in China. Although reassortment was not detected in this co-infection, a potential risk for emergence of a new pandemic strain by reassortment between these 2 viruses (with humans as mixing vessels) should not be ignored. To reduce the risk for emergence of new viral subtypes, the public health and scientific communities should enhance surveillance for co-infection with influenza (H7N9) virus and other influenza virus subtypes.

References

1. Zhu Y, Qi X, Cui L, Zhou M, Wang H. Human co-infection with novel avian influenza A H7N9 and influenza A H3N2 viruses in Jiangsu Province, China. Lancet. 2013;381:2134. http://dx.doi.org/10.1016/S0140-6736(13)61135-6
2. Li J, Kou Y, Yu X, Sun Y, Zhou Y, Pu X, et al. Human co-infection with avian influenza and seasonal influenza viruses, China. Emerg Infect Dis. 2014;20:1953–5. http://dx.doi.org/10.3201/eid2011.140897
3. Ramakrishnan MA, Tu Z, Singh S, Chockalingam A, Gramer M, Wang P, et al. The feasibility of using high resolution genome sequencing of influenza A viruses to detect mixed infections and quasispecies. PLoS ONE. 2009;4:e7105. http://dx.doi.org/10.1371/journal.pone.0007105
4. Chan KH, To K, Hung I, Zhang A, Chan J, Cheng V, et al. Differences in antibody responses of individuals with natural infection and those vaccinated against pandemic H1N1 2009 influenza. Clin Vaccine Immunol. 2011;18:867–73. http://dx.doi.org/10.1128/CVI.00555-10
5. Dong L, Bo H, Bai T, Gao R, Dong J, Zhang Y, et al. Combination of serological assays to detect human antibodies to the avian influenza A H7N9 virus. PLoS ONE. 2014;9:e95612. http://dx.doi.org/10.1371/journal.pone.0095612
6. Zhang W, He Y, Xu L, Dai F, Mei Z, Qian L, et al. Full-genome analysis of influenza A(H7N9) virus from Shanghai, China, 2014. Genome Announc. 2014;2:e00578–14. http://dx.doi.org/10.1128/genomeA.00578-14

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Nairobi Sheep Disease Virus RNA in Ixodid Ticks, China, 2013

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To the Editor: Nairobi sheep disease virus (NSDV; genus *Nairovirus*, family *Bunyaviridae*) causes acute hemorrhagic gastroenteritis in sheep and goats (*1,2*). First identified in Nairobi, Kenya, in 1910, it is considered endemic in East Africa (*1,3*). Ganjam virus, a variant of NSDV, is found in India and Sri Lanka (*2*). NSDV has a limited effect on animals bred in areas to which the virus is endemic but can cause large losses of animals during introduction of new livestock or transport of animals through these areas (*4*). In humans, NSDV infection can cause febrile illness, headache, nausea, and vomiting (*5*).

Ticks are the main transmission vectors of NSDV and many other viral pathogens and therefore pose a major threat to public health (*6,7*). Here, we describe a newly discovered NSDV, named NSDV (China), identified by viral metagenomic analysis of ticks collected from the northeast region of the People's Republic of China (Liaoning, Jilin, and Heilongjiang provinces) during May–July, 2013, and divided into 9 groups according to tick species and sampling sites. Four tick species were morphologically identified: *Haemaphysalis longicornis* (84.8%); *Dermacentor silvarum* (7.2%); *D. nuttalli* (5.5%); and *Ixodes persulcatus* (2.5%) (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/4/14-1602-Techapp1.pdf).

Of the 6,427 ticks collected, 3,410 were divided into 9 groups (average 379 ticks/group, range 163–512); each group was homogenized in SM buffer (50 mmol/L Tris, 10 mmol/L $MgSO_4$, 0.1 mol/L NaCl, pH 7.5). Viral RNA extraction, Solexa sequencing, and analysis are described in the online Technical Appendix. Among the sequences annotated to mammalian viruses, 65 contigs were found to target the small (n = 15), medium (n = 27), and large (n = 23) segments of the NSDV genome (online Technical Appendix Tables 2–4).

[1]These authors contributed equally to this article.

