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

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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.

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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

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## COMMENT LETTER

### Transmission and Surveillance of Rat Hepatitis E Virus in Swine

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**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.

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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.

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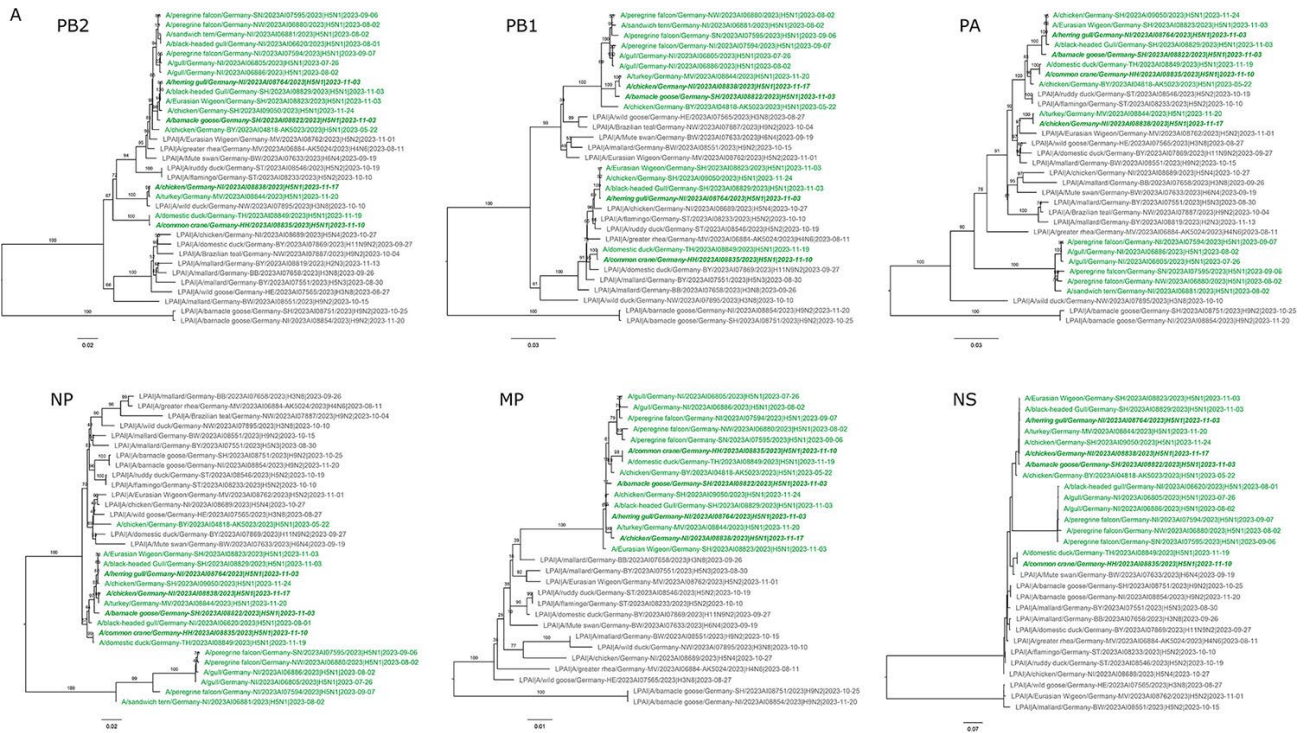
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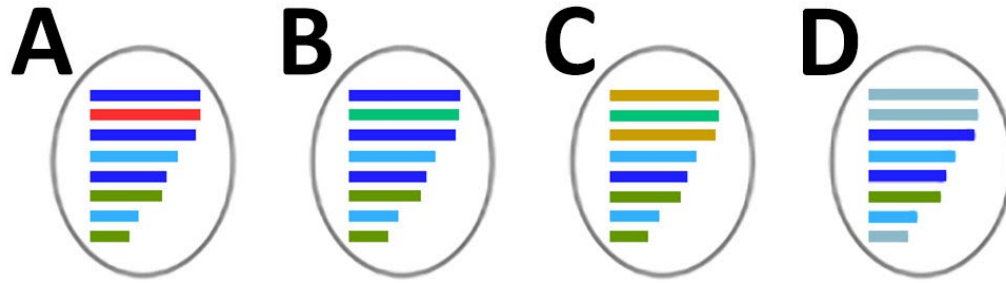
# Novel Genotypes of Highly Pathogenic Avian Influenza H5N1 Clade 2.3.4.4b Viruses, Germany, November 2023

## Appendix



**Appendix Figure 1.** Phylogeny of genome segments coding for polymerase (PB2, PB1, PA), nucleoprotein (NP), matrix protein (MP), and nonstructural (NS) bearing segments of highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) viruses collected late May to late November 2023 in Germany. Sequences from HPAI viruses are highlighted in green and reference strains for the new genotypes are shown in bold italics. Sequences of older HPAIV genotypes identified in summer are included for comparison.





**Appendix Figure 2.** Overview of segment composition of the newly detected genotypes identified in November 2023 with the respective reference strains. A) Ger-11-23-N1.1 (DB) A herring gull/Germany-NI/2023AI08764/2023. B) Ger-11-23-N1.2 (AB) A barnacle goose/Germany-SH/2023AI08822/2023. C) Ger-11-23-N1.3 (DG) A/chicken/Germany-NI/2023AI08838/2023. D) Ger-11-23-N1.4 (DA) A/common crane/Germany-HH/2023AI08835/2023.