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 reevaluation 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. Virol J. 2011;8:49. http://dx.doi.org/10.1186/1743-422X-8-49
2. Binns MM, Boursnell 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
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

with encephalitis have increased dramatically, with reports of cases in humans (*1*), mink (*2*), and cattle (*3*–*6*).

The most common causes for viral encephalitis in sheep include maedi-visna virus, Borna disease virus, and rabies virus. In a high proportion of cases of nonsuppurative encephalitis cases (which is indicative of a viral infection) in sheep, however, the etiologic agent remains unknown (*7*). To investigate that matter, we subjected 3 ovine encephalitis samples from our archives to next-generation sequencing and a bioinformatics pipeline for virus discovery (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/9/17-0168-Techapp1.pdf). In 1 animal (ID 41669), 1 of the contigs obtained had high similarity (>98%) to bovine astrovirus CH15 (BoAstV-CH15), a virus found recently in 2 cases of nonsuppurative encephalitis in cattle (*5*). The affected sheep was a 7-year-old Swiss white alpine ewe that was culled for reasons other than human consumption. No other information about the clinical history of the animal was available. Histological diagnosis consisted of severe nonsuppurative meningoencephalitis. Routine diagnostic tests for Borna, rabies, and maedi-visna viruses were all negative.

We used primers based on the BoAstV-CH15 genome sequence (GenBank accession no. KT956903) and the Sanger method to sequence the complete genome of the ovine strain (online Technical Appendix Figure 1), which we named ovine astrovirus CH16 (OvAstV-CH16). The sequence we obtained shared >98% identity with BoAstV-CH15 on the nucleotide and amino acid level (Genbank accession no. KY859988; online Technical Appendix Table 1). The virus reported here is genetically clearly distinct from intestinal OvAstV strains described previously (OvAstV-1 and OvAstV-2; online Technical Appendix Table 1).

A phylogenetic comparison confirmed the close relationship of OvAstV-CH16 with BoAstV-CH15 (*5*) and BoAstV-BH89/14, another astrovirus detected in association with encephalitis in a cow in Germany (*6*). Recently, 2 astroviruses were reported in association with encephalitis in sheep in Scotland (OvAstV UK/2013/ewe/lib01454 and UK/2014/lamb/lib01455) (*8*), and we included their genomic data in the study comparison. All these strains clustered in the same branch of the phylogenetic tree, with >95% amino acid sequence similarity in the viral capsid protein (online Technical Appendix Figure 2) and, therefore, should be considered 1 genotype species (*9*). When we compared all these viruses more closely on the amino acid level, we were not able to find any sequence variant that could be specifically associated with a tropism for sheep or cattle (online Technical Appendix Table 3).

We then analyzed brain samples of sheep with nonsuppurative encephalitis of unknown etiology (n = 47), which had been identified within the framework of active disease surveillance in Switzerland (*7*), by RT-PCR specific for BoAstV-CH15 (online Technical Appendix). None reacted positively, implying a low incidence of OvAstV-CH16 infection associated with encephalitis in the sheep population in Switzerland.

To confirm the presence of OvAstV-CH16 in situ, we used polyclonal antisera targeted at the putative capsid protein of BoAstV-CH15 and tested formalin-fixed, paraffin-embedded brain tissues of sheep 41669 by immunohistochemistry (online Technical Appendix). We observed positive staining of neurons, assessed as such on the basis of morphological criteria, in all brain regions examined (e.g., medulla oblongata, cerebellum, thalamus, hippocampus, cortex, caudate nucleus), in particular in some areas (Figure; online Technical Appendix Figure 3). This finding supports a plausible biological association of OvAstV-CH16 infection and encephalitis in the sheep under investigation and underlines again the close relationship between OvAstV-CH16 and BoAstV-CH15. The identification of similar astroviruses in sheep and cattle with comparable diseases, by different methods and in distinct geographic areas, further strengthens these findings. We consider it unlikely that the ovine cases reported in Scotland and Switzerland are epidemiologically related and speculate that the respective viruses were already geographically widely spread but were undetected until recently, which also seems to be the case for neurotropic astroviruses in cattle.

