indicate the risk of its occurrence in these regions, where *H. longicornis* is widely distributed (*10*). More extensive investigation to clarifty the natural circulation of NSDV among ticks should be conducted and surveillance of sheep improved to prevent outbreaks of Nairobi sheep disease in China and East Asia.

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# Avian Influenza A(H10N7) Virus–Associated Mass Deaths among Harbor Seals

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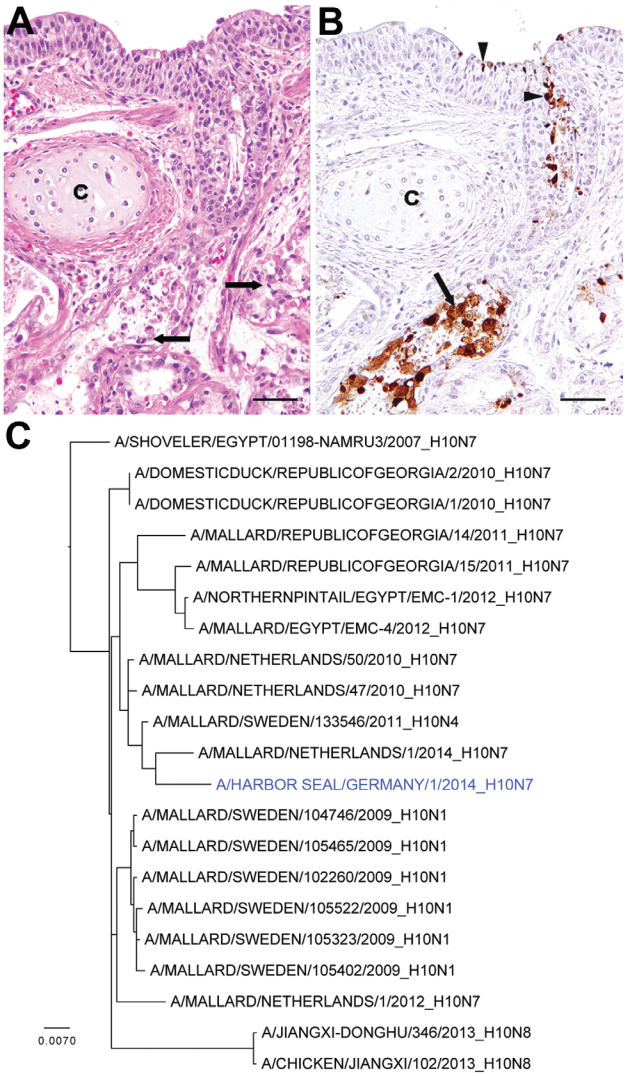
**To the Editor:** Avian influenza A viruses occasionally cross the species barrier; influenza A(H5N1) virus and the recently emerged influenza A(H7N9) virus are prime examples of bird-to-human transmission (*1*,*2*). In addition, avian influenza A viruses can cross to various other mammalian species, including pinnipeds (e.g., seals) (*3*,*4*).

Recently, mass deaths have occurred among harbor seals (*Phoca vitulina*); hundreds of carcasses washed up the shores of Sweden (March 2014), Denmark (July 2014), and Germany (October 2014). Approximately 1,400 dead harbor seals were seen in the coastal waters of Schleswig-Holstein in Germany alone, where the population is ≈12,000 animals.

We report the investigation of the deaths of 17 seals from different age groups that were found dead on the islands of Helgoland and Sylt, Germany, during the second week of October 2014. Complete necropsies were performed on the carcasses, which were in variable nutritional conditions, ranging from very poor to good. Necropsy results showed consistently poorly retracted lungs with severe congestion, occasional diffuse consolidation, and multifocal firm nodular areas of gray-yellow discoloration with varying numbers of metazoic parasites. Histologic examinations showed acute necrotizing bronchitis and adenitis of bronchial glands with sloughing of epithelial cells (Figure, panel A). Occasionally, mild interstitial pneumonia was found. Multifocal pyogranulomatous to necrotizing pneumonia was associated with parasite infestation. A few animals had suppurative to necrotizing or nonsuppurative rhinitis and tracheitis.

