Triple Reassortant Swine Influenza A (H3N2) Virus in Waterfowl

To the Editor: In 1998, a new lineage of triple reassortant influenza A (H3N2) virus (TR-H3N2) with genes from humans (hemmaglutinin [HA], neuraminidase [NA], and polymerase basic 1 [PB1]), swine (matrix [M], nonstructural [NS], and nucleoprotein [NP]), and birds (polymerase acidic [PA] and PB2) emerged in the U.S. swine population. Subsequently, similar viruses were isolated from turkeys (*1*,*2*), minks, and humans in the United States and Canada (*3*,*4*). In 2007, our national influenza surveillance resulted in isolation of 4 swine-like TR-H3N2 viruses from migratory waterfowl (3 from mallards [*Anas platyrrhynchos*] and 1 from a northern pintail [*Anas acuta*] of 266 birds sampled) in north-central South Dakota. We report on the characterization of these TR-H3N2 viruses and hypothesize about their potential for interspecies transmission.

Two of these isolates, A/mallard/South Dakota/Sg-00125/2007 (H3N2) and A/northern pintail/South Dakota/Sg-00126/2007 (H3N2), were recovered from the birds sampled in north-central South Dakota, 45°44′30″N, 98°16′30″W; 2 isolates, A/mallard/South Dakota/Sg-00127/2007(H3N2) and A/mallard/South Dakota/Sg-00128/2007(H3N2), were sampled at 45°46′30″N, 98°15′30″W. Viral RNA was extracted, reverse transcribed, and amplified; all segments were sequenced in entirety and submitted to GenBank under the identified virus names. Phylogenetic analysis showed significant nucleotide identities (99%–100%), differing only in 4 nucleotide positions: 1 each from PB1, PA, NP, and NS genes. Among 4 substitutions, 3 were nonsynonymous (PA, NP, and NS), and 1 (PB1) was synonymous. A1725G substitution in PB1 was identified in 2 isolates. C419T change was identified in 3 isolates (Sg-00125, Sg-00126, and Sg-00128), resulting in substitution of threonine by phenylalanine. Three isolates (Sg-00125, Sg-00126, and Sg-00127) carried an A at residue 149 of the NP gene (leading to S50N change) and 1 isolate (Sg-00128) had a G at that position (encoding serine). G809A change was present in the NP gene of 3 isolates (Sg-00125, Sg-00127, and Sg-00128). Genomes of the 4 isolates had high nucleotide and amino acid identities (>98%) with North American swine TR-H3N2 virus (A/Swine/Iowa/533/99 [H3N2]). Phylogenetic analysis indicated that TR-H3N2 waterfowl and North American TR-H3N2 swine isolates belonged to a single cluster. The H3N2 subtypes from avian and swine isolates of our sequencing projects belonged to different clusters (Figure). Deduced amino acid sequences of all segments showed that these virus isolates shared common themes in virulence determinants to those previously reported for swine-like TR-H3N2 viruses (*5*).

Inasmuch as we identified a swine lineage virus in waterfowl, we first investigated laboratory contamination by using trace back and history of swine virus isolations during the time the surveillance samples were processed. No H3N2 subtype were isolated from swine sources in the Minnesota Veterinary Diagnostic Laboratory during this period. Furthermore, phylogenetic analysis of all HA segment sequences from isolates obtained in that 4-month period confirmed no contamination. We then investigated whether an ecologic niche existed for potential exposure of waterfowl to pigs. We identified a swine herd near the wildlife refuge area where the waterfowl sampling occurred. Pigs were housed outdoors, and the owner of this swine herd reported that geese and ducks inhabit the water ponds/stock dams/slough area to which the pigs had access. Contact with the local veterinarian and the South Dakota Veterinary Diagnostic Laboratory indicated no recent reports of influenza A (H3N2) episodes in the swine herd. In addition, this herd was not vaccinated for swine influenza.

The mode of transmission of swine-origin virus to waterfowl is not clear. In previously published cases, where swine influenza viruses have been identified in turkeys, the flocks were in close proximity to swine herds (*2*). Similarly, we identified a swine herd in north-central South Dakota where all 4 waterfowl were sampled. Respiratory secretions from the pigs possibly could have spread to birds through aerosols or droplets. It is also likely that swine and waterfowl shared common water sources, which contained feces from influenza-infected waterfowl or respiratory secretions from influenza-infected swine. This mode of influenza virus transmission from birds to pigs has been documented (*6*–*9*). Indeed, a waterborne source for transmission is most likely because influenza A virus can persist in water for several months depending on environmental factors such as pH, temperature, and salinity (*10*). Finally, because the swine herd in this area was housed outdoors in open pens, direct interaction with waterfowl was possible.

