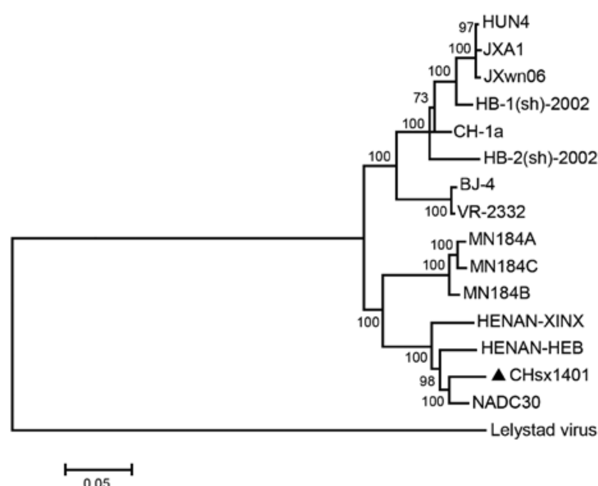

Figure. Phylogenetic analysis of whole genomes of porcine reproductive and respiratory syndrome virus (PRRSV) CHsx1401 (triangle) (GenBank accession no. KP861625); representative prototype strain VR-2332 (U87392); isolates BJ-4 (AF331831), CH-1a (AY032626), HB-1(sh)/2002 (AY150312), and HB-2(sh)/2002 (AY262352) from China; highly pathogenic strains JXA1 (EF112445), JXwn06 (EF641008), and HUN4 (EF635006); strains MN184A (DQ176019), MN184B (DQ176020), MN184C (EF488739), and NADC30 (JN654459) from the United States; and recent strains HENAN-HEB (KJ143621) and HENAN-XINX (KF611905) from China. Prototype Lelystad virus (M96262) was used as the outgroup. The phylogenetic tree was constructed by using the distance-based neighbor-joining method with 1,000 bootstrap replicates in MEGA6 (http://www.megasoftware.net/). Numbers along branches are bootstrap values. Scale bar indicates nucleotide substitutions per site.

Recent widespread outbreaks of PPRS in China were associated with a novel NADC30-like strain of PPRSV. Whole genomic analysis showed that the strain differed from previously identified PRRSV strains in China, but had an overall genetic similarity and a unique deletion in the NSP2-coding region that was identical to that of NADC30, which originated in the United States. We propose that the NADC30 strain was introduced into China in recent years by importing of breeding pigs and has since undergone mutations, resulting in variant viruses.

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References

1. Lunney JK, Benfield DA, Rowland RR. Porcine reproductive and respiratory syndrome virus: an update on an emerging and re-emerging viral disease of swine. Virus Res. 2010;154:1–6. http://dx.doi.org/10.1016/j.virusres.2010.10.009
2. Wensvoort G, Terpstra C, Pol JM, ter Laak EA, Bloemraad M, de Kluyver EP, et al. Mystery swine disease in The Netherlands: the isolation of Lelystad virus. Vet Q. 1991;13:121–30. http://dx.doi.org/10.1080/01652176.1991.9694296
3. Benfield DA, Nelson E, Collins JE, Harris L, Goya SM, Robison D, et al. Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC-VR2332). J Vet Diagn Invest. 1992;4:127–33. http://dx.doi.org/10.1177/104063879200400202
4. Han J, Wang Y, Faaberg KS. Complete genome analysis of RFLP 184 isolates of porcine reproductive and respiratory syndrome virus. Virus Res. 2006;122:175–82. http://dx.doi.org/10.1016/j.virusres.2006.06.003
5. Tian K, Yu X, Zhao T, Feng Y, Cao Z, Wang C, et al. Emergence of fatal PRRSV variants: unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark. PLoS ONE. 2007;2:e526. http://dx.doi.org/10.1371/journal.pone.0000526
6. Zhou L, Yang H. Porcine reproductive and respiratory syndrome virus in China. Virus Res. 2010;154:31–7. http://dx.doi.org/10.1016/j.virusres.2010.07.016
7. Karniychuk UU, Geldhof M, Vanhee M, Van Doorsselaere J, Saveleva TA, Nauwynck HJ. Pathogenesis and antigenic characterization of a new East European subtype 3 porcine reproductive and respiratory syndrome virus isolate. BMC Vet Res. 2010;6:30–9. http://dx.doi.org/10.1186/1746-6148-6-30
8. Brockmeier SL, Loving CL, Vorwald AC, Kehrli ME Jr, Baker RB, Nicholson TL, et al. Genomic sequence and virulence comparison of four type 2 porcine reproductive and respiratory syndrome virus strains. Virus Res. 2012;169:212–21. http://dx.doi.org/10.1016/j.virusres.2012.07.030
9. Zhou L, Chen S, Zhang J, Zeng J, Guo X, Ge X, et al. Molecular variation analysis of porcine reproductive and respiratory syndrome virus in China. Virus Res. 2009;145:97–105. http://dx.doi.org/10.1016/j.virusres.2009.06.014
10. Zhou L, Yang X, Tian Y, Yin S, Geng G, Ge X, et al. Genetic diversity analysis of genotype 2 porcine reproductive and respiratory syndrome viruses emerging in recent years in China. Biomed Res Int. 2014;2014:748068. Epub 2014 Feb 25. http://dx.doi.org/10.1155/2014/748068