To confirm the Solexa results, a 376-nt fragment of the NSDV small gene segment was amplified by reverse transcription PCR (RT-PCR) by using primers P1 (5′-AG-CAAAGAGCACATTGACTGGGC-3′) and P2 (5′-CTGT-CACACCTGCCTTCCAA-3′). Ticks in 3 *H. longicornis* groups were positive for NSDV: group 1 from sheep in Jian, Jilin Province (125°34′E, 40°52′N); group 2 from cattle in Jinxing, Jilin Province (130°38′E, 42°25′N); and group 5 from sheep in Dandong, Liaoning Province (124°23′E, 40°07′N). Ticks in the other groups were negative. The obtained sequences shared 92% identity with NSDV from *H. intermedia* in India.

The full-length sequence of NSDV was then obtained from group 2 by RT-PCR by using primers based on the Solexa sequences or the conserved sequences of nairoviruses (online Technical Appendix Table 5). The complete sequences of the small, medium, and large segments of NSDV (China) (GenBank accession nos. KM464724–KM464726) contained 1,590, 5,077, and 12,081 nt, respectively; that is, they were similar to other NSDVs. Sequence comparisons showed 75.1%–89.6% identity with other NSDVs at the nucleotide level and 81.3%–96.7% at the deduced amino acid level (online Technical Appendix Table 6). Compared with other member species within the genus *Nairovirus* (Dugbe, Kupe, Hazara, and Crimean Congo hemorrhagic fever viruses), low identities (37.5%–68.6%) were observed at both nucleotide and amino acid levels (online Technical Appendix Table 6). Phylogenetic analysis based on the amino acid sequences grouped the virus together with NSDVs from Africa and South Asia (Figure).

The remaining tick samples of the NSDV-positive groups were used to determine the infection frequency by using RT-PCR to analyze primers P1 and P2. We assayed 104 tick pools (average 15 ticks/pool, range 8–40), 13 pools of 416 ticks in Jian Province and 91 pools of 1,095 ticks in Jinxing Province; 12.5% (13/104) tested positive, 38.5% (5/13) in Jian and 8.8% (8/91) in Jinxing. The higher prevalence in Jian Province may result from more ticks in the pools. Attempts to isolate virus from the positive samples in cell lines (Vero and BHK-21) and suckling mice were unsuccessful; thus, its pathogenicity could not be determined.

In Africa, NSDV is primarily transmitted by *R. appendiculatus* ticks (*5*). In South Asia (India and Sri Lanka), NSDV has been isolated from ticks (*H. intermedia, H. wellingtoni,* and *R. haemaphysaloides*), mosquitoes, sheep and humans; *H. intermedia* ticks are considered the main vector for the virus (*5,8,9*). NSDV had not previously been reported from East Asia. The isolate we identified, NSDV (China), is genetically divergent from the NSDVs of South Asia and Africa and is therefore a novel strain, with *H. longicornis* likely the main vector. Nairobi sheep disease has not been reported in China and East Asia, but our results
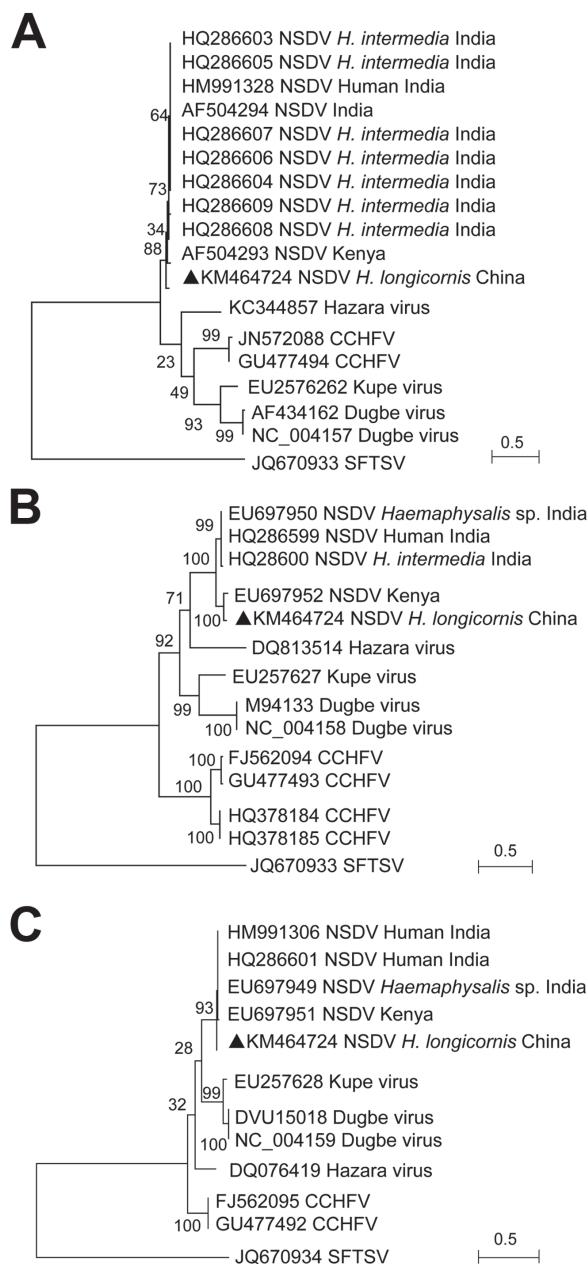
Figure. Phylogenetic analysis of Nairobi sheep disease virus (China) and other nairoviruses. The phylogenetic trees were generated in MEGA5.2 software (http://www.megasoftware.net). The complete coding regions for nucleocapsid protein in the small segment (A), glycoprotein precursor in the medium segment (B), and RNA dependent RNA polymerase in the large segment (C) were analyzed by the maximum-likelihood method. An emergent severe fever thrombocytopenia syndrome virus (SFTSV; family *Bunyaviridae,* genus *Phlebovirus*) was used as the outgroup. Bootstrap testing (1,000 replicates) was performed, and the bootstrap values are indicated. Sequences are identified by their GenBank accession numbers, followed by the virus name, host, and country. Black triangles indicate novel strain NSDV (China). Scale bars indicate substitutions per site. CCHFV, Crimean-Congo hemorrhagic fever virus.