The importance of the link between astroviruses and encephalitis is increasingly being brought to light, but the factors determining their tropism and neuroinvasion are still unknown. Deeper epidemiologic, genetic, and molecular investigations will help to clarify these aspects of astrovirus pathology. Astroviruses were traditionally considered to be host specific, but in recent years, several reports challenged this assumption; for instance, when human astroviruses were found in fecal samples of primates (*10*). In such cases, however, effective infection of atypical hosts was never shown. In this study, we demonstrated the presence of the virus in situ, a finding that strengthens the likelihood of such an infectious event. The fact that a virus of the same genotype was described in cattle with similar pathologic findings also challenges this concept of host specificity and suggests that astroviruses can cross the species barrier and, therefore, represent a zoonotic threat as not only a gastroenteritic agent but also a potential cause of encephalitis.

Acknowledgments

The authors thank Muriel Fragnière and Camille Monney for excellent technical support, Anna Oevermann for neuropathology expertise, and the Next Generation Sequencing Platform of the University of Bern, which is supervised by Cord Drögemüller, for performing the high-throughput sequencing experiments. Thanks also to L. Boujon for proofreading the article.

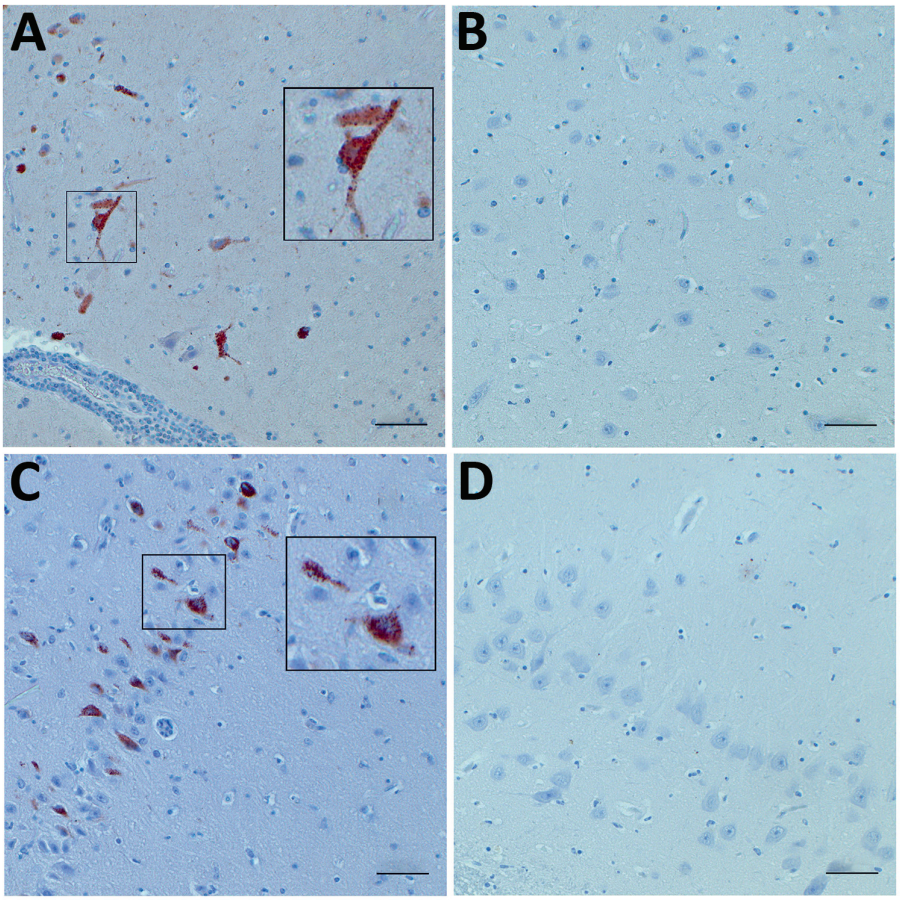

Figure. Immunohistochemistry (IHC) for ovine astrovirus CH16 in brain tissues (hippocampus) of a sheep (ID 41669) with encephalitis using 2 polyclonal antisera targeted at the putative capsid protein of bovine astrovirus CH15. A) IHC using antiserum against the conserved region of the capsid protein showing positive staining (box at left; box at right shows area at higher magnification); B) negative control. C) IHC using antiserum against the variable regions of the capsid protein n showing positive staining (box at left; box at right shows area at higher magnification); D) negative control. Scale bars indicate 50 μm.