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Serologic Evidence of Influenza A (H14) Virus Introduction into North America

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To the Editor: Although a diverse population of influenza A viruses (IAVs) is maintained among ducks, geese, shorebirds, and gulls, not all of the 16 avian

hemagglutinin (HA) subtypes are equally represented (*1*). The 14th HA subtype, commonly known as the H14 subtype, was historically limited to isolates from the former Soviet Union in the 1980s (*2*) and was not subsequently detected until 2010, when isolated in Wisconsin, USA from long-tailed ducks and a white-winged scoter (*3–5*). In the United States, the H14 subtype has since been isolated in California (*6*), Mississippi, and Texas (*7*); and has been reported in waterfowl in Guatemala (*7*). In this study, we examined whether there was serologic evidence of H14 spread among ducks in North America before (2006–2010) and after (2011–2014) the initial detection of the H14 subtype virus on this continent.

This report was reviewed and approved by United States Geological Survey under the Fundamental Science Practices policy (http://www.usgs.gov/fsp/). Serum samples from blue-winged teal, American green-winged teal, and mallard ducks were screened by using blocking ELISA (FlockCheck AI MultiS-Screen antibody test kit; IDEXX Laboratories, Westbrook, ME, USA) to detect antibodies against the influenza virus nucleoprotein. Positive samples were tested by microneutralization assays as described (*7*) against viruses representing H14 and H3 subtypes. H3 is commonly detected in ducks found in North America (*8*) (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/12/15-0413-Techapp1.pdf).

Antibodies against H3 were detected during 2006–2014 in Michigan, Minnesota, New Jersey, Texas, and Louisiana (Table); titers ranged from 20 to 320. Antibodies against H14 were detected in 1 duck in 2007 and in 24 ducks sampled in 2012 after August. H14 antibodies were detected in all years and most locations studied after 2012; antibody titers ranged from 20 to 160. Thus, antibody prevalence was consistent with the relative prevalence of H3 reported among ducks in North America (*1*,*4*,*8*) and the timing of initial detection of H14 viruses.

To address the possibility of cross-neutralizations between HA subtypes, we tested the 2007 H14-positive serum samples and 22 of the H14-positive serum samples from 2012–2014 against HA subtypes 1–12 (online Technical Appendix Table 1) by virus neutralization (online Technical Appendix Table 2). Among humans, broadly neutralizing antibodies within HA groups targeting conserved regions in the HA stalk have been described (*9*), and if present in samples from mallards, these could contribute to cross-neutralizations. The H14-positive serum samples from 2007 reacted to subtypes H3, H4, H7, and H11, and high titers were identified for H3 and H4, which are within the same clade. Samples from 17 of these birds tested antibody-positive for additional HA subtypes and 5 tested positive only to H14. An H14 virus was recovered by virus isolation from the same blue-winged teal population sampled in March 2013, from which serum samples were obtained (*7*); however, although H14 antibodies have been detected in Minnesota, an H14 virus has not yet been isolated in that state.

