References

1. Ledford H. Autopsy slowdown hinders quest to determine how coronavirus kills. Nature. 2020 May 7. https://doi.org/10.1038/d41586-020-01355-z
2. Fineschi V, Aprile A, Aquila I, Arcangeli M, Asmundo A, Bacci M, et al.; Scientific Society of Hospital Legal Medicine of the National Health System (COMLAS); Italian Society of Anatomical Pathology and Cytology (SIAPEC). Management of the corpse with suspect, probable or confirmed COVID-19 respiratory infection—Italian interim recommendations for personnel potentially exposed to material from corpses, including body fluids, in morgue structures and during autopsy practice. Pathologica. 2020;112:64–77.
3. Kritselis M, Remick DG. Universal precautions provide appropriate protection during autopsies of patients with infectious diseases. Am J Pathol. 2020;190:2180–4. https://doi.org/10.1016/j.ajpath.2020.08.005
4. Plenzig S, Bojkova D, Held H, Berger A, Holz F, Cinatl J, et al. Infectivity of deceased COVID-19 patients. Int J Legal Med. 2021;135:2055–60. https://doi.org/10.1007/s00414-021-02546-7
5. Jurek T, Rorat M, Szleszkowski Ł, Tokarski M, Pielka I, Małodobra-Mazur M. SARS-CoV-2 viral RNA is detected in the bone marrow in post-mortem samples using RT-LAMP. Diagnostics (Basel). 2022;12:515. https://doi.org/10.3390/diagnostics12020515
6. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25:2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045
7. Brandner JM, Boor P, Borcherding L, Edler C, Gerber S, Heinemann A, et al. Contamination of personal protective equipment during COVID-19 autopsies. Virchows Arch. 2022;480:519–28. https://doi.org/10.1007/s00428-021-03263-7

Address for correspondence: Jonas N. Kantonen, University of Helsinki, Haartmaninkatu 3 C 323, 00290 Helsinki, Finland; email: jonas.kantonen@helsinki.fi

Novel Genotypes of Highly Pathogenic Avian Influenza H5N1 Clade 2.3.4.4b Viruses, Germany, November 2023

Ann Kathrin Ahrens, Anne Pohlmann, Christian Grund, Timm Harder, Martin Beer

Author affiliation: Friedrich-Loeffler-Institut, Greifswald–Insel Riems, Germany

DOI: https://doi.org/10.3201/eid3008.240103

Several subtypes and many different genotypes of highly pathogenic avian influenza viruses of subtype H5 clade 2.3.4.4b have repeatedly caused outbreaks in Germany. Four new highly pathogenic avian influenza genotypes emerged in November 2023 after reassortment with low pathogenicity precursors, replacing genotype BB, which had dominated in Europe since 2022.

Germany has experienced repeated outbreaks of highly pathogenic avian influenza (HPAI) viruses (HPAIVs) of clade 2.3.4.4b of the H5 goose/Guangdong lineage since 2016, causing devastating losses to wild bird biodiversity and the poultry production sector (*1*). Since 2016, seasonal outbreaks or cases increased during the winter season and decreased to zero in summer. Seasonality terminated in 2021, when HPAIV H5 became endemic in wild birds in Germany and the rest of Europe (*2*). Along with an increasing incidence, genetic diversity expanded, resulting in a high number of new genotypes (*3*).

During summer 2023, genotype Ger-02-23-N1.1 (BB based on the European Union nomenclature [*4*,*5*]), a reassortment with a gull-derived H13 virus, dominated HPAI cases caused by outbreaks in colony breeders (*6*). Sporadically, older genotypes (Ger-10-21-N1.5 and Ger-12-22-N1.1) were identified, accompanied by some viruses that could not be assigned to a proper genotype because of incomplete genome covering. After the breeding season ended, incidence decreased (84 cases in July, 16 in August, 10 in September, and 3 in October). In addition, increasing numbers of low pathogenicity avian influenza (LPAI) viruses (LPAIVs) were detected during active and passive wild bird monitoring, representing the autumnal, bird migration–related upsurge of avian influenza virus infections in Germany. Since November, the number of HPAIV H5 cases has increased to a still moderate but substantially higher level (29 in November).

We analyzed the genotypes of HPAI and LPAI viruses by using full-genome sequencing. Sequencing

procedures of HPAI and LPAI viruses (*7*) and methods for genotype differentiation including the reference sequences have been described previously (*8*). We analyzed 244 sequences from 33 viruses collected from late May to late November 2023, of which 22 originated from wild birds, 5 from poultry, and 6 from captive birds. We found various LPAIVs and 16 HPAIVs of H5N1 subtype in the resulting sequences (Table).

