autoimmune diseases: an update. BMC Med. 2013;11:88 http://dx.doi.org/10.1186/1741-7015-11-88.
6. Casulo C, Maragulia J, Zelenetz AD. Incidence of hypogammaglobulinemia in patients receiving Rituximab and the use of intravenous immunoglobulin for recurrent infections. Clin Lymphoma Myeloma Leuk. 2013;13:106–11. http://dx.doi.org/10.1016/j.clml.2012.11.011
7. Goede V, Fischer K, Busch R, Engelke A, Eichhorst B, Wendtner CM, et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. N Engl J Med. 2014. [Epub ahead of print]. http://dx.doi.org/10.1056/NEJMoa1313984
8. Wildenbeest JG, van den Broek PJ, Benschop KS, Koen G, Wierenga PC, Vossen AC, et al. Pleconaril revisited: clinical course of chronic enteroviral meningoencephalitis after treatment correlates with in vitro susceptibility. Antivir Ther. 2012;17:459–66. http://dx.doi.org/10.3851/IMP1936
9. Abzug MJ. The Enteroviruses: problems in need of treatments. J Infect. 2014;68:S108–14. http://dx.doi.org/10.1016/j.jinf.2013.09. 020
10. Hincks JR, Collet MS. Safety and pharmacokinetics of pocapavir, an oral antiviral candidate against poliovirus. In: Abstracts of the 53th Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, Colorado, Sep 10–13, 2013. Washington (DC): American Society for Microbiology; 2013. Abstract no. A-017d.

Address for correspondence: Johan Maertens, Department of Hematology, University Hospitals Leuven, Campus Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium; email: johan.maertens@uzleuven.be

Serologic Evidence of Influenza A(H1N1) pdm09 Virus Infection in Northern Sea Otters

To the Editor: Sporadic epizootics of pneumonia among marine mammals have been associated with multiple animal-origin influenza A virus subtypes (*1–6*); seals are the only known nonhuman host for influenza B viruses (*7*). Recently, we reported serologic evidence of influenza A virus infection in free-ranging northern sea otters (*Enhydra lutris kenyoni*) captured off the coast of Washington, USA, in August 2011 (*8*). To investigate further which influenza A virus subtype infected these otters, we tested serum samples from these otters by ELISA for antibody-binding activity against 12 recombinant hemagglutinins (rHAs) from 7 influenza A hemagglutinin (HA) subtypes and 2 lineages of influenza B virus (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/article/20/5/13-1890-Techapp1.pdf). Estimated ages for the otters were 2–19 years (online Technical Appendix Table 2); we also tested archived serum samples from sea otters of similar ages collected from a study conducted during 2001–2002 along the Washington coast (*9*).

Of the 30 sea otter serum samples collected during 2011, a total of 21 (70%) had detectable IgG (≥200) for rHA of influenza A(H1N1)pdm09 virus (pH1N1) strain A/Texas/05/2009. Four of 7 serum samples that showed IgG ≥6,400 against pH1N1 rHA also showed low cross-reactivity (IgG 200) against rHA of A/Brisbane/59/2007, a previous seasonal influenza A(H1N1) virus (Figure, panel A; online Technical Appendix Table 1). No IgG was detected in any samples for any of the other 11 rHAs tested (IgG ≤100), and the sea otter serum samples collected during 2001–2002 did not react with any of the rHAs tested, including pH1N1 (IgG ≤100; Figure, panel A).

Next, we tested serum samples by using a hemagglutination inhibition (HI) assay with whole influenza virus to detect strain-specific antibodies that inhibit receptor binding. Of the 30 samples collected during 2011, a total of 22 (73%) showed HI antibody titers of ≥40 against pH1N1 virus. Titers against all other human and avian viruses tested were ≤10 for all samples by HI assay using turkey red blood cells (RBCs) (Figure, panel B; online Technical Appendix Table 3). No influenza A or B virus–specific HI antibodies were detected in the samples collected during 2001–2002 (data not shown). Although nasal swab specimens were collected from sea otters in the 2011 study, all specimens were negative for influenza virus by testing in embryonated eggs and by real-time PCR for detection of influenza A viral RNA (data not shown). These results suggest that sea otters were infected with influenza A virus sometime before the August 2011 sample collection date.

