

multiple organ failure within 5 days. This patient did not show any signs or symptoms of rabies or encephalitis.

None of the recipients had been exposed to potentially rabid animals or had been vaccinated previously for rabies (online Technical Appendix). Both kidney recipients tested positive for rabies virus (online Technical Appendix Table 2). The genome sequences of the rabies virus isolates from the right kidney recipient (isolate no. CCS1501H) were  $\approx$ 11 kb nucleotides in length and belonged to the China I lineage. No testing for rabies was done on the donor or on the liver recipient.

In the past 10 years, rabies transmission by solid organ transplantation has been described occasionally worldwide (2–4). Hence, rabies transmission through organ transplantation is a clinical and public health concern. To prevent future cases such as this, we recommend that patients with unexplained encephalitis or mental status changes should not be used as organ donors even if tests for some infectious causes of encephalitis are negative. In addition, if rabies is suspected in the donor after organs have been transplanted, the recipients should also not be used as organ donors. An antibody test is not the ideal choice for the diagnosis of rabies virus and by itself cannot reliably exclude rabies from the differential diagnosis. For this reason, a combination of multiple techniques, preferably direct fluorescent antibody test and reverse transcription PCR, should be used before organ transplantation, especially when the donor is suspected of having rabies or a potential exposure to rabies. In addition, if a patient has meningoencephalitis of unknown cause, a specific epidemiologic and laboratory evaluation should be performed to conclusively rule out rabies as a cause of illness before organ donation.

Dr. Shuilian Chen is a medical doctor in the Changsha Center for Disease Control and Prevention in Changsha, China. Her main field of research is emerging infectious disease control and prevention.

## References

- Zhou H, Zhu W, Zeng J, He J, Liu K, Li Y, et al. Probable rabies virus transmission through organ transplantation, China, 2015. *Emerg Infect Dis.* 2016;22:1348–52. <http://dx.doi.org/10.3201/eid2208.151993>
- Srinivasan A, Burton EC, Kuehnert MJ, Rupprecht C, Sutker WL, Ksiazek TG, et al.; Rabies in Transplant Recipients Investigation Team. Transmission of rabies virus from an organ donor to four transplant recipients. *N Engl J Med.* 2005;352:1103–11. <http://dx.doi.org/10.1056/NEJMoa043018>
- Bronnert J, Wilde H, Tepsumethanon V, Lumlertdacha B, Hemachudha T. Organ transplantations and rabies transmission. *J Travel Med.* 2007;14:177–80. <http://dx.doi.org/10.1111/j.1708-8305.2006.00095.x>
- Vora NM, Basavaraju SV, Feldman KA, Paddock CD, Orciari L, Gitterman S, et al.; Transplant-Associated Rabies Virus Transmission Investigation Team. Raccoon rabies virus variant transmission through solid organ transplantation. *JAMA.* 2013;310:398–407. <http://dx.doi.org/10.1001/jama.2013.7986>

Address for correspondence: Tianmu Chen, Changsha Center for Disease Control and Prevention, 509 North Wanjiali Road Section 2, Kaifu District, Changsha, China; email: 13698665@qq.com

## Identification of Clade E Avipoxvirus, Mozambique, 2016

Lourenço P. Mapaco, Zeiss Lacerda, Iolanda V.A. Monjane, Esayas Gelaye, Afonso H. Sussuro, Gerrit J. Viljoen, William G. Dundon, Sara J. Achá

Author affiliations: Agrarian Research Institute of Mozambique, Maputo, Mozambique (L.P. Mapaco, I.V.A. Monjane, A.H. Sussuro, S.J. Achá); Eduardo Mondlane University, Maputo (Z. Lacerda); National Veterinary Institute, Debre Zeit, Ethiopia (E. Gelaye); International Atomic Energy Agency, Vienna, Austria (G.J. Viljoen, W.G. Dundon)

DOI: <https://doi.org/10.3201/eid2309.161981>

Analysis of scab samples collected from poultry during outbreaks of fowlpox in Mozambique in 2016 revealed the presence of clade E avipoxviruses. Infected poultry were from flocks that had been vaccinated against fowlpox virus. These findings require urgent reevaluation of the vaccine formula and control strategies in this country.