Because mass deaths among seals were caused by phocine distemper virus in the same area in 1988 and 2002,

we tested lung and throat swab samples for morbillivirus using reverse transcription PCR (RT-PCR) and immunohistochemical analysis (*5*). In addition, real-time RT-PCR targeting the influenza A virus matrix gene was performed (*6*). No indications for the morbillivirus were detected by RT-PCR and immunohistochemistry; however, in lung lesions and throat swab samples of 11 animals, a positive signal was observed by the influenza A matrix gene real-time RT-PCR (cycle threshold values 15.0–33.9). Influenza A virus (A/harbor seal/Germany/1/2014) was subsequently isolated from lung and throat swab samples; the virus replicated to high titers in 11-day-old embryonated chicken eggs and on MDCK cells. By PCR using specific primers and subsequent Sanger sequencing of the hemagglutinin and neuraminidase genes, this virus was characterized as an influenza A virus of the H10N7 subtype, commonly found in migratory waterfowl (*6*). In addition, genetic analyses of all other gene segments indicated that the influenza virus A/harbor seal/Germany/1/2014 was most closely related to various influenza A viruses detected in wild birds. Specifically, the hemagglutinin and neuraminidase genes were genetically most closely related to subtype H10N7 viruses recently found in migratory ducks in Georgia, Egypt, and the Netherlands (Figure, panel C) (*7*). Genetic analyses were based on BLAST analyses using public databases available as of October 17, 2014 (http://www.ncbi.nlm.nih.gov, http://www.gisaid.com) and supplemented with H10 and N7 sequences from the international wild bird surveillance program of Erasmus Medical Center (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/4/14-1675-Techapp1.pdf). A maximum-likelihood phylogenetic tree of the hemagglutinin gene was generated by using PhyML version 3.1 (*8*) with the general time reversible +I+G model of nucleotide substitution; a full heuristic search and subtree pruning and regrafting searches were performed. The tree was visualized by using Figtree version 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree).




Histopathologic and phylogenetic analyses of necropsy samples from harbor seals infected with avian influenza A(H10N7) virus, Germany, 2014. A) Lung of harbor seal showing marked necrosis and sloughing of epithelial cells in bronchial glands (arrows); c = bronchial cartilage; hematoxylin and eosin stain. Scale bar indicates 50 μm B) Immunohistochemical labeling of influenza A nucleoprotein in bronchial epithelial cells (arrowheads) and glandular epithelial cells (arrows); c = bronchial cartilage; avidin-biotin-peroxidase complex method. Scale bar indicates 50 μm. C) Maximum-likelihood phylogenetic tree of the partial hemagglutinin gene (1,577 nt) of the influenza A/harbor seal/Germany/1/2014 (H10N7) isolate and various other closely related viruses. GenBank accession numbers are provided in online Technical Appendix Table 1 (http://wwwnc.cdc.gov/EID/article/21/4/14-1675-Techapp1.pdf). Scale bar indicates nucleotide substitutions per site.

To further elucidate the role of influenza A(H10N7) virus in the pathogenesis of the disease causing deaths among the seals, we conducted immunohistochemical analysis on the lungs using an influenza A virus nucleoprotein-specific monoclonal antibody (*9*). Evaluation of the lung tissues of the dead seals showed viral antigen in cytoplasm and nuclei of epithelial cells of bronchi and bronchial glands of affected lung areas (Figure, panel B), which suggests that this virus played a major role in the deaths. Immunohistochemical analysis performed on various organs (including brain and olfactory bulb) indicated that viral antigen was restricted to the respiratory tract.

Although avian influenza A virus infections previously have caused mass deaths in seals (*3*,*4*,*10*), subtype H10N7 has not been associated with such events. We can speculate that the ongoing deaths could eventually affect all harbor seal populations of northwestern Europe and have consequences for wildlife management and seal rehabilitation activities. In addition, preliminary analysis of the hemagglutinin sequence of the influenza A(H10N7) virus suggests the presence of molecular determinants that indicate

mammalian adaptation. Various analyses are ongoing to answer questions about the route of transmission among seals and possible transmissibility to humans.

Note added in proof: Zohari et al. also recently reported the involvement of avian influenza A(H10N7) virus in mass deaths of harbor seals in Sweden (Euro Surveill. 2014;19:pii: 20967).

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# Zika Virus Infection, Philippines, 2012

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**To the Editor:** Zika virus (ZIKV), a mosquitoborne flavivirus, was first isolated from a rhesus monkey in Uganda in 1947 (*1*). This positive-sense, single-stranded RNA virus (family *Flaviviridae*, genus *Flavivirus*) has a 10,794-nt genome and is most closely related to Spondweni virus (*2*,*3*). Phylogenetic analyses have revealed 2 major lineages: Asian and African (*2*–*4*).

