




# Enterovirus Sequence Data Obtained from Primate Samples in Central Africa Suggest a High Prevalence of Enteroviruses

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**ABSTRACT** Enteroviruses infect humans and animals and can cause disease, and some may be transmitted across species barriers. We tested Central African wildlife and found *Enterovirus* RNA in primates (17) and rodents (2). Some sequences were very similar, while others were dissimilar to known species, highlighting the underexplored enterovirus diversity in wildlife.

The genus *Enterovirus* (family *Picornaviridae*) contains many diverse viruses that infect humans and cause disease, including poliomyelitis (human poliovirus), hand, foot, and mouth disease (human enterovirus 71), and the common cold (human rhinoviruses) (1). Enteroviruses associated with many other mammalian species have also been discovered, but their diversity, distribution, and roles in disease are overall poorly understood (2, 3). As zoonotic transmission from animals in close contact with humans is of concern, we were interested in the diversity of enteroviruses in wildlife in Cameroon and the Democratic Republic of the Congo (DRC).

Samples from 1,450 bats, 488 rodents, 86 nonhuman primates (NHPs), and 65 shrews were collected in Cameroon and the DRC from 2003 to 2014. The samples included primarily oral and rectal swabs, liver and spleen tissue, as well as feces, and were obtained from animals that were trapped and released, animals in captivity, and animals hunted for consumption. RNA was isolated and reverse transcribed (4), before the samples were screened for enterovirus RNA using a family level consensus PCR targeting the 5' non-coding region (5). Both strands of the PCR amplicons were sequenced (Sanger), aligned (ClustalW, Geneious 11.1.3), and subjected to phylogenetic analysis using MrBayes 3.2, employing default parameters and 4 chains of 1,000,000 generations, with final split frequencies below 0.01 (6). The first 10% of the trees was discarded, and the remaining trees were combined using TreeAnnotator (BEAST 2.5.1) and displayed using FigTree

**Editor** Jelle Matthijssens, KU Leuven

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**Received** 26 September 2021

**Accepted** 5 November 2021

**Published** 9 December 2021

**TABLE 1** Sequencing and phylogenetic analysis data

Sample	GenBank accession no.	Amplicon size (nt) <sup>a</sup>	BLASTN search results <sup>b</sup>			
			Similarity (%)	Reference strain (GenBank accession no.)	Host (sample type)	Country (interface)
CD116032	<a href="#">MK215161</a>	360	93	Coxsackievirus A13 ( <a href="#">MG571836</a> )	<i>Pan troglodytes</i> (feces)	Democratic Republic of the Congo (captive)
CD116033	<a href="#">MK215162</a>	363	93	Coxsackievirus A17 ( <a href="#">JF260925</a> )	<i>Pan troglodytes</i> (feces)	Democratic Republic of the Congo (captive)
CD116035	<a href="#">MK215163</a>	358	94	Coxsackievirus A17 ( <a href="#">JF260925</a> )	<i>Pan troglodytes</i> (feces)	Democratic Republic of the Congo (captive)
CD116037	<a href="#">MK215164</a>	360	93	Coxsackievirus A13 ( <a href="#">MG571836</a> )	<i>Pan troglodytes</i> (feces)	Democratic Republic of the Congo (captive)
CD116038	<a href="#">MK215165</a>	360	93	Coxsackievirus A13 ( <a href="#">MG571836</a> )	<i>Pan troglodytes</i> (feces)	Democratic Republic of the Congo (captive)
CD116040	<a href="#">MK215167</a>	358	93	Coxsackievirus A13 ( <a href="#">MG571836</a> )	<i>Pan troglodytes</i> (feces)	Democratic Republic of the Congo (captive)
CD116055	<a href="#">MK215168</a>	358	95	Human enterovirus strain B ( <a href="#">JX129469</a> )	<i>Pan troglodytes</i> (feces)	Democratic Republic of the Congo (captive)
CD116064	<a href="#">MK215173</a>	358	96	Human enterovirus strain B ( <a href="#">HM209138</a> )	<i>Pan troglodytes</i> (feces)	Democratic Republic of the Congo (captive)
CD116066	<a href="#">MK215174</a>	359	94	Coxsackievirus A24 ( <a href="#">EF026081</a> )	<i>Pan troglodytes</i> (feces)	Democratic Republic of the Congo (captive)
CD116072	<a href="#">MK215175</a>	359	93	Coxsackievirus A13 ( <a href="#">MG571836</a> )	<i>Pan troglodytes</i> (feces)	Democratic Republic of the Congo (captive)
CD116079	<a href="#">MK215176</a>	358	93	Coxsackievirus A13 ( <a href="#">JF260920</a> )	<i>Pan troglodytes</i> (feces)	Democratic Republic of the Congo (captive)
CD116084	<a href="#">MK215177</a>	358	95	Human enterovirus strain B ( <a href="#">JX129469</a> )	<i>Pan troglodytes</i> (feces)	Democratic Republic of the Congo (captive)
CD116086	<a href="#">MK215178</a>	358	95	Human enterovirus strain B ( <a href="#">JX129469</a> )	<i>Pan troglodytes</i> (feces)	Democratic Republic of the Congo (captive)
ECO05844	<a href="#">MK215179</a>	321	76	<i>Picornaviridae</i> sp. ( <a href="#">KF614478</a> )	<i>Praomys</i> sp. (liver, spleen)	Cameroon (free-ranging)
ECO05846	<a href="#">MK215180</a>	309	74	<i>Apodemus agrarius</i> picornavirus strain Longquan-Aa118 ( <a href="#">MF352426</a> )	<i>Praomys</i> sp. (liver, spleen)	Cameroon (free-ranging)
ECO50936	<a href="#">MK215181</a>	358	83	Human enterovirus A ( <a href="#">HM209159</a> )	<i>Cercopithecus nictitans</i> (colon)	Cameroon (captive)
ECO50937	<a href="#">MK215184</a>	358	83	Human enterovirus A ( <a href="#">HM209159</a> )	<i>Allochrocebus preussi</i> (small intestine)	Cameroon (captive)
ECO50938	<a href="#">MK215188</a>	358	82	Uncultured enterovirus clone 0626416 ( <a href="#">EU672963</a> )	<i>Allochrocebus preussi</i> (colon)	Cameroon (captive)
ECO50939	<a href="#">MK215192</a>	358	82	Uncultured enterovirus clone 0626416 ( <a href="#">EU672963</a> )	<i>Cercopithecus nictitans</i> (small intestine)	Cameroon (captive)