All HPAIV H5 sequences from viruses collected in November clustered differently from genotype Ger-02-23-N1.1 (BB) that dominated during the summer of 2023. None of those genotypes, including genome segments from viruses that could not be sequenced to completion, were detected after November 2023. Instead, we identified 4 new genotypes. Four viruses grouped with the HPAIV H5N1 genotype Ger-11-23-N1.1 (DB) (reference A/herring gull/Germany-NI/2023AI08764/2023) with a new reassorted polymerase basic 1 (PB1) gene similar to LPAIVs detected in a zoo in Germany (LPAI A/flamingo/Germany-ST/2023AI08233/2023 [H5N2]). One virus (reference A/barnacle goose/Germany-SH/2023AI08822/2023) was associated with genotype Germany Ger-11-23-N1.2 (AB) with a reassorted PB1 gene. Two viruses from Germany clustered with Ger-11-23-N1.3 (DG) (reference A/chicken/Germany-NI/2023AI08838/2023) containing the PB1 gene of Ger-11-23-N1.2 and a new reassorted polymerase basic 2 and polymerase acidic genes, which were also found in LPAIVs from a wild duck in Germany in October 2023 (type strain LPAI A/wild duck/Germany-NW/2023AI07895/2023 [H3N8]) and November 2023 (LPAI A/Eurasian Wigeon/Germany-MV/2023AI08762/2023 [H5N2]). Two sequences formed a new reassortant for Germany, Ger-11-23-N1.4 (DA) (reference A/common crane/Germany-HH/2023AI08835/2023) with new PB1, polymerase basic 2, and nonstructural gene segments (Appendix Figures 1, 2, https://wwwnc.cdc.gov/EID/article/30/8/24-0103-App1.pdf).

Table. Number of HPAI and LPAI virus subtypes identified from 244 sequences from 33 viruses collected in Germany, late May to late November 2023*

Subtype	Wild birds	Poultry	Captive birds
HPAI H5N1†	12	4	
LPAI H11N9			1
LPAI H2N3	1		
LPAI H3N8	3		
LPAI H4N6			2
LPAI H5N2†	1		2
LPAI H5N3	1		
LPAI H5N4		1	
LPAI H6N4	1		
LPAI H9N2	3		1

*HPAI, highly pathogenic avian influenza; LPAI, low pathogenicity avian influenza.

In conclusion, our study shows a high number of emerging new HPAIV H5 clade 2.3.4.4b genotypes in November 2023 and identified related LPAIVs circulating at the same time in the same area, which may have served as reassortment partners. These findings highlight the continued promiscuity of currently circulating HPAI H5 strains of clade 2.3.4.4b and the need for genotypic surveillance of both HPAIVs and LPAIVs.

This work was funded in part by the German Federal Ministry of Education and Research within the project PREPMEDVET (grant no. 13N15449), by the European Union under the Horizon 2020 Programme (grant agreement VEO no. 874735) and under the Horizon Europe Programme (grant agreement KAPPA-FLU no. 101084171).

All sequences from this study are available in the public GISAID (https://www.gisaid.org) EpiFlu database (accession nos. 18432788–9, 18432793, 18435608–12, 18458714–6, 18458718, 18458720, 18458722, 18463193–4, 18526630–5, 18745067–74, 18745076–7, and 18745086).

The authors declare no conflicts of interest in this work.

About the Author

Dr. Ahrens is a junior scientist at the Institute of Diagnostic Virology at the German Federal Institute for Animal Health (Friedrich-Loeffler-Institut). Her research interests include avian influenza viruses, from virus isolation up to real-time sequencing.

References

1. King J, Harder T, Conraths FJ, Beer M, Pohlmann A. The genetics of highly pathogenic avian influenza viruses of subtype H5 in Germany, 2006–2020. Transbound Emerg Dis. 2020.
2. Pohlmann A, King J, Fusaro A, Zecchin B, Banyard AC, Brown IH, et al. Has epizootic become enzootic? Evidence for a fundamental change in the infection dynamics of highly pathogenic avian influenza in Europe, 2021. MBio. 2022;13:e0060922. https://doi.org/10.1128/mbio.00609-22
3. Kandeil A, Patton C, Jones JC, Jeevan T, Harrington WN, Trifkovic S, et al. Rapid evolution of A(H5N1) influenza viruses after intercontinental spread to North America. Nat Commun. 2023;14:3082. https://doi.org/10.1038/s41467-023-38415-7
4. European Food Safety Authority, European Centre for Disease Prevention and Control, European Reference Laboratory for Avian Influenza, Adlhoch C, Fusaro A, Gonzales JL, et al. Avian influenza overview March–April 2023. EFSA J. 2023;21:8039. https://doi.org/10.2903/j.efsa.2023.8039
5. European Food Safety Authority, European Centre for Disease Prevention and Control, European Reference Laboratory for Avian Influenza, Adlhoch C, Fusaro A, Gonzales JL, et al. Avian influenza overview September–December 2023. EFSA J. 2023;21:8539. https://doi.org/10.2903/j.efsa.2023.8539