Although none of the 2011 samples showed HI titers to influenza A/duck/New York/96 (H1N1) virus (dk/NY/96) by testing using turkey RBCs (online Technical Appendix Table 2), titers against this strain were detected when using horse RBCs, which is a more sensitive means for the detection of mammalian antibodies against some avian influenza subtypes (*10*). Of the 22 samples that had HI titers ≥40 to pH1N1 virus, 16 also had HI titers ≥40 against dk/NY/96 by horse RBC HI assay (online Technical Appendix Table 2). However, titers against this strain were on average ≈4–8-fold lower than those for the pH1N1 virus strain, which suggests that that the titers against dk/NY/96 were the result of serologic cross-reactivity with avian- and swine-origin pH1N1 viruses.

To further test for cross-reactivity, 4 sea otter serum samples were

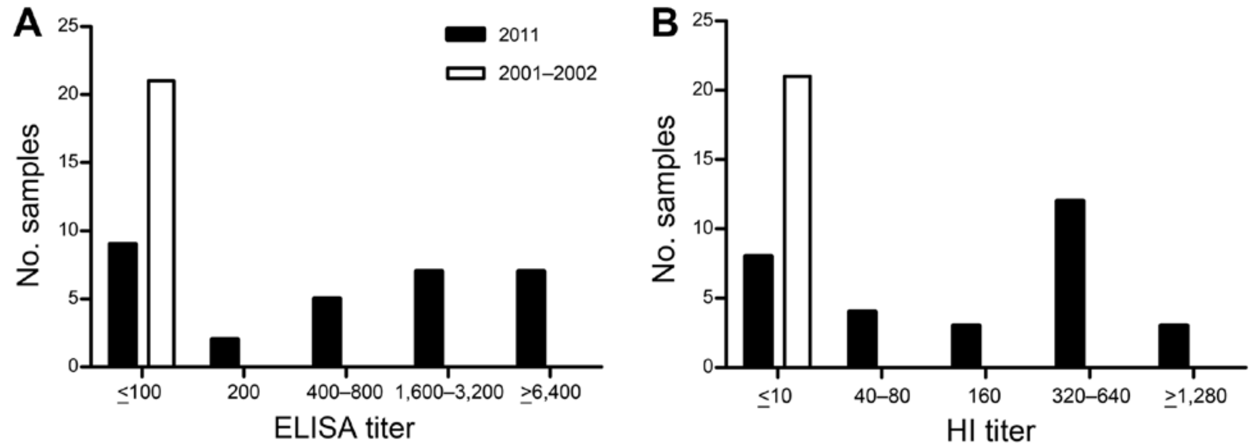

Figure. Results of ELISA and hemagglutination inhibition (HI) testing for influenza viruses in serum samples from northern sea otters captured off the coast of Washington, USA, during studies conducted in August 2011 (n = 30) and 2001–2002 (n = 21). A) IgG for influenza A(H1N1) pdm09 strain A/Texas/05/2009 detected by using standard indirect ELISA techniques with HRP-Protein A (Sigma, St. Louis, MO, USA). The ELISA titer was read as the reciprocal of the highest dilution of serum with an OD_{450nm} of $\geq$0.2 and 2-fold higher than the OD_{450nm} of control wells lacking serum. B) HI for influenza A(H1N1)pdm09 strain A/Mexico/4108/2009. HI titers were determined by using 0.5% turkey red blood cells (RBCs) for influenza A(H1N1)pdm09, seasonal influenza A(H1N1), influenza (H3N2), and influenza B viruses that circulated in North America during 2000–2011 and by using 1% horse RBCs supplemented with 0.5% BSA for avian influenza A(H1N1) virus strain A/duck/New York/96. HI assay was performed as described (www.who.int/influenza/gisrs_laboratory/manual_diagnosis_surveillance_influenza/en). OD, optical density.

adsorbed with purified pH1N1 and dk/NY/96 virions. Adsorption with pH1N1, but not dk/NY/96, removed HI antibodies to pH1N1, whereas adsorption with either virus removed HI antibodies against dk/NY/96 (online Technical Appendix Table 4). A comparison of amino acid sequences comprising the known HA antigenic sites on the pH1N1 structure confirmed high sequence identity and structural similarity with dk/NY/96 HA in Sa (12/13 aa residues) and Sb (8/12 aa residues) antigenic sites (data not shown). These results indicate that HI antibodies detected in sea otters are the result of pH1N1 virus infection but cross-react with the avian influenza A(H1N1) virus.