Our serologic results are temporally consistent with H14 isolation reports and suggest that H14 subtype viruses were not circulating among ducks in North America before initial virus isolation. However, there are potential challenges with serologic-based investigations. For

Table. H3 and H14 microneutralization assay data from ducks sampled during 2006–2014, North America*

Year	Month of sampling	State	Species	No.	H3N8, no. (%)	H14N5, no. (%)
2006	Aug	Michigan	Mallard	29	6 (21)	0
	Aug/Sep	Minnesota	Mallard	39	3 (8)	0
2007	Aug/Sep	Minnesota	Mallard	46	8 (17)	1 (2)
2008	Aug/Sep	Minnesota	Mallard	44	8 (18)	0
2009	Aug/Sep	Minnesota	Mallard	29	10 (34)	0
	Aug	New Jersey	Domestic and wild mallard	36	1 (3)	0
2010	Aug/Sep	Minnesota	Mallard	29	6 (21)	0
	Aug	New Jersey	Domestic and wild mallard	20	5 (25)	0
2011	Aug/Sep	Minnesota	Mallard	124	37(30)	0
2012	Feb/Mar	Texas	Blue-winged teal	19	3 (16)	0
	Aug/Sep	Minnesota	Mallard	188	11 (6)	2 (1)
2013	Feb/Mar	Texas/Louisiana	Blue-winged teal	120	13 (11)	12 (10)
	Feb/Mar	Texas/Louisiana	American green-winged teal	91	5 (5)	2 (2)
	Aug/Sep	Minnesota	Mallard	65	8 (12)	7 (11)
2014	Feb/Mar	Texas	Blue-winged teal	22	1 (5)	1 (5)
	Sep	Minnesota	Mallard	41	4 (10)	0
Totals						
2006–2010	NA	NA	All ducks	272	47 (17)	1 (0.3)
	NA	NA	Mallards only	272	47 (17)	1 (0.3)
2011–2014	NA	NA	All ducks	670	82 (12)	24 (3.5)
	NA	NA	Mallards only	418	60 (14)	9 (2.1)
	NA	NA	Blue-winged teal and American green-winged teal	252	22 (9)	15 (6)

*NA, not applicable.

example, the overall prevalence of H14 antibodies after the initial detection of H14 viruses (2011–2014) was low (3.5% of blocking ELISA positive samples), thus requiring a large sample size (n = 670) for H14 antibody detection. However, an even lower prevalence was observed by using virus isolation; we isolated only 1 H14 IAV during parallel sampling of these sites (n = 8,875) during 2011–2014.

Differences in pre- and post-H14 detection also varied between species, location, and season. Differences in H14 antibody prevalence were observed in all ducks sampled pre- and post- (0.3%–3.5%, p = 0.0103) H14 detection, but not in the mallard-only subset (0.3%–2.1%, p = 0.0963). A significant difference in seroprevalence also was detected between species (mallard [2%] vs teal [6%]) in the 2011–2014 samples (p = 0.0104). IAV show strong seasonal patterns in prevalence, and the observed differences in antibodies may be associated with the probability of IAV infection before sampling and the persistence of antibody responses in these species. Mallards (primarily hatch-year birds) were sampled at the beginning of fall migration (≈3–4 months of potential IAV exposure for hatch-year birds), whereas teal were sampled later, during spring migration (≈9–10 months of potential IAV exposure for birds hatched the previous spring or summer). It is apparent that the sampling approach used can affect results.

Interpretation of subtype-specific serologic data can be complex, especially in birds that are normally infected with several IAV subtypes during their lives. Nevertheless, this study demonstrates the value of a subtype-specific serologic approach to detect even relatively minor changes in subtype diversity and clearly shows that new viruses can establish in duck populations in North America. Serologic techniques also can be optimized to detect incursions of novel viruses such as the highly pathogenic Eurasian H5 viruses (*10*) among wild birds.

