DOI: 10.7589/2016-09-205

Journal of Wildlife Diseases, 53(3), 2017, pp. 616–620

Surveillance for Highly Pathogenic Avian Influenza in Wild Turkeys (*Meleagris gallopavo*) of Minnesota, USA during 2015 Outbreaks in Domestic Poultry

Christopher S. Jennelle,[1,5] Michelle Carstensen,[1] Erik C. Hildebrand,[1] Paul C. Wolf,[2] Daniel A. Grear,[3] Hon S. Ip,[3] and Louis Cornicelli[4] [1]Minnesota Department of Natural Resources, 5463 C West Broadway Ave., Forest Lake, Minnesota 55025, USA; [2]US Department of Agriculture, Animal Plant Health Inspection Service – Wildlife Services, 3503 Old Highway 99 South, Roseburg, Oregon 97471, USA; [3]US Geological Survey, National Wildlife Health Center, 6006 Schroeder Rd., Madison, Wisconsin 53711, USA; [4]Minnesota Department of Natural Resources, 500 Lafayette Rd., St. Paul, Minnesota 55155, USA; [5]Corresponding author (email: christopher.jennelle@state.mn.us)

ABSTRACT: An outbreak of a novel reassortant of highly pathogenic avian influenza A (H5N2) virus (HPAIV) decimated domestic turkeys (*Meleagris gallopavo*) from March through mid-June, 2015 in the state of Minnesota, US. In response, as part of broader surveillance efforts in wild birds, we designed a pilot effort to sample and test hunter-harvested Wild Turkeys (*Meleagris gallopavo*) for HPAIV in Minnesota counties with known infected poultry facilities. We also collected opportunistic samples from dead Wild Turkeys or live Wild Turkeys showing neurologic signs (morbidity and mortality samples) reported by the public or state agency personnel. Cloacal and tracheal samples were collected from each bird and screened for avian influenza virus (AIV) RNA by real-time reverse transcription PCR. From 15 April to 28 May 2015, we sampled 84 hunter-harvested male Wild Turkeys in 11 Minnesota counties. From 7 April 2015 through 11 April 2016, we sampled an additional 23 Wild Turkeys in 17 Minnesota counties. We did not detect type A influenza or HPAIV from any samples, and concluded, at the 95% confidence level, that apparent shedding prevalence in male Wild Turkeys in central Minnesota was between 0% and 2.9% over the sampling period. The susceptibility of wild turkeys to HPAIV is unclear, but regular harvest seasons make this wild gallinaceous bird readily available for future AIV testing.

Key words: Avian influenza, H5N2, highly pathogenic avian influenza, *Meleagris gallopavo*, Minnesota, surveillance, wild turkey, wildlife disease.

Type A influenza viruses (AIVs) occur regularly in reservoir hosts, including waterfowl, gulls, and shorebirds (Webster et al. 1992), and most can be considered as low pathogenicity subtypes because of benign clinical effects of infection in chickens. There is great diversity in AIVs with 144 known subtypes possible–all combinations of viral glycoproteins hemagglutinin and neuraminidase with numerous strains (Fouchier et al. 2005; Alexander 2007). Typically, only H5 and H7 subtypes have the capability to mutate into highly pathogenic forms, which quickly kill domestic poultry and, in some cases, wild birds (Horimoto et al. 1995; Duan et al. 2007).

The novel strain of Eurasian/North American highly pathogenic avian influenza A (H5N2) virus (HPAIV) detected in North America in 2014 (World Health Organization 2015) was responsible for 50 million domestic poultry deaths in the US in 2015 (US Department of Agriculture 2016). It is hypothesized to be dispersed in the environment by movements of wild birds, although recent evidence suggests that HPAIV is not generally maintained in wild bird populations (Krauss et al. 2016). In Minnesota, US, from 3 March through 4 June 2015, the virus was detected in 23 counties with 104 poultry farms confirmed infected, along with six dangerous contacts (i.e., suspicion of exposure, facility depopulated; US Department of Agriculture 2016). Most of the nine million domestic birds killed or euthanized in Minnesota during the outbreaks were turkeys, and the peak of infection was coincidentally near the opening of the spring Wild Turkey (*Meleagris gallopavo*) hunting season, which begins the Saturday closest to 15 April. We initiated a pilot surveillance effort in central Minnesota to determine if hunter-harvested Wild Turkeys were infected and shedding HPAIV during the outbreak period, and whether hunter-harvested Wild Turkeys could constitute a viable AIV surveillance sampling stream.

