with an equal volume of isopropanol for 10 min at room temperature. The precipitated RNA was washed with 70% ethyl alcohol and then dried. Total RNA was dissolved in 20 μL RNase-free water for analysis.

* Corresponding author. Tel.: +81 466 84 3375; fax: +81 466 84 3380.
E-mail address: itou.takuya@nihon-u.ac.jp (T. Itou).

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Table 1
Analyzed Ugandan RABV isolates in 2003.

Sample name	Host species	Place of isolation	RT-PCR results	Accsession No.
UGdg1	Dog	Nakasongola	+[a]	AB505874
UGdg2	Dog	Wakiso	–[b]	–
UGbv3	Cattle	Nakasongola	–	–
UGgt4	Goat	Wakiso	–	–
UGgt5	Goat	Wakiso	–	–
UGgt6	Goat	Wakiso	+	AB505875
UGgt7	Goat	Nakasongola	+	AB505876
UGdg8	Dog	Wakiso	+	AB505877
UGdg9	Dog	Kibaale	+	AB505878
UGdg10	Dog	Adjumani	+	AB505879
UGdg11	Dog	Adjumani	+	AB505880
UGgt12	Goat	Wakiso	+	AB505881

[a] Positive.
[b] Negative.

As the long-fragment (606 bp) targeting RT-PCR using the Jw12 (5′-ATGTAACACCYCTACAATG-3′) and Jw6 (5′-CAATTCGCACACATTTTGTG-3′) primer pair was unsuccessful, RT-PCR and sequencing were performed using the short-fragment (306 bp) targeting primer pair: Jw12 and NesC (5′-GCWATCAGGATTCCATAGCT-3′), as described previously (Sato et al., 2004). Jw12, Jw6 and NesC correspond to the sequences at 55–73, 660–641 and 360–341, respectively, of the genome of PV rabies virus strain (Acc. No., M13215).

Nucleotide sequences were determined by ATGC program version 4.02 (GENETYX Co., Tokyo, Japan). Multiple alignments were performed using ClustalW 1.83 (Thompson et al., 1994). Phylogenetic analysis was performed to determine the genetic relationship among rabies virus isolates based on the partial sequences of the N gene (Arai et al., 1997). Phylogenetic trees were generated by the neighbor joining method with bootstrap analysis (1000 replicates) under the Kimura 2 parameter model, after aligning sequences using MEGA ver. 4.0 (Tamura et al., 2007). To confirm the relationship among the African lineages, Ugandan field RABVs were analyzed phylogenetically with rabies-related lyssaviruses and representative classical rabies isolates from each African lineage. Bootstrap value greater than 70% were regarded as evidence for phylogenetic grouping (Hills and Bull, 1993).

Amplified products were detected in 8 of 12 samples (Table 1). To elucidate the lineages to which the field RABVs from Uganda belonged, isolates were analyzed phylogenetically with reference RABVs using a 195-nt region of the N gene (Fig. 1a). The radial phylogenetic tree branched into the classical rabies viruses and the rabies-related lyssaviruses. The classical rabies clade consists of the Cosmopolitan, Africa 2 and Viverrid (mongoose) lineages, and all field RABVs from Uganda belonged to the Cosmopolitan lineage. A further study was performed using phylogenetic analysis of 203 nt of the N gene in order to confirm where in the Cosmopolitan lineage the Ugandan field RABVs belong. The 203 nt phylogenetic tree showed that the field RABVs belonged to the Africa 1a or Africa 1b lineage (Fig. 1b). Isolates belonging to the Africa 1a or Africa 1b lineage were distributed widely in northern and southern Uganda (Fig. 2). The goat isolates were grouped with the dog isolates in the Nakasongola and Wakiso districts.

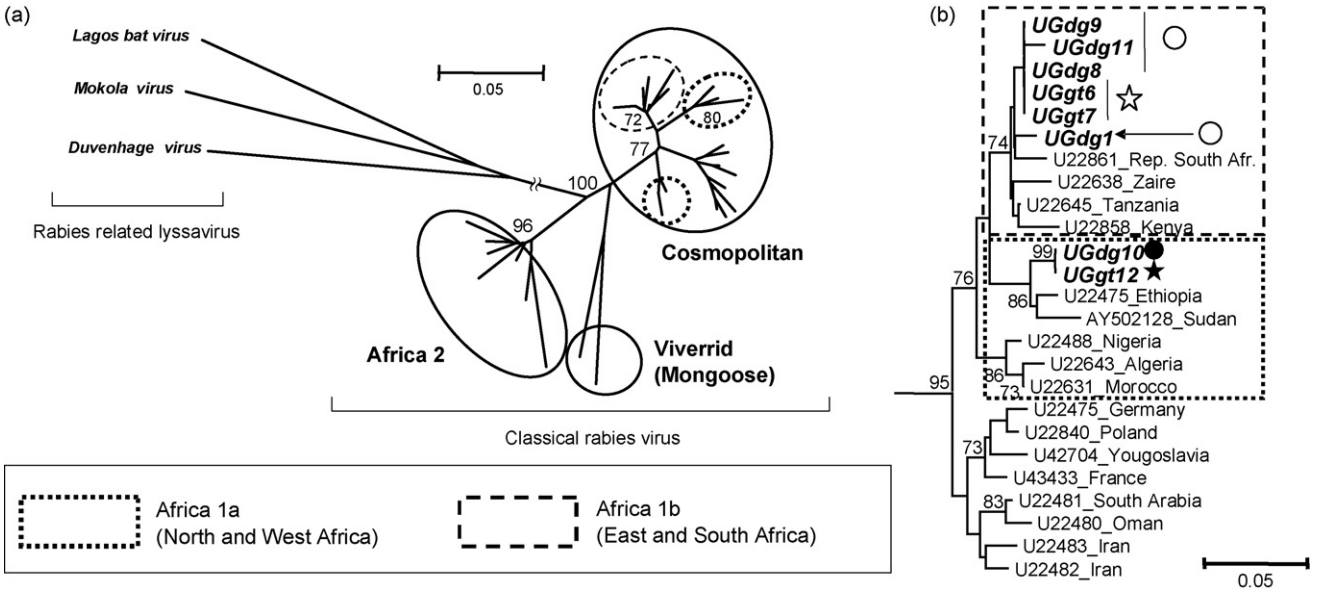

Fig. 1. Partial N gene-based phylogenetic trees: (a) radial phylogenetic tree of RABV and rabies-related lyssavirus isolates from Africa based on 195-nt region of the N gene; (b) more detailed phylogenetic tree of Cosmopolitan lineage based on 203-nt region of the N gene. Significant bootstrap values (>70%) are included. Bold italics indicate analyzed Ugandan isolates. Full and open circles indicate dog isolates belonging to the Africa 1a lineage and the Africa 1b lineage, respectively. Full and open stars indicate goat isolates belonging to the Africa 1a lineage and the Africa 1b lineage, respectively.