Although we cannot exclude the possibility that sea otters were infected with classical swine influenza A(H1N1) virus, which shares high HA genetic and antigenic similarity with pH1N1 virus, our serologic evidence is consistent with isolation of pH1N1 virus from northern elephant seals (*1*). Therefore, we conclude that these sea otters were infected with pH1N1 virus. The origin or transmission route of pH1N1 virus infection in sea otters remain unknown. Potential contact between northern elephant seals and sea otters is one possibility; elephant seals' summer feeding ranges and breeding areas along the Northeast Pacific coast overlap with areas where the Washington sea otter population is distributed (*1*).

In conclusion, our results show that sea otters are susceptible to infection with influenza A virus and highlight the complex nature of interspecies transmission of influenza viruses in the marine environment. Further surveillance, especially in other sea otter populations, is required to determine virus origin, potential pathogenesis, and consequences for the marine ecosystem.

Acknowledgments

We thank Heather Tatum, Leilani Thomas, and Peter Browning for specimen management and Tina Egstad, Katy Griffin, Renee Long, and Zac Najacht for technical assistance.

Sample collection was done in collaboration with United States Geological Survey–Alaska Science Center, Monterey Bay Aquarium, and Seattle Aquarium. Recombinant HAs with Histidine Tag from A/Japan/305/57 (H2N2), FR-700; A/Netherlands/219/2003 (H7N7), FR-71; A/Hong Kong/1073/99 (H9N2), FR-88; A/shorebird/DE/68/2004 (H13N9), FR-73; globular head domain HA1 rHAs of B/Brisbane/60/2008, FR-836; and B/Wisconsin/1/2010 FR-843, were obtained through the Influenza Reagent Resource, Influenza Division, World Health Organization Collaborating Center for Surveillance, Epidemiology and Control of Influenza, Centers for Disease Control and Prevention.

Zhu-Nan Li, Hon S. Ip, Jessica F. Trost, C. LeAnn White, Michael J. Murray, Paul J. Carney, Xiang-Jie Sun, James Stevens, Min Z. Levine, and Jacqueline M. Katz

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (Z.-N. Li, J.F. Trost, P.J. Carney, X.-J. Sun, J. Stevens, M.Z. Levine, J.M. Katz); United States Geological Survey National Wildlife Health Center, Madison, Wisconsin, USA (H.S. Ip, C.L. White); and Monterey Bay Aquarium, Monterey, California, USA (M.J. Murray)

DOI: http://dx.doi.org/10.3201/eid2005.131890

References

1 Goldstein T, Mena I, Anthony SJ, Medina R, Robinson PW, Greig DJ, et al. Pandemic H1N1 influenza isolated from free-ranging Northern Elephant Seals in 2010 off the central California coast. PLoS ONE. 2013;8:e62259. http://dx.doi.org/10.1371/journal.pone.0062259
2. Hinshaw VS, Bean WJ, Geraci J, Fiorelli P, Early G, Webster RG. Characterization of two influenza A viruses from a pilot whale. J Virol. 1986;58:655–6.
3. Hinshaw VS, Bean WJ, Webster RG, Rehg JE, Fiorelli P, Early G, et al. Are seals frequently infected with avian influenza viruses? J Virol. 1984;51:863–5.
4. Geraci JR, St Aubin DJ, Barker IK, Webster RG, Hinshaw VS, Bean WJ, et al. Mass mortality of harbor seals: pneumonia associated with influenza A virus. Science. 1982;215:1129–31. http://dx.doi.org/10.1126/science.7063847
5. Anthony SJ, St Leger JA, Pugliares K, Ip HS, Chan JM, Carpenter ZW, et al. Emergence of fatal avian influenza in New England harbor seals. MBio. 2012;3:e00166–12. http://dx.doi.org/10.1128/mBio.00166-12
6. Callan RJ, Early G, Kida H, Hinshaw VS. The appearance of H3 influenza viruses in seals. J Gen Virol. 1995;76:199–203. http://dx.doi.org/10.1099/0022-1317-76-1-199
7. Osterhaus AD, Rimmelzwaan GF, Martina BE, Bestebroer TM, Fouchier RA. Influenza B virus in seals. Science. 2000;288:1051–3. http://dx.doi.org/10.1126/science.288.5468.1051
8. White CL, Schuler KL, Thomas NJ, Webb JL, Saliki JT, Ip HS, et al. Pathogen exposure and blood chemistry in the Washington, USA population of northern sea otters (*Enhydra lutris kenyoni*). J Wildl Dis. 2013;49:887–99. http://dx.doi.org/10.7589/2013-03-053
9. Brancato MS, Milonas L, Bowlby CE, Jameson R, Davis JW. Chemical contaminants, pathogen exposure and general health status of live and beach-cast Washington sea otters (*Enhydra lutris kenyoni*). Marine Sanctuaries Conservation Series ONMS-09–01. Silver Spring (MD): US Department of Commerce, National Oceanic and Atmospheric Administration, Office of National Marine Sanctuaries; 2009. p. 181.
10. Stephenson I, Wood JM, Nicholson KG, Zambon MC. Sialic acid receptor specificity on erythrocytes affects detection of antibody to avian influenza haemagglutinin. J Med Virol. 2003;70:391–8. http://dx.doi.org/10.1002/jmv.10408