This work was funded in part by the Federal Food Safety and Veterinary Office (grant MON-108), by the Swiss National Science Foundation (grant 31003A_163438), and by the Bangerter-Rhyner-Foundation.

Dr. Boujon is a veterinarian and PhD student at the Vetsuisse Faculty in Bern. Her primary research lies in the study of neurotropic astroviruses in cattle.

References

1. Vu DL, Cordey S, Brito F, Kaiser L. Novel human astroviruses: Novel human diseases? J Clin Virol. 2016;82:56–63. http://dx.doi.org/10.1016/j.jcv.2016.07.004
2. Blomström AL, Widén F, Hammer AS, Belák S, Berg M. Detection of a novel astrovirus in brain tissue of mink suffering from shaking mink syndrome by use of viral metagenomics. J Clin Microbiol. 2010;48:4392–6. http://dx.doi.org/10.1128/JCM.01040-10
3. Li L, Diab S, McGraw S, Barr B, Traslavina R, Higgins R, et al. Divergent astrovirus associated with neurologic disease in cattle. Emerg Infect Dis. 2013;19:1385–92. http://dx.doi.org/10.3201/eid1909.130682
4. Bouzalas IG, Wüthrich D, Walland J, Drögemüller C, Zurbriggen A, Vandevelde M, et al. Neurotropic astrovirus in cattle with nonsuppurative encephalitis in Europe. J Clin Microbiol. 2014;52:3318–24. http://dx.doi.org/10.1128/JCM.01195-14
5. Seuberlich T, Wüthrich D, Selimovic-Hamza S, Drögemüller C, Oevermann A, Bruggmann R, et al. Identification of a second encephalitis-associated astrovirus in cattle. Emerg Microbes Infect. 2016;5:e5. http://dx.doi.org/10.1038/emi.2016.5
6. Schlottau K, Schulze C, Bilk S, Hanke D, Höper D, Beer M, et al. Detection of a novel bovine astrovirus in a cow with encephalitis. Transbound Emerg Dis. 2016;63:253–9. http://dx.doi.org/10.1111/tbed.12493
7. Oevermann A, Botteron C, Seuberlich T, Nicolier A, Friess M, Doherr MG, et al. Neuropathological survey of fallen stock: active surveillance reveals high prevalence of encephalitic listeriosis in small ruminants. Vet Microbiol. 2008;130:320–9. http://dx.doi.org/10.1016/j.vetmic.2008.01.015
8. Pfaff F, Schlottau K, Scholes S, Courtenay A, Hoffmann B, Höper D, et al. A novel astrovirus associated with encephalitis and ganglionitis in domestic sheep. Transbound Emerg Dis. 2017;64:677–82. http://dx.doi.org/10.1111/tbed.12623
9. Bosch A, Pintó RM, Guix S. Human astroviruses. Clin Microbiol Rev. 2014;27:1048–74. http://dx.doi.org/10.1128/CMR.00013-14
10. Karlsson EA, Small CT, Freiden P, Feeroz MM, Matsen FA IV, San S, et al. Non-human primates harbor diverse mammalian and avian astroviruses including those associated with human infections. PLoS Pathog. 2015;11:e1005225. http://dx.doi.org/10.1371/journal.ppat.1005225

Address for correspondence: Torsten Seuberlich, NeuroCenter, Division of Neurological Sciences, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109 a, CH-3001 Bern, Switzerland; email: torsten.seuberlich@vetsuisse.unibe.ch