Avipoxviruses are large, enveloped DNA viruses that belong to the genus *Avipoxvirus* in the *Chordopoxvirinae* subfamily of the family *Poxviridae*. These viruses cause disease in a large number of bird species and are generally named after the species from which the virus was first isolated and characterized (*1*). Fowlpox virus (FPV) has caused substantial economic losses in domestic poultry resulting from reduced egg production and growth, blindness, and death, with a death ratio that can reach as high as 50%.

Phylogenetic analyses of the *Avipoxvirus* genus are usually conducted with the segments of the genes encoding the 4b core-like protein (P4b) and the DNA polymerase, which are both highly conserved among poxviruses (2,3). Using these loci, researchers have seen that most strains cluster into 3 major clades, namely A, B, and C, with clade A being subdivided further into subclades A1–A7 and clade B into subclades B1–B3 (3–5). Two additional clades

(i.e., D and E), each with just a single isolate, have also been proposed (5,6).

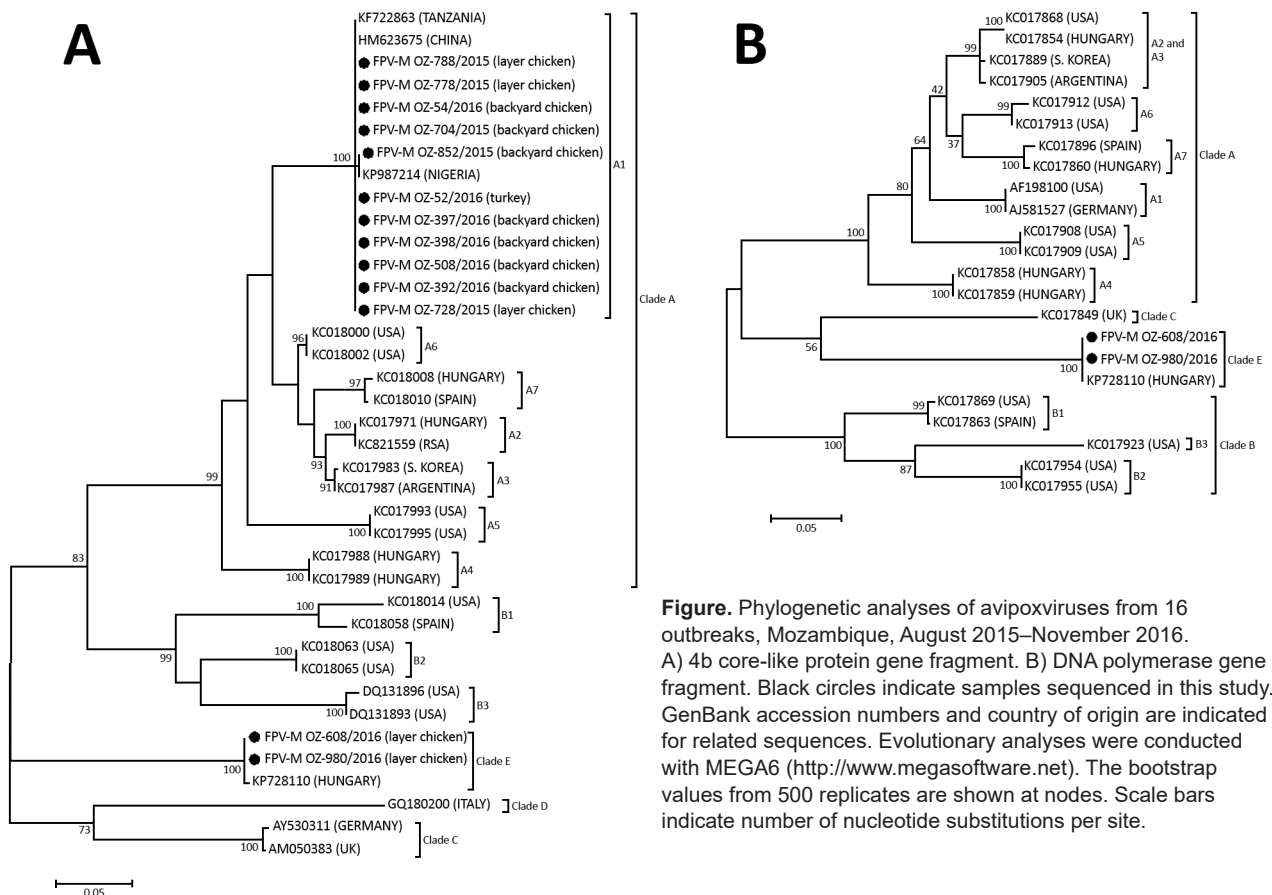
Little is known about avipoxviruses in Africa. Avipoxviruses isolated from chickens, turkeys, and pigeons in 2011 in northern Egypt belonged to either subclade A1 or A2 (7). In 2013, thirteen avipoxviruses from different bird species from different regions of South Africa grouped into either subclade A2 or A3 (8). Sequences generated from isolates from naturally infected chickens in Tanzania were also found to belong to clade A1 (9). Therefore, all avipoxviruses previously identified in Africa belonged to clade A.