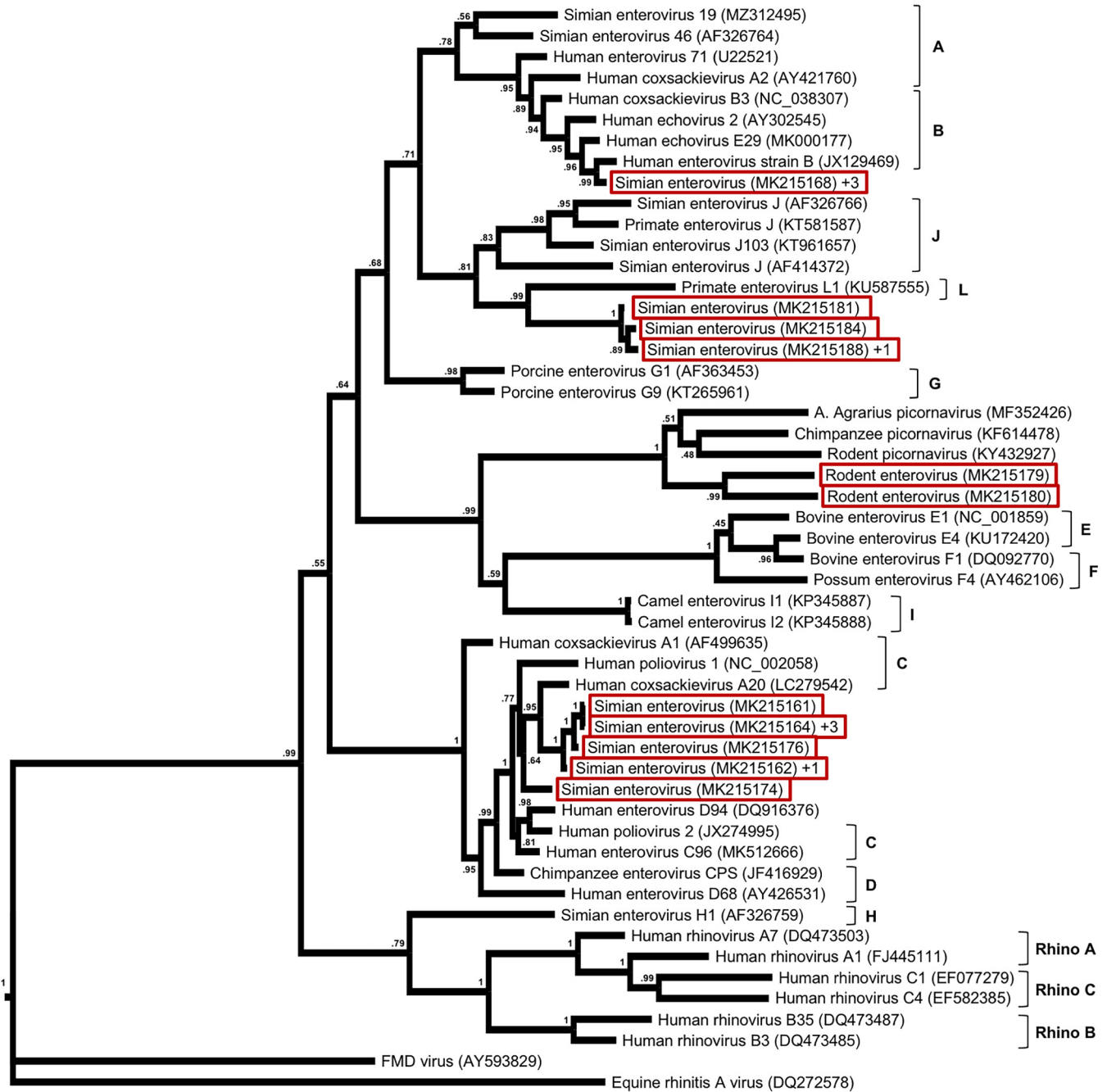
<sup>a</sup> nt, nucleotides.