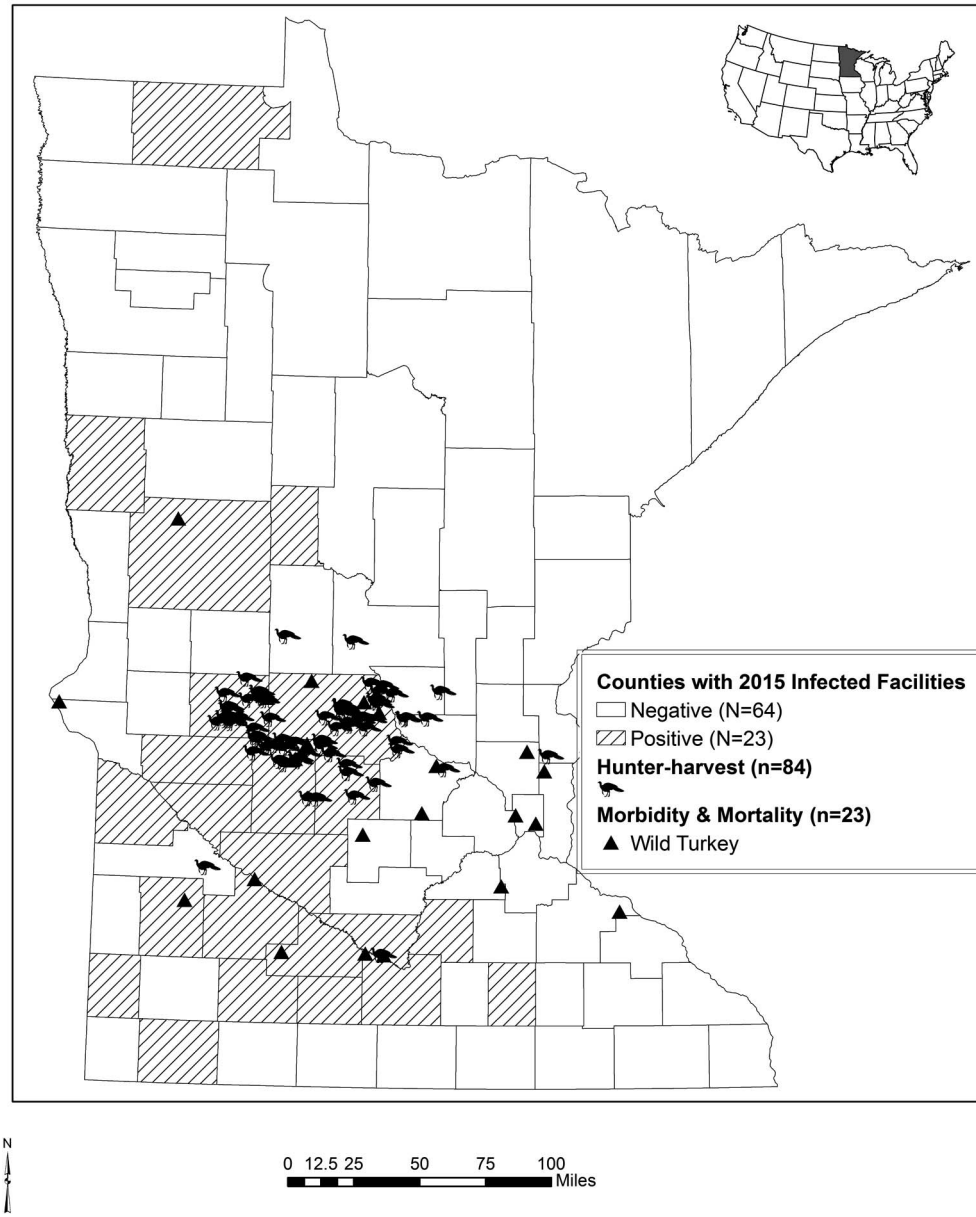

FIGURE 1. Collection sites in Minnesota, USA, for male Wild Turkeys (*Meleagris gallopavo*): hunter-harvested (n=84) and sick or found dead (n=23) were sampled for highly pathogenic avian influenza virus between March 2015 and April 2016.

The study area comprised all of central and southern Minnesota, with efforts focused in four counties (Kandiyohi, Meeker, Pope, and Stearns) where the majority of infected poultry facilities occurred during the 2015 outbreak (Fig. 1). Counties were categorized as either containing facilities with or without HPAIV infection, and we had an overall sample goal of 300 male Wild Turkeys. The Minnesota Department of Natural Resources (MNDNR) published a press release, conducted radio, television, and print interviews, and contacted the Minnesota chapter of the National Wild

Turkey Federation to solicit samples from successful turkey hunters.