Fowlpox is endemic in Mozambique and commonly reported. The effect of the disease is more severe in backyard production systems affecting mostly young chickens and turkeys. An official control program for FPV in Mozambique does not exist, and because the country does not have a poultry production system that meets the national demand, birds are often imported from neighboring countries, such as South Africa, Swaziland, and Zimbabwe.

During August 2015–November 2016, scab samples from 16 separate FPV outbreaks were collected by the Agrarian Research Institute of Mozambique from 4 locations: Gaza Province, Maputo Province, Maputo City, and Tete Province. The outbreaks primarily affected backyard chickens and

commercial laying hens, although a flock of broiler chickens and a flock of turkeys were also investigated. The clinical signs reported and postmortem examination findings included reduction of appetite; listlessness; nodules or scabs of different sizes on less-feathered body areas (e.g., wattles, comb, eye lids, ear lobes, limbs, and interdigit spaces); and pronounced ulcerations on the interdigit space. Different color tones and irregular wrinkled shells were also observed on eggs.

The 16 scab samples were positive for FPV P4b gene following PCR amplification with specific primers (online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/23/9/16-1981-Techapp1.pdf>); the amplicons were purified and sequenced. A phylogenetic analysis of the P4b gene sequences revealed that most of the samples collected contained virus that clustered in subclade A1 (Figure, panel A). However, the 2 samples that did not cluster in subclade A1 (FPV-MOZ-608/2016 and FPV-MOZ-980/2016) were of interest; these samples were obtained from 2 separate outbreaks in layer chickens 3 months apart (i.e., in Maputo City [25°55'55.5"S, 32°32'53.4"E] on July 7, 2016, and in Maputo Province [25°53'22.3"S, 32°27'16.6"E] on October 25, 2016). To characterize FPV-MOZ-608/2016 and FPV-MOZ-980/2016 further, we amplified and sequenced a segment of the DNA polymerase gene (online Technical



**Figure.** Phylogenetic analyses of avipoxviruses from 16 outbreaks, Mozambique, August 2015–November 2016. A) 4b core-like protein gene fragment. B) DNA polymerase gene fragment. Black circles indicate samples sequenced in this study. GenBank accession numbers and country of origin are indicated for related sequences. Evolutionary analyses were conducted with MEGA6 (<http://www.megasoftware.net>). The bootstrap values from 500 replicates are shown at nodes. Scale bars indicate number of nucleotide substitutions per site.

Appendix). The maximum-likelihood analysis using both P4b and DNA polymerase gene fragments showed that FPV-MOZ-608/2016 and FPV-MOZ-980/2016 clustered in clade E with the APV isolated in Hungary in 2011 (TKPV-HU1124/2011) (Figure, panels A and B) (6).