<sup>b</sup> BLASTN search conducted on 26 October 2021.

1.4.4 (7, 8). Samples for which no RNA of the expected size could be amplified and sequenced were counted as negative.

Enterovirus RNA was detected in 17 NHPs and 2 rodents (Table 1). The sequences fall into four phylogenetic clusters, one of them coinciding with the species enterovirus B, one clustering with enterovirus C and D sequences, one related to enterovirus L, and one clustering with unclassified enteroviruses from rodent and primate hosts (Fig. 1; Table 1).

The detection of Enterovirus RNA in almost 20% of the sampled NHPs supports previous findings that suggest a high prevalence of enteroviruses among primates (9–16). Even though attempts with multiple assays failed to produce sequence beyond the 5' noncoding region, the results suggest that the diversity of NHP enteroviruses needs further exploration. Enteroviruses can be transmitted between humans and NHPs, and contact between these two is not uncommon across many parts of Central Africa, which is of concern (12, 13). The RNAs detected in the rodents suggests the presence of two novel enterovirus species, given their low sequence similarity and phylogenetic placement; however, in the absence of full genomic sequence information, classification is not possible. Despite having tested many bats in the study, we did not detect enterovirus RNA in any of them. Bats, which are hosts



**FIG 1** Maximum likelihood phylogenetic tree of *Enterovirus* sequences, based on the PCR-targeted 362-nucleotide sequence of the 5' untranslated region (UTR). The tree includes the sequences detected during the project (red boxes) and the sequences of known species. The latter were selected to represent all classified species and include sequences with the highest similarities to the novel ones. As the tree is based on the partial 5' UTR, its structure differs from trees based on the full genome or individual coding sequences. The numbers at the nodes indicate the bootstrap support. Novel sequences with high similarity (nucleotide identities of >97%) to other novel sequences are not included but are represented by a single sequence and "+N." These are the sequences with GenBank accession numbers MK215173, MK215177, and MK215178 (represented by MK215168); MK215192 (represented by MK215188); MK215161, MK215165, and MK215167 (represented by MK215164); and MK215163 (represented by MK215162). The ICTV classification of species within the genus *Enterovirus* is indicated where applicable.

of many zoonotic viruses, including rabies and coronaviruses, can be experimentally infected with enteroviruses, but reports of genuine bat enteroviruses are sparse, unlike reports of other bat picornaviruses (4, 8, 17–22). We conclude that Central African bats may either not host many enteroviruses or that the enteroviruses that infect bats are genetically divergent enough from the known species to evade PCR detection with the primers used in this study.

**Data availability.** The partial genomic sequences described are deposited in GenBank under accession numbers [MK215161](#) to [MK215165](#), [MK215167](#), [MK215168](#), [MK215173](#) to [MK215181](#), [MK215184](#), [MK215188](#), and [MK215192](#). The raw data from the collected samples and sampling maps are available at the Zenodo repository (<https://zenodo.org/record/5528104>).

## ACKNOWLEDGMENTS

We thank the governments of Cameroon and the Democratic Republic of the Congo for permission to conduct this study, as well as the JACK Sanctuary in Lubumbashi and the National Institute of Biomedical Research (DRC) and the Limbe Wildlife Centre and the Military Health Research Center (Cameroon) for their engagement. We also thank Prime Mulembakani Mangombo, Kimberly Dodd, Christine Hercik, Joseph Fair, and all other involved members of the PREDICT-1 consortium for their contributions (<https://ohi.sf.ucdavis.edu/programs-projects/predict-project/authorship>).

The study was undertaken as part of the global Emerging Pandemic Threats (EPT) PREDICT project, which focuses on enhancing the global capacity for the detection and discovery of potentially zoonotic viruses at the human-animal interface. It was made possible by the generous support of the American people through the U.S. Agency for International Development (USAID) (cooperative agreement number GHN-A-00-09-00010-00).

The contents are the responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government.

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