Molecular Epidemiology of Paramyxoviruses in Frugivorous *Eidolon helvum* Bats in Zambia

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(Received 23 October 2013/Accepted 18 December 2013/Published online in J-STAGE 31 December 2013)

**ABSTRACT.** In this study, we describe the detection of novel paramyxoviruses from the *Eidolon helvum* species of fruit bats. We extracted RNA from 312 spleen samples from bats captured in Zambia over a period of 4 years (2008–2011). Semi-nested RT-PCR detected a total of 25 (8%) positive samples for paramyxoviruses which were then directly sequenced and analyzed using phylogenetic analysis. Among the positive samples, seven novel paramyxoviruses were detected. Five viruses were closely related to the genus *Henipavirus*, while two viruses were related to the unclassified Bat paramyxoviruses from Ghana and Congo Brazzaville. Our study identified novel Henipavirus-related and unrelated viruses using RT-PCR in fruit bats from Kansaka National Park and indicated the presence of similar Bat paramyxoviruses originating from wide geographic areas, suggesting the ability of bats to harbor and transmit viruses. The presence of these viruses in fruit bats might pose a public health risk.

**KEY WORDS:** bat viruses, epidemiology, paramyxoviruses, virus, Zambia.

**NOTE** Virology

In the past 10 years, a lot of attention has been given to bats as reservoirs of emerging zoonotic viruses. This has been as a result of the high detection rate of previously unknown viral sequences in bats coupled with the emergence of pathogens, such as Hendra, Nipah, Severe acute respiratory syndrome (SARS)-Corona, Ebola and Marburg viruses, all of which are highly virulent and pose a great zoonotic risk [2, 3, 8, 9, 17]. Bats, being the only flying mammals with ancient evolutionary origins and long life span, are capable of covering great distances during migrations, rendering them suitable hosts and reservoirs for various viruses. Paramyxoviruses from the family *Paramyxoviridae* have been implicated in several human epidemics and mortalities [6, 10, 19]. Several studies have indicated bats as potential natural reservoirs of Paramyxoviruses, such as *Henipavirus*, *Respirovirus* and *Morbillivirus*-related viruses [1, 4]. This undoubtedly presents a threat to the health of the human population in areas where human beings live in close proximity to fruit bat species [7]. In Zambia, straw-colored fruit bats (*Eidolon helvum*) annually converge in Kasanka National Park (KNP).

In this study, we investigated the presence of paramyxoviruses in the *Eidolon helvum* bats captured over a period of 4 years (2008–2011) from KNP ($S15:34.688 \, E28:16.513$). During that period, a total of 312 spleen samples were collected from the same number of bats (Table 1). Appropriate research permits and hunting licenses were obtained from the Zambia Wildlife Authority (ZAWA).