6. Pohlmann A. HPAIV genotypes in Germany. 2022 Sep 30 [cited 2024 May 21]. https://zenodo.org/records/7078789
7. King J, Harder T, Beer M, Pohlmann A. Rapid multiplex MinION nanopore sequencing workflow for influenza A viruses. BMC Infect Dis. 2020;20:648. https://doi.org/10.1186/s12879-020-05367-y
8. Pohlmann A, Harder T. Genotype differentiation of highly pathogenic avian influenza viruses (HPAIV) of the goose/Guangdong lineage in Germany—derivation and deployment of reference sequences. 2023 Aug 10 [cited 2021 May 21]. https://zenodo.org/records/8233815

Address for correspondence: Martin Beer, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald–Insel Riems, Germany; email: martin.beer@fli.de

COMMENT LETTER

Transmission and Surveillance of Rat Hepatitis E Virus in Swine

Matheus Filgueira Bezerra, Maria Gabriela Oliveira da Paz, Edmilson Ferreira de Oliveira-Filho, Christian Robson de Souza Reis

Author affiliations: Oswaldo Cruz Foundation, Recife, Pernambuco, Brazil (M.F. Bezerra, M.G.O. da Paz, C.R.D.S. Reis); Humboldt-Universität zu Berlin, Berlin, Germany (E.F. de Oliveira-Filho).

DOI: https://doi.org/10.3201/eid3008.240484

To the Editor: The study by Rios-Muñoz et al. reporting rat hepatitis E virus (HEV) RNA in swine feces contains intriguing findings with the potential to change our understanding of rat HEV transmission routes (*1*). One relevant aspect highlighted by the authors is that limited prior evidence of rat HEV infection in swine might be partially explained by the lack of rat HEV serology tests for pigs. The low genomic homology between rat HEV and other HEV (<60%) makes most of the commercially available HEV-based tests ineffective in detecting rat HEV (*2*,*3*).

Such findings are exciting but must be interpreted with caution because some gaps remain to be addressed. Pigs can feed on small mammal remains and even prey on rodents, which means detecting rat HEV RNA in pig feces does not conclusively indicate an infection. To rule out the possibility of viral detection because of contaminated food, it is necessary to detect the virus in other tissues, such as blood or liver (*3*). Of note, a substantial proportion of the positive samples in the study by Rios-Muñoz et al. exhibited high cycle threshold values, which might be suggestive of residual viral RNA. Furthermore, considering the hypothesis that both viruses could be transmitted through contaminated swine meat, it remains unclear why rat HEV infection in humans is uncommon when compared with other HEV.

Nevertheless, from our perspective, these findings suggest the possibility of approaching swine as a sentinel species. If results are confirmed in additional eco-epidemiologic studies, sampling swine stools could offer valuable public health information. Insights from other rodentborne diseases, such as bubonic plague, underscore the benefits of a surveillance strategy focused on sentinel species rather than primary hosts (*4*). Sampling rodents is logistically more challenging and expensive than for domestic animals, but surveillance of sentinel species is typically more efficient in predicting the dissemination of zoonotic diseases at early stages.

About the Author

Dr. Bezerra currently works a technologist at the Plague National Reference Laboratory, Aggeu Magalhães Institute, Recife, Brazil. His research interests include bubonic plague, epidemiology, laboratory diagnosis of zoonotic diseases, and genomic surveillance.

References

1. Rios-Muñoz L, Gonzálvez M, Caballero-Gomez J, Castro-Scholten S, Casares-Jimenez M, Agulló-Ros I, et al. Detection of rat hepatitis E virus in pigs, Spain, 2023. Emerg Infect Dis. 2024;30:823–6. https://doi.org/10.3201/eid3004.231629
2. Chen Z, Li G, Situ J, Li Z, Guo S, Huang Y, et al. Redeveloping antigen detection kits for the diagnosis of rat hepatitis E virus. J Clin Microbiol. 2023;61:e0071023. https://doi.org/10.1128/jcm.00710-23
3. Johne R, Plenge-Bönig A, Hess M, Ulrich RG, Reetz J, Schielke A. Detection of a novel hepatitis E-like virus in faeces of wild rats using a nested broad-spectrum RT-PCR. J Gen Virol. 2010;91:750–8. https://doi.org/10.1099/vir.0.016584-0
4. Rajerison M, Andrianaivoarimanana V, Ratsitorahina M, Rahelinirina S, Chanteau S, Telfer S, et al. Field assessment of dog as sentinel animal for plague in endemic foci of Madagascar. Integr Zool. 2021;16:886–92. https://doi.org/10.1111/1749-4877.12541

Address for correspondence: Matheus Bezerra, Oswaldo Cruz Foundation, Avenida Professor Moraes Rego, s/n, Recife/PE 50740-465, Brazil; email: matheus.bezerra@fiocruz.br