University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

USDA National Wildlife Research Center - Staff Publications | Wildlife Damage Management, Internet Center for

1-1-2009

Serologic Evidence of Avian Influenza (H4N6) Exposure in a Wild-Caught Raccoon

Nathan M. Roberts
USDA/APHIS/WS/NWRC

David J. Henzler

Larry Clark
USDA/APHIS/WS/NWRC

Follow this and additional works at: http://digitalcommons.unl.edu/icwdm_usdanwrc

Part of the Environmental Sciences Commons

Roberts, Nathan M.; Henzler, David J.; and Clark, Larry, "Serologic Evidence of Avian Influenza (H4N6) Exposure in a Wild-Caught Raccoon" (2009). *USDA National Wildlife Research Center - Staff Publications.* Paper 878.
http://digitalcommons.unl.edu/icwdm_usdanwrc/878

This Article is brought to you for free and open access by the Wildlife Damage Management, Internet Center for at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in USDA National Wildlife Research Center - Staff Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

AVIAN DISEASES 53:455–457, 2009

Research Note—

Serologic Evidence of Avian Influenza (H4N6) Exposure in a Wild-Caught Raccoon

Nathan M. Roberts,[ACE] David J. Henzler,[BD] and Larry Clark[A]

[A]United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, 4101 Laporte Avenue, Fort Collins, CO 80521

[B]Commonwealth of Pennsylvania Department of Agriculture, Bureau of Animal Health and Diagnostic Services, 2301 North Cameron Street, Harrisburg, PA 17110

Received 3 September 2008; Accepted and published ahead of print 16 March 2009

SUMMARY. Growing concerns about avian influenza, and its effect on agriculture and human health, have highlighted the need to understand the role of wildlife in maintaining and spreading the virus. We surveyed the wildlife inhabiting a poultry farm with recent H3N6 and H4N6 avian influenza virus exposure in Pennsylvania, U.S.A. One raccoon (*Procyon lotor*) tested positive for H4N6 antibodies. This is the first recorded incident of avian influenza exposure in a wild raccoon. We suggest that raccoons may play a role in the transmission of avian influenza viruses and in compromising biosecurity efforts at poultry operations.

RESUMEN. *Nota de Investigación*—Evidencia serológica de la exposición a influenza aviar (H4N6) en un mapache silvestre. El interés creciente acerca de la influenza aviar y su efecto en la agricultura y en la salud humana, ha destacado la necesidad de entender el papel de la especies silvestres en la preservación y diseminación de este virus. En este estudio, se muestreó la fauna silvestre que habitaba una granja avícola en la que había ocurrido recientemente una exposición a los subtipos H3N6 y H4N6 del virus de la influenza aviar en el estado de Pennsylvania en los Estados Unidos. Un mapache común (*Procyon lotor*) mostró un resultado positivo a la presencia de anticuerpos para H4N6. Este es el primer incidente reportado acerca de la exposición de un mapache silvestre al virus de la influenza aviar. En este estudio, se sugiere que los mapaches pudieran tener un papel en la transmisión de los virus de influenza aviar y también pudieran vulnerar los esfuerzos de bioseguridad en las operaciones avícolas.

Key words: avian influenza, avian flu, bird flu, biosecurity, wildlife, raccoon, H4N6, *Procycon lotor*, poultry, wild animals, furbearer

Abbreviations: AGID = agar gel immunodiffusion; AIV = avian influenza viruses; HI = hemagglutination inhibition; NVSL = National Veterinary Services Laboratory

Biosecurity efforts on poultry operations typically focus on reducing the potential of human activity, including the movement of poultry and poultry products, in controlling the spread of disease and the containment of infected flocks. Although the role of wildlife in compromising biosecurity is not well understood, the role of wildlife as reservoirs and vectors for influenza viruses has been widely recognized. This is especially true in the case of avian influenza viruses (AIV). Wild bird species have been identified as potential AIV hosts and reservoirs (5,18,20). Unfortunately, very little is known about AIV in wild mammals, despite the fact that numerous wild mammalian species are commonly found in or near poultry facilities.