In late 2008, serum samples were collected from this swine herd. Hemagglutination inhibition test (*1*) showed that 10 of 19 samples reacted with all 4 waterfowl isolates; titers ranged from 10 to ≥640. Although low titers may have occurred because pigs were exposed to heterologous cross-reactive viruses, the high titers in most animals with positive serum samples suggest exposure to an influenza (H3N2) virus similar to that recovered from the waterfowl. Our data emphasize the need to investigate the possible role of waterfowl in the maintenance and transmission of influenza A viruses to humans and to lower mammalian species.

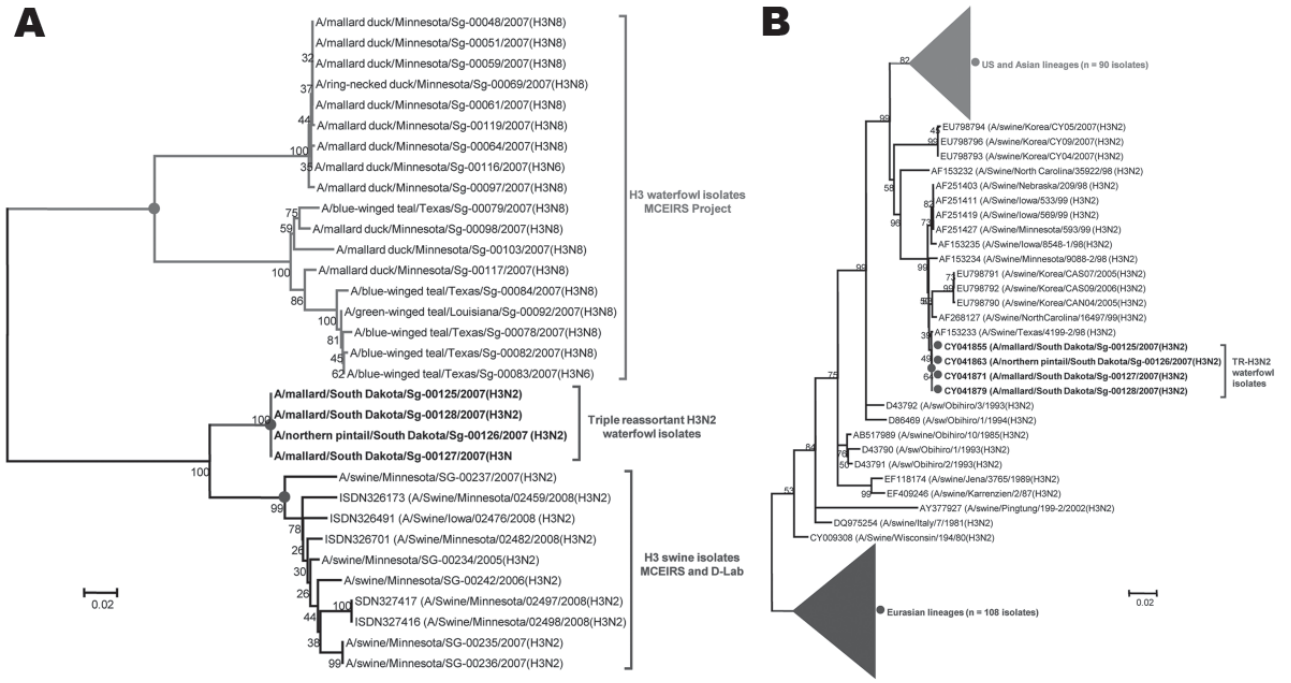

Figure. Phylogenetic analysis of hemagglutinin (HA) sequences from waterfowl strains isolated in this study (**boldface**), based on the HA gene sequences. The evolutionary associations were inferred in MEGA4.0 (www.megasoftware.net) by using the neighbor-joining algorithm with the Kimura 2-parameter gamma model and 1,000 bootstrap replications (shown on branch bifurcations). A) Evolutionary distances of waterfowl isolates from swine and avian HA (H3) sequences from the Minnesota Center of Excellence for Influenza Research and Surveillance (MCEIRS) sequencing project or Minnesota Veterinary Diagnostic Laboratory (D-Lab) database. B) Phylogeny of 230 strains, including Eurasian and North American lineages of influenza A (H3N2) viruses. Data suggest swine influenza virus (H3N2) ancestry in the waterfowl strains. GenBank accession numbers are shown. Scale bars indicate nucleotide substitutions per site.