The first human infection with ZIKV was reported in Nigeria in 1954 (*5*). The virus caused only sporadic infections until 2007, when a large outbreak occurred on Yap, an island in the Federated States of Micronesia (*6*). In October 2013, ZIKV was detected in French Polynesia; since then, >400 laboratory-confirmed cases have been reported (*7*). ZIKV has spread across the South Pacific, and autochthonous cases have been reported in New Caledonia, Easter Island, and the Cook Islands. Several cases of ZIKV infections have been reported in travelers to Southeast Asia (*4*,*8*) and French Polynesia (*3*,*7*).

In March 2012, a prospective longitudinal cohort study, which included active surveillance for acute febrile illness, was initiated in Cebu City, Philippines (I. Yoon, unpub. data). Participants contacted study staff to report fever and were also contacted weekly by staff to determine if they had fever during the previous 7 days. Fever episodes triggered an acute-illness visit by a study nurse, who performed a clinical assessment of the patient and collected an acute-phase blood sample. During the first year of surveillance, 270 acute febrile

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## Technical Appendix

Technical Appendix Table. Details of hemagglutinin sequences shown in the Figure*

| Isolate name | Accession no. | Online database |
|---|---|---|
| A/seal/Germany/EMC-1/2014(H10N7) | EPI544351 | GISAID EpiFlu |
| A/mallard/Netherlands/1/2014(H10N7) | EPI552751 | GISAID EpiFlu |
| A/mallard/Sweden/133546/2011(H10N4) | CY183991.1 | GenBank |
| A/domestic duck/RepublicofGeorgia/2/2010(H10N7) | CY185457.1 | GenBank |
| A/domestic duck/RepublicofGeorgia/1/2010(H10(N7) | CY185449.1 | GenBank |
| A/mallard/Sweden/104746/2009(H10N1) | CY183855.1 | GenBank |
| A/mallard/Sweden/105465/2009(H10N1) | CY183927.1 | GenBank |
| A/mallard/Sweden/102260/2009(H10N1) | CY183847.1 | GenBank |
| A/mallard/Sweden/105323/2009(H10N1) | CY183887.1 | GenBank |
| A/mallard/Sweden/105402/2009(H10N1) | CY183911.1 | GenBank |
| A/mallard/Sweden/105522/2009(H10N1) | JX566079.1 | GenBank |
| A/mallard/Netherlands/50/2010(H10N7) | EPI552752 | GISAID EpiFlu |
| A/mallard/Netherlands/47/2010(H10N7) | EPI552753 | GISAID EpiFlu |
| A/mallard/Republic of Georgia/14/2011(H10N7) | CY185689.1 | GenBank |
| A/mallard/Republic of Georgia/15/2011(H10N7) | CY185385.1 | GenBank |
| A/northern pintail/Egypt/EMC-1/2012(H10N7) | EPI552754 | GISAID EpiFlu |
| A/mallard/Egypt/EMC-4/2012(H10N7) | EPI552755 | GISAID EpiFlu |
| A/mallard/Netherlands/1/2012(H10N7) | EPI552756 | GISAID EpiFlu |
| A/shoveler/Egypt/01198-NAMRU3/2007(H10N7) | EPI372402† | GISAID EpiFlu |
| A/Jiangxi-Donghu/346/2013(H10N8) | EPI497477‡ | GISAID EpiFlu |
| A/chicken/Jiangxi/102/2013(H10N8) | EPI530542§ | GISAID EpiFlu |

*We gratefully acknowledge the authors, originating and submitting laboratories of the sequences from the Global Initiative on Sharing Avian Influenza Data (GISAID) EpiFluTM database on which this research is based. All submitters of data may be contacted directly through the GISAID website.
†Originating laboratory: US Naval Medical Research Unit No.3, Egypt; Submitting laboratory: Centers for Disease Control and Prevention, Atlanta, GA, USA (*1*).
‡Originating and submitting laboratory: World Health Organization Chinese National Influenza Center, Beijing, China (*2*).
§Originating laboratory: South China Agricultural University, Guangzhou, China; Submitting laboratory: South China Agricultural University, Guangzhou, China (*3*).

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