Mammals that have been recognized as hosts for AIVs include seals, whales, ferrets, and swine (4,7,8,11,12,15). AIV H4N6, most likely of avian origin, was isolated from morbid seals in January of 1991 in Massachusetts, U.S.A. (7). Bailey (2) identified AIV antibodies in a raccoon following an experimental exposure to a H1N1 virus. Similarly, H1N1 virus was isolated from a skunk (*Mephitis mephitis*) following an experimental exposure to this virus (2). Additionally, mice and rats experimentally infected with AIV have demonstrated successful replication of H5N1 and H7N2 viruses (10,17). However, AIV could not be isolated from wild house mice (*Mus musculus*) on poultry farms during an outbreak of low-pathogenic AIV subtype H7N2 (10). Recently, the African–Eurasian highly pathogenic AIV subtype H5N1 has been identified in tigers (*Panthera tigris*), leopards (*Panthera pardus*), and domestic cats (*Felis silvestris catus*) (1,14,19,22). While the primary route of infection in these feline species is thought to be associated with consumption of bird carcasses, horizontal transmission among tigers was also suspected (1,14,19,22). This report documents AIV subtype H4N6 exposure in a wild raccoon (*Procyon lotor*) present on an AIV-infected domestic duck farm in Pennsylvania, U.S.A.

MATERIALS AND METHODS

An avian influenza serologic survey of wildlife inhabiting a poultry operation in Franklin County, Pennsylvania, U.S.A. was conducted in September of 2004. This poultry operation consisted of various duck species, domestic turkeys, and chickens. AIV activity had been identified through routine serologic surveillance in ducks at this farm 4 wk earlier, and again 1 wk prior to our visit, by the Pennsylvania Department of Agriculture. Subsequent subtyping of these seropositive sera identified the H3N6 and H4N6 subtypes.

Wild birds were collected with mist nets. Mammals were captured using Sherman™ (H. B. Sherman Trap, Tallahassee, FL) and Tomahawk ™ (Tomahawk Live Trap Co., Tomahawk, WI) live-capture traps. Wild birds were euthanatized by cervical dislocation, and mammals were euthanatized in a CO_2 chamber. Blood was collected immediately following euthanasia. Serum samples were tested for exposure to AIV by agar gel immunodiffusion (AGID) (21).

[C]Present address: Cornell University, College of Agriculture and Life Sciences, Department of Natural Resources, Fernow Hall, Ithaca, NY 14853.

[D]Present address: North Central West Virginia Veterinary Emergency Clinic, Route 3, Box 336-H12, Fairmont, WV 26554.

[E]Corresponding author. E-mail: nmr25@cornell.edu

Avian Diseases avdi-53-03-04.3d 21/7/09 13:38:44 455 Cust # 8459-082708-RESNOTER

This article is a U.S. government work, and is not subject to copyright in the United States.

Confirmation of positive results, and subtyping of antibodies, was conducted at the United States Department of Agriculture's National Veterinary Services Laboratory (NVSL) in Ames, Iowa, U.S.A. following the protocol outlined by Swayne (21). This protocol tests for the 15 known hemagglutin and 9 neuraminidase subtypes and eliminates the potential for nonspecific inhibition by using a hemagglutination inhibition (HI) reference reagent that has a heterologous neuraminidase subtype.

RESULTS

A total of 25 house sparrows (*Passer domesticus*), 10 European starlings (*Sturnus vulgaris*), two house mice, one woodchuck (*Marmota monax*), and one raccoon (*Procyon lotor*) were collected. Only 1 specimen, the raccoon, tested positive for exposure to AIV. This specimen tested positive for AIV antibodies using the AGID test, and these results were confirmed by the United States Department of Agriculture's NVSL. The H4 hemagglutin was positive at a dilution of ≥1:32. Serologic subtyping further identified these antibodies as specific to the H4N6 AIV virus.

