Highly Pathogenic Avian Influenza A(H5N8) Virus, Cameroon, 2017

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Highly pathogenic avian influenza A(H5N8) viruses of clade 2.3.4.4 spread into West Africa in late 2016 during the autumn bird migration. Genetic characterization of the complete genome of these viruses detected in wild and domestic birds in Cameroon in January 2017 demonstrated the occurrence of multiple virus introductions.

Since the first detection in China in 1996, highly pathogenic avian influenza (HPAI) viruses of the H5 subtype descendent of the H5N1 virus A/goose/Guangdong/1/1996 (Gs/GD/96) have evolved into multiple genetic clades (1,2) that have been threatening poultry worldwide. Since 2010, clade 2.3.4 has demonstrated an unusual propensity to replace its N1 subtype and acquire different neuraminidase (NA) genes from unrelated avian influenza viruses through reassortment, which has resulted in the emergence of new viral subtypes within the Gs/GD/96 H5 lineage (e.g., N2, N5, N6, N8). In late spring 2016, reassortant HPAI A(H5N8) clade 2.3.4.4 (group B) virus was detected in migratory wild birds in Qinghai Lake, China (3), and in the salt lake system of Uvs Nuur, on the border between Mongolia and the Russian Federation (4). Since then, this newly emerged virus has caused multiple outbreaks of the disease in poultry and wild birds across Europe, Asia, and the Middle East and has extended not only to countries in northern, central, and western Africa, as did the previous Gs/GD/96 H5 lineage outbreaks, but also to the eastern and southern parts of Africa (5). We investigated the epidemiology of the outbreaks in early 2017 of HPAI H5N8 virus in domestic and wild birds in Cameroon and determined the possible origin of this virus through whole-genome analyses.

The Study

On January 2, 2017, high death rates were reported in Indian peafowl (Pavo cristatus) in a backyard exotic poultry farm located in the town of Makilingaye (Tokombéré district of Mayo-Sava division), a village neighboring Nigeria in the far-north region of Cameroon. Samples were collected by the Cameroon Epidemio-Surveillance Network (Reseau d’Epidemio-Surveillance au Cameroun [RESCAM]) of the Ministry of Livestock, Fisheries and Animal Industries and were sent to the National Veterinary Laboratory (LANAVET) in Garoua, where the H5N8 subtype was diagnosed. Almost all the peafowl (103/107) died within ≈2 weeks. Death in chickens (Gallus gallus domesticus, 24/24) housed in the same compound was delayed and appeared 5 days later than in peafowl. Following the laboratory confirmation of the first outbreak, the Cameroon government, through the Ministry of Livestock, Fisheries, and Animal Industries, implemented prompt and strong control measures to stop the spread of the virus and reduce the risk of human infections. Stampinging out was deployed together with movement restrictions and virological surveillance; disinfection of premises and contact materials was intensified. The RESCAM team conducted a routine avian influenza survey in the Maroua, Yagoua, and Guidiguis central poultry markets in the far-north region. All the samples were analyzed at LANAVET Garoua and Annex Yaounde; H5N8 virus was detected in 5 birds (1 pigeon, 1 chicken, 2 guinea fowls, and 1 duck) out of 122 birds.

We sequenced the hemagglutinin (HA) and NA gene segments of the virus A/Indian peafowl/Cameroun/17RS1661-6/2017, identified from an Indian peafowl in Makilingaye, at the Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy (B. Zecchin, A. Fusaro, A. Bianco, A. Salomoni, G. Zamperin, I. Monne); Food and Agriculture Organization of the United Nations, Rome, Italy (L. Wiersma); Animal Production and Health Laboratory, Seibersdorf, Austria (G. Cattoli)

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a wild pigeon, all identified in the Maroua and Yagoua markets (Table). A detailed description of the methods used for sequencing and genetic analyses is provided in online Technical Appendix 1 (https://wwwnc.cdc.gov/EID/article/24/7/17-2120-Techapp1.pdf) and details on the HA gene segments used for the analysis are given in Technical Appendix 2 (https://wwwnc.cdc.gov/EID/article/24/7/17-2120-Techapp2.xlsx). We submitted consensus sequences to GenBank (accession nos. MG650618–41).

Topology of the phylogenetic tree based on the HA gene segment showed that the 4 H5N8 viruses detected in Cameroon in 2017 fell within genetic clade 2.3.4.4 group