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References

1. Olsen B, Munster VJ, Wallensten A, Waldenstrom J, Osterhaus ADME, Fouchier RAM. Global patterns of influenza A virus in wild birds. Science. 2006;312:384–8 http://dx.doi.org/10.1126/science.1122438.
2. Kawaoka Y, Yamnikova S, Chambers TM, Lvov DK, Webster RG. Molecular characterization of a new hemagglutinin, subtype-H14, of influenza-A virus. Virology. 1990;179:759–67 http://dx.doi.org/10.1016/0042-6822(90)90143-F.
3. Fries AC, Nolting JM, Bowman AS, Killian ML, Wentworth DE, Slemons RD. Genomic analyses detect Eurasian-lineage H10 and additional H14 influenza A viruses recovered from waterfowl in the Central United States. Influenza Other Respir Viruses. 2014;8:493–8 http://dx.doi.org/10.1111/irv.12250.
4. Fries AC, Nolting JM, Danner A, Webster RG, Bowman AS, Krauss S, et al. Evidence for the circulation and inter-hemispheric movement of the H14 subtype influenza A virus. PLoS ONE. 2013;8 [cited 2015 Jun 22]. http://dx.doi.org/10.1371/journal.pone.0059216
5. Nolting JFA, Slemons RD, Courtney C, Hines N, Pedersen J. Recovery of H14 influenza A virus isolates from sea ducks in the Western Hemisphere. PLOS Currents Influenza 2012 (Edition 1).
6. Boyce WM, Schobel S, Dugan VG, Halpin R, Lin X, Wentworth DE, et al. Complete genome sequence of a reassortant H14N2 avian influenza virus from California. Genome Announc. 2013;1:e00543–13. http://dx.doi.org/10.1128/genomeA.00543-13
7. Ramey AM, Poulson RL, Gonzalez-Reiche AS, Perez DR, Stallknecht DE, Brown JD. Genomic characterization of H14 subtype influenza A viruses in new world waterfowl and experimental infectivity in mallards (*Anas platyrhynchos*). PLoS ONE. 2014;9 [cited 2015 Jun 22]. http://dx.doi.org/10.1371/journal.pone.0095620
8. Wilcox BR, Knutsen GA, Berdeen J, Goekjian V, Poulson R, Goyal S, et al. Influenza-A viruses in ducks in northwestern Minnesota: fine scale spatial and temporal variation in prevalence and subtype diversity. PLoS ONE. 2011;6:e24010 http://dx.doi.org/10.1371/journal.pone.0024010.
9. Ekiert DC, Friesen RHE, Bhabha G, Kwaks T, Jongeneelen M, Yu WL, et al. A highly conserved neutralizing epitope on group 2 influenza A viruses. Science. 2011;333:843–50 http://dx.doi.org/10.1126/science.1204839.
10. Gilbert M, Koel BF, Bestebroer TM, Lewis NS, Smith DJ, Fouchier RAM. Serological evidence for non-lethal exposures of Mongolian wild birds to highly pathogenic avian influenza H5N1 virus. PLoS ONE. 2014;9 [cited 2015 Jun 22]. http://dx.doi.org/10.1371/journal.pone.0113569

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Technical Appendix

Technical Appendix Table 1. List of antigens used for microneutralization assays of samples from ducks, North America

Antigen
A/mallard/MN/AI0-4823/2010 (H1N1)
A/mallard/MN/AI08-2755/2008 (H2N3)
A/mallard/MN/AI0-2593/2010 (H3N8)
A/mallard/MN/AI10-3208/2010 (H4N6)
A/mallard/MN/AI11-3933/2011 (H5N1)
A/mallard/MN/AI08-2721/2008 (H6N1)
A/mallard/MN/AI08-3770/2009 (H7N9)
A/mallard/MN/SG-01048/2008 (H8N4)
A/RUTU/DE/AI11-809/2011 (H9N2)
A/mallard/MN/SG-00999/2008 (H10N7)
A/mallard/MN/SG-00930/2008 (H11N9)
A/mallard/MN/SG-3285/2007 (H12N5)
A/blue-winged teal/TX/AI13-1028/2010 (H14N5)

Technical Appendix Table 2. Subtype-specific virus neutralization data for H1–H12 from 23 birds positive for H14, North America*