We used polyester-tipped swabs to separately sample the trachea and cloaca of male Wild Turkeys. We pooled both swab samples from each bird in a single vial of Brain Heart Infusion media (Docherty and Slota 1988) and kept samples refrigerated until testing, which generally occurred within 1 wk after collection. Hunter provision of harvested turkeys was voluntary, and we recorded harvest location, date, and age.

Through outreach on the MNDNR and Minnesota Board of Animal Health websites and official press releases, we solicited the public and agency personnel (state and federal employees) to report to the MNDNR and the US Geological Survey National Wildlife Health Center (NWHC) any live Wild Turkeys exhibiting neurological symptoms consistent with AIV infection or dead Wild Turkeys that were not associated with roadways. We emphasized the need to report Wild Turkeys as soon as possible to ensure collection of viable tissue samples; generally, we only collected samples from birds that were dead for <24 h. Birds were sampled by MNDNR staff in the same manner as hunter-harvested birds, with separate swabs used for sampling the tracheal and cloacal cavities. These swabs were placed in the same viral transport media vial, and kept cool in a portable cooler with ice packs or a refrigerator until testing. When viral transport media was not available, whole carcasses were collected, double bagged, and refrigerated until samples could be collected.

As per NWHC instructions, swabs from hunter-harvested Wild Turkeys were shipped overnight in coolers with ice packs to Madison, Wisconsin, US for diagnostic testing. Tissue samples for AIV testing were homogenized in viral transport media and centrifuged at 1,000 × G for 30 min at 4 C. The RNA from 50 μL of the supernatant of the tissue homogenate or swab material was recovered and tested for AIV by the current National Animal Health Laboratories Network reverse transcriptase PCR (rRT-PCR) protocols based on Spackman et al. (2002) and Pedersen et al. (2010).

For morbidity and mortality samples, whole carcasses were shipped overnight to the NWHC for necropsy and AIV screening, as mentioned earlier. Swab samples were submitted to the US Department of Agriculture National Wildlife Research Center (Fort Collins, Colorado, USA) or the University of Minnesota Veterinary Diagnostic Lab (St. Paul, Minnesota, USA) for AIV screening using the National Animal Health Laboratories Network protocol as mentioned earlier.

From 15 April through 28 May 2015, we collected tracheal and cloacal swab samples from 84 hunter-harvested male Wild Turkeys in 11 counties of central Minnesota (Fig. 1). From 7 April 2015 through 11 April 2016, we collected tracheal and cloacal swab samples from 23 Wild Turkeys in 17 Minnesota counties as part of our morbidity and mortality sampling efforts (Fig. 1). All 107 turkeys were negative for AIV using rRT-PCR, and we calculated the 95% detection threshold of AIV using methods of Cameron and Baldock (1998). For our calculations, we used a sensitivity of 0.95 and specificity of 1.0. The data suggested that the apparent shedding prevalence in male Wild Turkey was approximately between zero and 2.9% at a 95% confidence level.

Given the large number of domestic turkeys in Minnesota affected by the H5N2 HPAIV outbreak in 2015, it was important to investigate whether Wild Turkeys were infected and shedding this or other AIV. The outbreak coincided with the spring hunting season, which afforded a unique opportunity to collect samples from hunter-harvested Wild Turkeys in counties with high numbers of infected poultry facilities. Although our pilot effort did not achieve the sampling goal, we did not detect AIV from any samples and could conclude that AIV shedding in male turkeys during the study period was no more than 3%. We cannot make strong conclusions about Wild Turkey susceptibility to AIV infection, but previous serosurveys of Wild Turkeys in the US suggest exposure to AIV is uncommon (Davidson et al. 1988; Hopkins et al. 1990;

Charlton 2000; Peterson et al. 2002). Sampling was limited by the number of successful hunters who chose to participate in the surveillance. For future Wild Turkey AIV sampling efforts during harvest seasons, we suggest that agencies make early contact with turkey hunters through press releases and local chapters of organizations such as the National Wild Turkey Federation in order to increase awareness and participation. We also suggest agencies collect data from multiple independent data sources including hunter-harvested (apparently healthy), sick, and found-dead Wild Turkeys to increase the likelihood of detecting AIV in this potential wild gallinaceous host, if it is present. Based on our results, we could not conclude that hunter-harvested Wild Turkeys constitute a valuable surveillance stream for detecting HPAIV in wild birds. However, if agencies can encourage hunter participation, spring and fall turkey harvest seasons lend themselves to a readily available source of this wild gallinaceous bird for AIV testing. Given the ability of AIVs to reassort into novel subtypes (Webster et al. 1992), we recommend consideration of hunter-harvested turkeys for future surveillance. The major challenges to address are how best to encourage hunter participation and provide a mechanism(s) for accessibility to successful hunters in the field (e.g., targeting public access parking areas on public lands).