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Ventilator-associated Pneumonia and MRSA ST398, Italy

To the Editor: Methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type (ST)398 has become increasingly common in livestock, particularly pigs, in some countries in Europe, such as Spain and Germany (*1*). In Italy, prevalences as high as 14% and 21.6% in pig-breeding facilities and meat-processing sites, respectively, have been recently reported (*1*).

Possible association of MRSA in animals with infection in humans has been investigated. One study showed a strong relationship between contact with pigs or calves and carriage by persons having direct contact with animals and families of persons who handle animals (*2*). Moreover, an MRSA prevalence ≥11.9% has been described by de Boer et al. (*3*) in meat, with 85% of isolates belonging to the ST398 lineage.

MRSA ST398 has been described as a lineage with limited virulence and ability to spread between humans, but severe clinical manifestations, such as wound infections and endocarditis, have been recently attributed to this clone (*1*,*4*). Cases of nosocomial ventilator-associated pneumonia have also been reported in Germany (*1*). Moreover, an outbreak of infection with MRSA ST398 occurred in a surgical ward of a hospital in the Netherlands in 2007 (*5*).

MRSA ST398 is an infrequent cause of human infections in Italy. No isolates belonged to this lineage in 2 studies of MRSA in Italy during 2006–2007 (*6*) or in hospitals during 1990–2007 (*7*). Only 1 invasive infection has been recently reported in a pig farm worker (*8*). We report a case of ventilator-associated pneumonia caused by MRSA ST398 in a patient in Palermo, Italy. The patient and his household members did not report any exposure to companion or livestock animals.

The case-patient was a 78-year-old man admitted to a cardiac intensive care unit (ICU) of ARNAS Ospedale Civico Di Cristina e Benfratelli in Palermo on January 31, 2009, because of a recent history of unstable angina pectoris and acute anemia caused by duodenal ulcers. After cardiocirculatory arrest, he was transferred to a general ICU on February 3. The patient had type 2 diabetes and ischemic-hypertensive cardiomyopathy. MRSA nasal colonization at admission was not investigated because the patient lacked risk factors for screening at admission, e.g., antimicrobial drug therapy, hospitalization for >48 hours or time in a long-term care facility within the past 6 months, need for long-term nursing care, presence of indwelling devices, or chronic skin lesions.

The clinical course of the patient's illness was characterized by serious hemodynamic instability and difficulty in weaning from mechanical ventilation. Two bronchial aspirate specimens were cultured on February 4 and 9, when he was being treated with a third-generation cephalosporin (ceftriaxone). These cultures showed *Staphylococcus epidermidis* and *S. saprophyticus*. On the 14th day in the ICU, clinical signs of ventilator-associated pneumonia developed in the patient. He had increased sputum production, fever (38.8°C), leukocytosis, and infiltrates were seen on a chest radiograph.

Empiric antimicrobial drug therapy with glycopeptides and a β-lactam/β-lactamase inhibitor combination was started. Culture of bronchial secretions yielded MRSA that was susceptible to glycopeptides, rifampin, linezolid, macrolides, and sulfamethoxazole and resistant to fluoroquinolones and tetracyclines. Three days later, linezolid was given, but the patient died after an acute myocardial infarction.

The isolate was identified genetically by *mec*A PCR. It was not typeable by pulsed-field gel electrophoresis after digestion with *Sma*I, negative for Panton-Valentine leukocidin, and carried staphylococcal cassette chromosome *mec* (SCC*mec*) type IVa (*9*). Multilocus sequence typing, performed according to a recommended procedure (http://saureus.mlst.net/misc/info.asp), identified the isolate as ST398.

A 1-year epidemiologic survey on MRSA isolates from 4 general hospitals in Palermo, which had begun on February 2009, did not identify any MRSA isolate carrying SCC*mec* type IV or V in patients admitted to the ICU until September 2009. However, colonization or infection by MRSA ST398 in the ICU patients before the study period could not be ruled out. Although an MRSA screening policy for the ICU staff members was not being carried out, a nosocomial chain of transmission appeared to be unlikely.

Our results indicate that a new zoonotic clone of MRSA is emerging as a potential cause of serious human infections. Screening at hospital admission would likely help efforts to determine whether exposure to pet animals and livestock had occurred. However, the absence of specific exposure to zoonotic clonal lineages, as in our case-patient, is a matter of concern in terms of screening and contact tracing policy for MRSA in-