Address for correspondence: Jacqueline M. Katz, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop G16, Atlanta, GA 30333, USA: email: jmk9@cdc.gov

New Variant of Porcine Epidemic Diarrhea Virus, United States, 2014

To the Editor: Porcine epidemic diarrhea (PED) was first reported in the United Kingdom in 1971 (*1*). The disease was characterized by severe enteritis, vomiting, watery diarrhea, dehydration, and a high mortality rate among swine. Subsequently, the causative agent of PED was identified as porcine epidemic diarrhea virus (PEDV), which belongs to the family *Coronaviridae* (*2*) and contains an enveloped, single-stranded positive-sense RNA genome. PEDV has been reported in many other countries, including Germany, France, Switzerland, Hungary, Italy, China, South Korea, Thailand, and Vietnam (*3*) and was first identified in the United States in May 2013. By the end of January of 2014, the outbreak had occurred in 23 US states, where 2,692 confirmed cases (www.aasv.org/news/story.php?id = 6989) caused severe economic losses. Recent studies have shown that all PEDV strains in the United States are clustered together in 1 clade within the subgenogroup 2a and are closely related to a strain from China, AH2012 (*4*,*5*).

In the state of Ohio, the first PED case was identified in June of 2013; since then, hundreds of cases have been confirmed by the Animal Disease Diagnostic Laboratory of the Ohio Department of Agriculture. In January of 2014, samples from pigs with unique disease, suspected to be PED, were submitted to this laboratory. Sows were known to be infected, but piglets showed minimal to no clinical signs and no piglets had died.

According to real-time reverse transcription PCR, all samples from the piglets were positive for PEDV. Subsequently, the full-length genome sequence of PEDV (OH851) was determined by using 19 pairs of oligonucleotide primers designed from alignments of the available genomes from PEDVs in the United States (*6*,*7*). On the basis of BLAST (http:blast.ncbi.nlm.nih.gov/Blast.cgi) searches, strain OH851 showed 99% and 97% nt identity to PEDVs currently circulating in the United States (Colorado, Iowa, Indiana, Minnesota) for the whole genome and the full-length spike (S) gene, respectively. By distinct contrast, strain OH851 showed only 89% or even lower nucleotide identity to PEDVs currently circulating in the United States in the first 1,170 nt of the S1 region. In that region, nucleotide similarity to that of a PEDV strain from China (CH/HBQX/10, JS120103) was 99%, suggesting that strain OH851 is a new PEDV variant. Phylogenetic analysis of the complete genome indicated that the novel OH851 PEDV is clustered with other strains of PEDV currently circulating in United States, including another stain from Ohio, OH1414 (Figure, panel A). However, phylogenetic analysis of the full-length S gene showed that strain OH851 is clustered with other strains of PEDV from China and most closely related to a PEDV strain from China, CH/HBQX/10 (*8*), but distantly related to other PEDV strains currently circulating in the United States and strain AH2012 (Figure, panel B). This finding strongly suggests that strain OH851 is a variant PEDV. In comparison with the S gene of other strains from the United States, the S gene of strain OH851 has 3 deletions (a 1-nt deletion at position 167, a 11-nt deletion at position 176, and a 3-nt deletion at position 416), a 6-nt insertion between positions 474 and 475,