We thank the wildlife staff from MNDNR who assisted with the sampling of hunter-harvested turkeys as well as the hunters that participated in this project. We also thank the members of the public and MNDNR that reported and collected sick or dead Wild Turkeys. We thank L. Minicucci for useful comments on an earlier draft. Funding was provided by the Minnesota Department of Natural Resources—Division of Fish and Wildlife, US Geological Survey, Ecosystems Mission Area, and US Department of Agriculture—Animal and Plant Health Inspection Service. The use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US government.

LITERATURE CITED

Alexander DJ. 2007. An overview of the epidemiology of avian influenza. *Vaccine* 25:5637–5644.

Cameron AR, Baldock FC. 1998. A new probability formula for surveys to substantiate freedom from disease. *Prev Vet Med* 34:1–17.

Charlton KG. 2000. Antibodies to selected disease agents in translocated wild turkeys in California. *J Wildl Dis* 36:161–164.

Davidson WR, Yoder HW, Brugh M, Nettles VF. 1988. Serological monitoring of eastern wild turkeys for antibodies to *Mycoplasma* spp. and avian influenza viruses. *J Wildl Dis* 24:348–351.

Docherty DE, Slota PG. 1988. Use of muscovy duck embryo fibroblasts for the isolation of viruses from wild birds. *J Tissue Cult Methods* 11:165–170.

Duan L, Campitelli L, Fan XH, Leung YHC, Vijaykrishna D, Zhang JX, Donatelli I, Delogu M, Li KS, Foni E, et al. 2007. Characterization of low-pathogenic H5 subtype influenza viruses from Eurasia: Implications for the origin of highly pathogenic H5N1 viruses. *J Virol* 81:7529–7539.

Fouchier RAM, Munster V, Wallensten A, Bestebroer TM, Herfst S, Smith D, Rimmelzwaan GF, Olsen B, Osterhaus ADME. 2005. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. *J Virol* 79:2814–2822.

Hopkins BA, Skeeles JK, Houghten GE, Slagle D, Gardner K. 1990. A survey of infectious diseases in wild turkeys (*Meleagridis gallopavo silvestris*) from Arkansas. *J Wildl Dis* 26:468–472.

Horimoto T, Rivera E, Pearson J, Senne D, Krauss S, Kawaoka Y, Webster RG. 1995. Origin and molecular changes associated with emergence of a highly pathogenic H5N2 influenza virus in Mexico. *Virology* 213:223–230.

Krauss S, Stallknecht DE, Slemons RD, Bowman AS, Poulson RL, Nolting JM, Knowles JP, Webster RG. 2016. The enigma of the apparent disappearance of Eurasian highly pathogenic H5 clade 2.3.4.4 influenza A viruses in North American waterfowl. *Proc Natl Acad Sci U S A* 113:9033–9038.

Pedersen J, Killian ML, Hines N, Senne D, Panigrahy B, Ip HS, Spackman E. 2010. Validation of a real-time reverse transcriptase-PCR assay for the detection of H7 avian influenza virus. *Avian Dis* 54:639–643.

Peterson MJ, Aguirre R, Ferro PJ, Jones DA, Lawyer TA, Peterson MN, Silvy NJ. 2002. Infectious disease survey of Rio Grande wild turkeys in the Edwards Plateau of Texas. *J Wildl Dis* 38:826–833.

Spackman E, Senne DA, Myers TJ, Bulaga LL, Garber LP, Perdue ML, Lohman K, Daum LT, Suarez DL. 2002. Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. *J Clin Microbiol* 40:3256–3260.

US Department of Agriculture. 2016. *Surveillance plan for highly pathogenic avian influenza in wild migratory birds in the United States*. https://www.

aphis.usda.gov/animal_health/downloads/animal_diseases/ai/2016-hpai-surveillance-plan.pdf. Accessed August 2016.

Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. 1992. Evolution and ecology of influenza A viruses. *Microbiol Rev* 56:152–179.

World Health Organization. 2015. *Summary of immediate notifications and follow-ups—2014. Highly pathogenic avian influenza*. http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=16660. Accessed October 2015.

Submitted for publication 2 September 2016.
Accepted 3 January 2017.