TKPV-HU1124/2011 was isolated from a flock of turkeys vaccinated with a commercial vaccine for FPV, and FPV-MOZ-608/2016 and FPV-MOZ-980/2016 were also obtained from vaccinated chickens. The laying pullets imported from South Africa had already been vaccinated for FPV on day 1 with the commercial fowlpox-vectored infectious laryngotracheitis vaccine and day 17 with the fowlpox-vectored infectious laryngotracheitis/avian encephalomyelitis vaccine. Our data suggest a possible failure of the vaccine to protect against clade E viruses, similar to what has been reported previously for TKPV-HU1124/2011 (6).

The identification of a clade E avipoxvirus in Mozambique requires further investigation to clarify how a virus that has only been reported once found its way to this country. Because the chickens in both infected flocks were purchased from the same pullet reseller who had (for both flocks) imported the birds from South Africa, it is likely that the source of infection was the same. However, the specific source has not been identified. FPVs are known to infect >230 species of wild birds, many of which are migratory (5); thus, introduction through migratory wild birds is a possibility.

Resolution of the full genome of these viruses might provide hints to their origin. The presence of fowlpox disease in birds vaccinated against FPV requires urgent re-evaluation of the vaccine formula and control strategies in Mozambique.

This work was supported through funding from the International Atomic Energy Agency Peaceful Uses Initiative (MOZ/14003) and the African Renaissance project, which is funded by the Republic of South Africa. Part of the field activities were supported by the Kyeema Foundation.

Dr. Mapaco is a researcher at the Central Veterinary Laboratory of the Agrarian Research Institute of Mozambique. His research interests are viral diseases of animals, including zoonoses.

## References

1. Weli SC, Tryland M. Avipoxviruses: infection biology and their use as vaccine vectors. *Virology*. 2011;8:49. <http://dx.doi.org/10.1186/1743-422X-8-49>
2. Binns MM, Bourns ME, Tomley FM, Campbell J. Analysis of the fowlpoxvirus gene encoding the 4b core polypeptide and demonstration that it possesses efficient promoter sequences. *Virology*. 1989;170:288–91. [http://dx.doi.org/10.1016/0042-6822\(89\)90380-2](http://dx.doi.org/10.1016/0042-6822(89)90380-2)
3. Gyuranecz M, Foster JT, Dán Á, Ip HS, Egstad KF, Parker PG, et al. Worldwide phylogenetic relationship of avian poxviruses. *J Virol*. 2013;87:4938–51. <http://dx.doi.org/10.1128/JVI.03183-12>
4. Jarmin S, Manvell R, Gough RE, Laidlaw SM, Skinner MA. Avipoxvirus phylogenetics: identification of a PCR length polymorphism that discriminates between the two major clades.

*J Gen Virol*. 2006;87:2191–201. <http://dx.doi.org/10.1099/vir.0.81738-0>

5. Manarolla G, Pisoni G, Sironi G, Rampin T. Molecular biological characterization of avian poxvirus strains isolated from different avian species. *Vet Microbiol*. 2010;140:1–8. <http://dx.doi.org/10.1016/j.vetmic.2009.07.004>
6. Bányai K, Palya V, Dénes B, Glávits R, Ivanics É, Horváth B, et al. Unique genomic organization of a novel avipoxvirus detected in turkey (*Meleagris gallopavo*). *Infect Genet Evol*. 2015;35:221–9. <http://dx.doi.org/10.1016/j.meegid.2015.08.001>
7. Abdallah FM, Hassanin O. Detection and molecular characterization of avipoxviruses isolated from different avian species in Egypt. *Virus Genes*. 2013;46:63–70. <http://dx.doi.org/10.1007/s11262-012-0821-y>
8. Offerman K, Carulei O, Gous TA, Douglass N, Williamson AL. Phylogenetic and histological variation in avipoxviruses isolated in South Africa. *J Gen Virol*. 2013;94:2338–51. <http://dx.doi.org/10.1099/vir.0.054049-0>
9. Mzula A, Masola SN, Kasanga CJ, Wambura PN. Existence of variant strains fowlpox virus integrated with reticuloendotheliosis virus in its genome in field isolates in Tanzania. *Trop Anim Health Prod*. 2014;46:711–6. <http://dx.doi.org/10.1007/s11250-014-0552-2>