Article DOI: http://dx.doi.org/10.3201/eid2005.131890

Serologic Evidence of Influenza A(H1N1)pdm09 Virus in Northern Sea Otters

Technical Appendix

Technical Appendix Table 1. HA specific IgG ELISA titer range detected in sea otter sera collected in 2011

	Number of sera with ELISA titer range				
rHA	≤100	200	400–800	1600–3200	≥6400
A/Texas/05/09 [A(H1N1)pdm09]	9	2	5	7	7
A/Brisbane/59/07 (H1N1)	26	4	0	0	0
A/Japan/305/57 (H2N2)	30	0	0	0	0
A/Hong Kong/1/68 (H3N2)	30	0	0	0	0
A/swine/Wisconsin/12/10 (H3N2v)	30	0	0	0	0
A/Wisconsin/67/05 (H3N2)	30	0	0	0	0
A/Vietnam/1203/04 (H5N1)	30	0	0	0	0
A/Netherlands/219/03 (H7N7)	30	0	0	0	0
A/Hong Kong/1073/99 (H9N2)	30	0	0	0	0
A/shorebird/Delaware/68/04 (H13N9)	30	0	0	0	0
B/Brisbane/60/08	30	0	0	0	0
B/Wisconsin/01/10	30	0	0	0	0

Technical Appendix Table 2. HI titers of sea otter sera collected in 2011 against A/Mexico/4108/09 [A(H1N1)pdm09] and A/duck/NY/1996 viruses

		A/Mexico/4108/09		A/duck/New York/96	
Sample	Age	tRBC HI	hRBC HI	tRBC HI	hRBC HI
1000	2	640	ND*	10	80
1001	6	320	ND	10	40
1002	7	320	ND	10	80
1003	8	1280	ND	10	160
1004	7	80	ND	10	10
1005	12	5	ND	10	10
1006	5	640	ND	10	160
1007	19	160	ND	10	160
1008	5	1280	ND	10	160
1009	5	80	ND	10	10
1010	10	5	ND	10	10
1011	3	160	ND	10	10
1012	9	640	ND	10	160
1013	6	5	ND	10	10
1014	10	80	ND	10	10
1015	7	5	ND	10	10
1016	6	320	ND	10	160
1017	10	5	ND	10	10
1018	10	40	ND	10	10
1019	11	320	ND	10	80
1020	7	5	ND	10	10
1021	7	160	ND	10	20
1022	6	640	ND	10	160
1023	6	320	ND	10	80
1024	12	5	ND	10	10
1025	4	640	ND	10	80
1026	13	5	ND	10	10
1027	6	640	ND	10	320
1028	3	640	ND	10	80
1029	7	1280	ND	10	160

*ND, not done. Horse red blood cell HAU<4 for A/Mexico/4108/09 virus

Technical Appendix Table 3. HI titer range against influenza A and B viruses detected in sea otter sera collected in 2011

Virus	Number of sera with HI titer range				
	≤10	40–80	160	320–640	≥1280
A/Mexico/4108/09 [A(H1N1)pdm09]	8	4	3	12	3
A/New Caledonia/20/99 (sH1N1)	30	0	0	0	0
A/Brisbane/59/07 (sH1N1)	30	0	0	0	0
A/duck/New York/96 (aH1N1)	30	0	0	0	0
A/Panama/2007/99 (H3N2)	30	0	0	0	0
A/Perth/16/09 (H3N2)	30	0	0	0	0
B/Beijing/184/93	30	0	0	0	0
B/Florida/4/06	30	0	0	0	0
B/Brisbane/60/08	30	0	0	0	0

Technical Appendix Table 4. Sea otter HI titers against A/Mexico/4108/09 [A(H1N1)pdm09] and A/duck/New York/96 viruses after serum adsorption with A(H1N1)pdm09 and A/duck/NY/96 virions.

Serum sample	HI titer to A(H1N1)pdm09* after adsorption with			HI titer to A/duck/NY/96† after adsorption with		
	Mock	A(H1N1)pdm09	A/duck/New York/96	Mock	A(H1N1)pdm09	A/duck/New York/96
1001	80	5	80	40	5	5
1007	80	5	40	80	5	5
1016	320	5	320	80	5	5
1019	160	5	160	40	5	5

*0.5% turkey RBCs were used
†1% horse RBCs supplemented with 0.5% BSA were used