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.

References

1. Montgomery E. On a tick-borne gastro-enteritis of sheep and goats occurring in East Africa. J Comp Pathol Ther. 1917;30:28–57. http://dx.doi.org/10.1016/S0368-1742(17)80002-3
2. Marczinke BI, Nichol ST. Nairobi sheep disease virus, an important tick-borne pathogen of sheep and goats in Africa, is also present in Asia. Virology. 2002;303:146–51. http://dx.doi.org/10.1006/viro.2002.1514
3. Weinbren MP, Gourlay RN, Lumsden WHR, Weinbren WM. An epizootic of Nairobi sheep disease in Uganda. J Comp Pathol Ther. 1958;68:174–87. http://dx.doi.org/10.1016/S0368-1742(58)80018-1
4. Lasecka L, Baron MD. The nairovirus Nairobi sheep disease virus/Ganjam virus induces the translocation of protein disulphide isomerase-like oxidoreductases from the endoplasmic reticulum to the cell surface and the extracellular space. PLoS ONE. 2014;9:e94656. http://dx.doi.org/10.1371/journal.pone.0094656
5. Yadav PD, Vincent MJ, Khristova M, Kale C, Nichol ST, Mishra AC, et al. Genomic analysis reveals Nairobi sheep disease virus to be highly diverse and present in both Africa, and in India in the form of the Ganjam virus variant. Infect Genet Evol. 2011;11:1111–20. http://dx.doi.org/10.1016/j.meegid.2011.04.001
6. Perera LP, Peiris JSM, Weilgama DJ, Calisher CH, Shope RE. Nairobi sheep disease virus isolated from *Haemaphysalis intermedia* ticks collected in Sri Lanka. Ann Trop Med Parasitol. 1996;90:91–3.
7. Liu Q, He B, Huang SY, Wei F, Zhu XQ. Severe fever with thrombocytopenia syndrome, an emerging tick-borne zoonosis. Lancet Infect Dis. 2014;14:763–72. http://dx.doi.org/10.1016/S1473-3099(14)70718-2
8. Rajagopalan PK, Sreenivasan MA, Paul SD. Isolation of Ganjam virus from the bird tick *Haemaphysalis wellingtoni* Nuttall and Warburton 1907. Indian J Med Res. 1970;58:1195–6.
9. Joshi MV, Geevarghese G, Joshi GD, Ghodke YS, Mourya DT, Mishra AC. Isolation of Ganjam virus from ticks collected of domestic animals around Pune, Maharashtra, India. J Med Entomol. 2005;42:204–6. http://dx.doi.org/10.1093/jmedent/42.2.204
10. Hoogstraal H, Roberts FH, Kohls GM, Tipton VJ. Review of *Haemaphysalis* (Kaiseriana) *longicornis* Neumann (resurrected) of Australia, New Zealand, New Caledonia, Fiji, Japan, Korea, and Northeastern China and USSR, and its parthenogenetic and bisexual populations (Ixodoidea, Ixodidae). J Parasitol. 1968; 54:1197–213. http://dx.doi.org/10.2307/3276992

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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,