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**Table.** Epidemiologic information of viruses characterized in study of highly pathogenic avian influenza A(H5N8) virus, Cameroon, January 2017*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Sample type</th>
<th>Location</th>
<th>EpiFlu accession numbers for 8 gene segments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/chicken/Cameroon/17RS1661-1/2017</td>
<td>Tracheal swab</td>
<td>Maroua market</td>
<td>HA, MG650619; MP, MG650622; NA, MG650626; NP, MG650630; NS, MG650632; PA, MG650635; PB1, MG650638; PB2, MG650641</td>
</tr>
<tr>
<td>A/duck/Cameroon/17RS1661-3/2017</td>
<td>Tracheal swab</td>
<td>Yagoua market</td>
<td>HA, MG650620; MP, MG650623; NA, MG650627; NP, MG650629; NS, MG650633; PA, MG650636; PB1, MG650637; PB2, MG650639</td>
</tr>
<tr>
<td>A/pigeon/Cameroon/17RS1661-4/2017</td>
<td>Cloacal swab</td>
<td>Maroua market</td>
<td>HA, MG650621; MP, MG650624; NA, MG650628; NP, MG650631; NS, MG650634; PA; PB1, na; PB2, MG650640</td>
</tr>
<tr>
<td>A/Indian peafowl/Cameroon/17RS1661-6/2017</td>
<td>Tracheal swab</td>
<td>Makilingaye</td>
<td>HA, MG650619; MP, NR; NA, MG650626; NP, NR; NS, NR; PA, NR; PB1, NR; PB2, NR</td>
</tr>
</tbody>
</table>

*HA, hemagglutinin; MP, matrix protein, NA, neuraminidase; NR, not reported; NP, nucleoprotein; NS, nonstructural proteins; PA, polymerase acidic protein; PB1, polymerase basic protein 1; PB2, polymerase basic protein 2.

**Figure.** Maximum-likelihood phylogenetic tree of the HA gene of highly pathogenic avian influenza A(H5N8) virus from Cameroon (bold) and reference viruses. Arrows indicate location where Cameroon viruses were obtained. Bootstrap supports >600/1,000 are indicated next to the nodes. Scale bar indicates numbers of nucleotide substitutions per site.

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cannot be excluded. The region contains several wetlands
spread of the virus. However, the involvement of wild birds
protected in the far-north region of Cameroon in Makilingaye,
Less than 2 months later, in January 2017, the virus was de
Nigeria was the first country in West Africa to report the
Conclusions
We confirmed the different clustering of the viruses
collected from distinct markets in Cameroon by the analy-
ses of the other gene segments, which suggests the occur-
ence of ≥2 distinct viral introductions in the country. Spe-
cifically, sequences of the 2 viruses from Maroua market
were identical for all the available gene segments (Table)
and clustered with viruses collected in Asia, Europe, and
Egypt (online Technical Appendix 1 Figures 2–8). On the
other hand, the virus A/duck/Cameroon/17RS1661-3/2017
grouped with the A/duck/India/10CA01/2016 virus (simi-
arity 94.3–99.9%), except for the nucleoprotein (NP) gene,
where it showed the highest identity (99.3%) with a virus
collected in 2016 in the Russian Federation (Uvs Nuur
Lake). The lack of genetic information on other H5N8 vi-
ruses detected in Africa makes it impossible to pinpoint how
these viruses entered Cameroon and spread, nor is it fea-
sible to determine where the 2 introductions occurred. The
time to the most recent common ancestor estimated for the
HA gene (online Technical Appendix 1) suggested that 2
introductions into Africa may have occurred almost simul-
taneously during March–December 2016 (online Technical
Appendix 1 Figure 9). However, analyses of the amino acid
sequences show that A/duck/Cameroon/17RS1661-3/2017
possesses the N319K mutation in the NP protein, which has
been reported to enhance polymerase activity and stimulate
vRNA synthesis in mammalian cells (6). In addition, A/
chicken/Cameroon/17RS1661-1/2017 contains the V100A
mutation in the PA protein, which is an amino acid sig-
ture typical of human influenza viruses (7).

Conclusions
Nigeria was the first country in West Africa to report the
presence of the HPAI H5N8 virus in November 2016 (8,9).
Less than 2 months later, in January 2017, the virus was de-
tected in the far-north region of Cameroon in Makilingaye,
close to the Nigerian border. Considering the extensive and
porous frontier between Cameroon and Nigeria, trade and
movement of poultry might have played a key role in the
spread of the virus. However, the involvement of wild birds
cannot be excluded. The region contains several wetlands
(Domayo River, Mayo Kani River, and Maga Lake) where
different wild birds congregate, in particular during the dry
season (December–April), when the virus was first detected.
The almost simultaneous detection (early January 2017) of
the H5N8 virus in poultry and wild birds in distant locations
in Africa, such as Tunisia and Uganda, suggests that the role
of wild birds in the introduction and/or dissemination of the
virus in the region should not be overlooked. Of note, the first
outbreak caused by the Gs/GD/96 H5 lineage in Cameroon,
in 2006, also occurred in the far-north region of Cameroon
at about the same time. This finding might suggest a common
pathway for introduction of the virus into this area and high-
lights the need to improve surveillance in this region.

Although the epidemiologic and genetic data are in-
sufficient to establish definite pathways and time of intro-
duction of H5N8 virus into West Africa, this study dem-
strates that ≥2 distinct H5N8 viruses entered Cameroon.
This finding, together with the evidence that this event rep-
resents the third incursion of a Gs/GD/96-lineage H5 HPAI
virus into Cameroon, again underlines the need to perform
avian influenza surveillance on an ongoing basis for rapid
identification and response to outbreaks in this area.

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EpiFlu database, on which this research is based in part (online
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References

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