Address for correspondence: William G. Dundon, APHL Joint FAO/IAEA Division, International Atomic Energy Agency Laboratories, Seibersdorf A-2444, Austria; email: w.dundon@iaea.org

## Indication of Cross-Species Transmission of Astrovirus Associated with Encephalitis in Sheep and Cattle

Céline L. Boujon, Michel C. Koch, Daniel Wüthrich, Simea Werder, Dennis Jakupovic, Rémy Bruggmann, Torsten Seuberlich

Author affiliation: University of Bern, Bern, Switzerland

DOI: <https://doi.org/10.3201/eid2309.170168>

We report the identification of a neurotropic astrovirus associated with encephalitis in a sheep. This virus is genetically almost identical to an astrovirus recently described in neurologically diseased cattle. The similarity indicates that astroviruses of the same genotype may cause encephalitis in different species.

Astroviruses are nonenveloped viruses with a single stranded, positive-sense RNA genome. They are best known to be associated with gastroenteritis, especially in humans. Recently, reports of these viruses in association

# Identification of Clade E Avipoxvirus, Mozambique, 2016

## Technical Appendix

### DNA Isolation, Amplification, and Sequencing

DNA was extracted directly from scab samples collected from different parts of birds (e.g., wattles, comb, eye lids, ear lobes, limbs, and interdigital spaces) by using a DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany). A 368-bp fragment of the 4b core-like protein gene was amplified by PCR with the primer pair FP-For (5'-CAGCAGGTGCTAAACAACAA-3') and FP-Rev (5'-CGGTAGCTTAACGCCGAATA-3') (1). A 1,000-bp fragment of the DNA polymerase was amplified by PCR with the primer pair PPolF (5'-GGCYAGTACKCTTATYAAAGG-3') and PPolR (5'-CGTCTCTACGTGTTTCGCT-3') (2).

The following thermal profile was used for both PCR amplifications: initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 35 s, and elongation at 72°C for 45 s; followed by a final elongation at 72°C for 7 min. Positive PCR amplicons were purified with a Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) and were sent to LGC Genomics (Berlin, Germany) for sequencing with the same primers used for the amplification. All 4b core-like protein and DNA polymerase gene sequences generated here were deposited in GenBank (accession nos. KX988302 and KY312501–KY312503).

### Phylogenetic Analysis

The Staden Package (<http://staden.sourceforge.net/>) was used to assemble the generated sequences. Multiple sequence alignment was performed by using MUSCLE (<http://www.ebi.ac.uk/Tools/msa/muscle/>) with default settings, incorporating all the sequences generated here combined with those available in GenBank. This resulted in a final data set of 41 sequences 368 bp in length for the 4b core-like protein gene fragment and 23 sequences 542 bp

in length for the DNA polymerase gene. A phylogenetic tree was estimated by using the maximum likelihood method available in MEGA6 (3), the Kimura 2-parameter model of nucleotide substitution, and 500 bootstrap replications.

## References

1. Jarmin S, Manvell R, Gough RE, Laidlaw SM, Skinner MA. Avipoxvirus phylogenetics: identification of a PCR length polymorphism that discriminates between the two major clades. *J Gen Virol.* 2006;87:2191–201. [PubMed http://dx.doi.org/10.1099/vir.0.81738-0](http://dx.doi.org/10.1099/vir.0.81738-0)
2. Gyuranecz M, Foster JT, Dán Á, Ip HS, Egstad KF, Parker PG, et al. Worldwide phylogenetic relationship of avian poxviruses. *J Virol.* 2013;87:4938–51. [PubMed http://dx.doi.org/10.1128/JVI.03183-12](http://dx.doi.org/10.1128/JVI.03183-12)
3. Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol.* 2013;30:2725–9. [PubMed http://dx.doi.org/10.1093/molbev/mst197](http://dx.doi.org/10.1093/molbev/mst197)