Total RNA was isolated from spleen tissues using TRizol (Life Technologies, Carlsbad, CA, U.S.A.) according to the manufacturer’s instructions. A semi-nested broad spectrum RT-PCR targeting the paramyxovirus polymerase *(L)* gene was used to screen total RNA samples (n=312) for paramyxoviruses using PAR-F1, PAR-F2 and PAR-R primers and PCR conditions described by Tong *et al.* [16]. A total of 25 samples out of 312 bat spleens (8%) were positive for paramyxoviruses on semi-nested PCR. The positive products (584 bp) were then purified using the monofas purification kit (GL sciences, Tokyo, Japan), according to the manufacturer’s instructions. The purified PCR products were then subjected to Cycle sequencing reactions using the Big Dye Terminator v3.1 system (Life Technologies) and the PAR-F2 and PAR-R inner primers. Ethanol precipitation was used to remove the
base substitutions per site as units [15]. The Composite Likelihood method was used with the number of
grams [12] with a 1,000 bootstrap replicate confidence level
neighbor joining method was used to generate the phylo-
MEGA file format created using MEGA ver.5.2 [14]. The
sequences using ClustalW1.6 followed by the creation of a
reference sequences and positive samples by aligning all
to electrophoresis in the ABI 3130 genetic analyzer (Life
labeled dNTPs from cycle sequence products and subjected
to electrophoresis in the ABI 3130 genetic analyzer (Life
Technologies). Phylogenetic analysis was performed using reference sequences and positive samples by aligning all
sequences using ClustalW1.6 followed by the creation of a
MEGA file format created using MEGA ver.5.2 [14]. The
neighbor joining method was used to generate the phylo-
grams [12] with a 1,000 bootstrap replicate confidence level
[5]. To compute the evolutionary distances, the Maximum
Composite Likelihood method was used with the number of
base substitutions per site as units [15].
Samples AB853101, AB853102, AB853104, AB853105
and AB853094 showed a nucleotide homology of 73%
with the Nipah virus (AF212302), while AB853106 and
AB853096 had a nucleotide homology of 74% with the
unclassified Bat paramyxovirus (Bat PV) (JN648087) from
Ghana. The relatively low nucleotide homology might indi-
cate that these sequences originate from novel paramyxovi-
ruses. The samples from Zambia formed clusters with the
Henipavirus-related viruses and with the unclassified Bat
paramyxoviruses (Fig. 1). Within the Henipavirus-related
virus cluster, two groups (A and B) were observed. Group
A comprised novel Zambian strains closely related to the
Nipah (NC002728, FN86955 and AF212302) and Hendra
(AFO17149 and NC001906) viral sequences, while Group B
comprised a cluster of Zambian strains, in close relationship
with an unclassified Bat PV from Ghana (JN648085) and
Cedar virus (JQ001776) (Fig. 1). The remaining Zambian
strains, including the novel AB853106 sequence, formed a
cluster with the unclassified Bat PV sequences from Ghana
(JN648078, JN648081, JN648087 and JN648089) and
Congo Brazzaville (HE647835 and HE647837) (Fig. 1).
The close relatedness of the viral sequence from Ghana and
Congo Brazzaville strains with those from Zambia might
imply the ability of bats to harbor and transmit similar vi-
ruses over long and diverse geographical distances. This is
facilitated by their ability to migrate, covering thousands
of kilometers to their hibernation and feeding sites [11]. Among
their migratory path, they interact directly or indirectly with
several terrestrial mammalian species in different geographi-
ical locations, thus enhancing the interspecies transmission
of viruses [11]. Humans can become exposed to these viruses
through environmental contamination with urine and feces
from bats. Although paramyxovirus infections derived from
bats have been reported in humans in Bangladesh [13], none
have been reported in Africa. The absence of cases might
be as a result of under-reporting. As such continued surveil-
ance and assessment of the zoonotic risk posed by these
viruses still remains important.
In order to isolate the detected viruses, spleens from
PCR positive bats were homogenized in minimum essential
media (MEM) followed by centrifugation at 1,000 × g for
3 min. The supernatant was then applied to Vero E6 cell
with 70–80% growth confluence. The Vero E6 cells were
cultured in MEM with 2% fetal bovine serum (FBS) and 2%
antibiotic-antimycotic (Life Technologies). The inoculated
Vero E6 cell cultures were then incubated at 37°C for 21
days, coupled with cell passage and microscopic examina-
Success isolation was only successfully carried out in two samples. Furthermore, serological examination of bat sera may pro-
provide important information about their exposure to specific
infections. Unfortunately, however, our study did not carry
out any serological test.
In conclusion, we report the identification of novel
Henipavirus-related (n=5) and unrelated (n=2) viruses in
fruit bats from the KNP using RT-PCR. The viruses identi-
fied in this study were shown to originate from wide geo-
ographical areas, and their presence in fruit bat species might
pose a public health risk and as such, continued surveillance
of these viruses in fruit bats is essential.
Fig. 1. Phylogenetic analysis of paramyxovirus samples based on a 530 nucleotide sequence of the polymerase L gene from Zambia (in bold) and other areas of the world. The neighbor joining method was used to construct the phylogram using a confidence level of 1,000 bootstrap replicates. Species names and accession numbers are used to identify both the Zambian samples and the reference isolates. Bootstrap values for 1,000 replicates are indicated as percentage (>90%) and nucleotide substitutions per site as scale bars.
ACKNOWLEDGMENTS. We thank Ms. Yamanouchi, Research Center for Zoonosis Control, Hokkaido University, for technical assistance. We also wish to thank the Zambia Wildlife Authority and the Kasanka Trust for providing permission and the enabling environment for sampling the fruit bats. This study was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology (MEXT); the Ministry of Health, Labour and Welfare, Japan; the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID), MEXT Japan.

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