					Group 1								Group 2				
					H9 Clade			H1 Clade				H11 Clade	H7 Clade		H3 Clade		
Date		Sample ID	Age†	Species‡	H9N2	H8N4	H12N5	H5N1	H2N3	H1N1	H6N1	H11N9	H7N9	H10N7	H3N8	H4N6	H14N5
2007																	
	Sep 17	AI07-4825	UA	MALL	<20§	<20	<20	<20	<20	<20	<20	**160**	**80**	<20	**640**	**640**	**40**
2012																	
	Sep 17	AI12-2725	HY	MALL	<20	<20	**80**	<20	<20	<20	<20	<20	<20	<20	<20	<20	**40**
2013																	
	Mar 1	AI13-112	AHY	BWTE	<20	<20	**40**	<20	<20	<20	<20	**20**	<20	**40**	<20	<20	**40**
	Mar 1	AI13-114	AHY	BWTE	**20**	**320**	<20	**20**	<20	<20	<20	20	<20	<20	**40**	<20	**40**
	Mar 1	AI13-137	AHY	BWTE	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	**20**
	Mar 1	AI13-6	AHY	BWTE	<20	<20	<20	<20	<20	<20	<20	**20**	<20	<20	<20	<20	**20**
	Mar 5	AI13-393	HY	BWTE	**80**	<20	**640**	**640**	**640**	**640**	**640**	<20	<20	**160**	<20	**40**	**160**
	Mar 5	AI13-433	AHY	AGWT	<20	<20	<20	<20	<20	20	<20	<20	<20	<20	<20	<20	**20**
	Mar 6	AI13-511	AHY	BWTE	<20	<20	<20	**40**	<20	<20	**40**	<20	<20	<20	<20	<20	**20**
	Mar 6	AI13-528	AHY	BWTE	<20	<20	**320**	<20	<20	<20	<20	<20	<20	<20	<20	<20	**40**
	Mar 7	AI13-538	AHY	BWTE	<20	<20	<20	<20	<20	<20	<20	<20	<20	**20**	<20	**160**	**160**
	Mar 7	AI13-541	AHY	BWTE	<20	**80**	**80**	**40**	<20	<20	**640**	**160**	<20	**80**	**160**	160	**20**
	Mar 7	AI13-554	AHY	BWTE	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	**20**
	Mar 15	AI13-1002	AHY	BWTE	<20	<20	<20	**20**	<20	<20	**40**	<20	<20	**20**	**40**	<20	**80**
	Mar 15	AI13-1072	AHY	AGWT	<20	<20	<20	<20	**20**	<20	<20	<20	<20	<20	<20	<20	**20**
	Aug 28	AI13-3025	AHY	MALL	<20	**20**	<20	<20	<20	<20	<20	**20**	<20	<20	**40**	<20	**20**
	Aug 28	AI13-3035	AHY	MALL	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	**40**	**20**
	Aug 28	AI13-3109	HY	MALL	<20	**20**	**80**	<20	<20	<20	<20	<20	<20	<20	<20	<20	**80**
	Sep 4	AI13-3115	AHY	MALL	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	**80**
	Sep 4	AI13-3123	AHY	MALL	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	**40**	**20**
	Sep 4	AI13-3125	HY	MALL	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	**20**
	Sep 12	AI13-3209	HY	MALL	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	**40**	**20**
2014																	
	Mar 7	AI14-49	AHY	BWTE	**20**	**80**	<20	<20	<20	<20	**640**	**20**	<20	**160**	**40**	**20**	**20**

*The volume of sera for 2 of the H14 positive samples was inadequate for testing against the complete panel of antigens. HA subtypes are organized based on phylogenetic relationships because patterns of cross-reactivity would be expected on the basis of clade membership. Case-patients are identified by sample ID.
†Abbreviations for age are: UA, unknown age; HY, hatched-year bird; AHY, after hatched-year bird.
‡Abbreviations for species are: MALL, mallard; BWT, blue-winged teal; AGWT, American green-winged teal.
§Virus neutralization antibody titers of <20 indicate negative results and titers of >20 are considered positive. Bold numbers indicate values >20.