DISCUSSION

Exposure to AIV has never before been documented in wild raccoons. Exposure to H4N6 has been documented in wild mallard ducks (*Anas platyrhnchos*) (23), harbor seals (*Phoca vitulina*) along the northeastern coast of the United States (3), and in pigs with pneumonia at a commercial swine operation in Canada (13). The lack of previous evidence of AIV exposure in wild raccoons may simply be an artifact of an absence of similar studies. Even so, subsequent work, initiated as a result of this finding, suggests that the host potential of wild raccoons may be more significant than was previously thought (9). However, it is possible that these results are evidence of disease transmission between species. The stomach of this raccoon contained a large quantity of poultry feed, confirming contact between the raccoon and the poultry facility. Furthermore, both the raccoon and the poultry on this farm were seropositive for the same AIV subtype, H4N6. The sample size of the present study makes extrapolation of these results problematic. While the host or reservoir capability of raccoons is unknown, similarly, it is unknown if raccoons can shed sufficient virus to spread disease. Even so, these results suggest that raccoons may play a role in compromising biosecurity methods aimed at controlling AIV.

Raccoon ecology and the behavior of raccoons may support the transmission of AIV and other pathogens across agricultural landscapes. Raccoons are semi-aquatic mesocarnivores that thrive in a number of rural and suburban settings, are highly mobile, and have home-ranges extending from 5–5000 ha (16). Pennsylvania has numerous duck flocks raised for live bird markets, and many of these flocks are reared in open pastures and with minimal confinement. Frequently, these flocks have access to small- and mid-sized streams and ponds on the premises. These environments are ideal for semi-aquatic carnivores, including raccoons and mink (*Mustela vison*). In addition, wild waterfowl also often inhabit these ecosystems. The combination of outdoor rearing of waterfowl and the resulting possibilities of water-source contamination with fecal material from infected ducks (either domestically reared, wild resident, or migrating), along with the direct access to poultry feed by mammals, including raccoons, provides ample opportunity for fecal–oral transmission of AIVs to mammals. Raccoons and other mammals can traverse home ranges that could encompass multiple duck flocks and poultry farms. This behavior could serve as a route of disease spread through either direct shedding or through horizontal mechanical routes.

Waterfowl are typically asymptomatic carriers of AIVs, making it difficult to detect viruses based on clinical signs and mortality. However, the highly pathogenic African-Eurasian avian influenza H5N1 has resulted in mortality in waterfowl and other migrating birds (6). Mortality in wild birds as a result of AIVs could result in scavenging of carcasses by mesocarnivores. This may be a cause for concern, as transmission has been documented in tigers, leopards, and a domestic cat through feeding on contaminated poultry and pigeon carcasses (14,19).

Continued interactions of mammals and birds increase the likelihood for receptor-binding changes, mixed infections, and a potential for genetic reassortment, thereby allowing for novel influenza viruses to emerge both in well-documented mammalian hosts and perhaps in newly identified mammals. Raccoons frequent domestic dog and cat feed bowls left outdoors and also frequent human dwellings, increasing the potential for human contact. Furthermore, raccoons are often considered a game animal and are frequently harvested for both fur and human consumption. Pennsylvania licenses over 20,000 individuals to harvest raccoons within specified hunting and trapping seasons (Pennsylvania Game Commission, pers. comm.).

The finding in the present study, and the ecologic considerations of potential host and vector species, suggests that collection of both nasal swabs and serum from raccoons for monitoring of AIVs on duck and other poultry farms may be warranted. Exclusion of raccoons and other mammals from farms should be emphasized as routine biosecurity measures to control AIV.

REFERENCES

1. Amonsin, A., S. Payungporn, A. Theamboonlers, R. Thanawongnuwech, S. Suradhat, N. Pariyothorn, R. Tantilertcharoen, S. Damrongwantanapokin, C. Buranathai, A. Chaisingh, T. Songserm, and Y. Poovorawan. Genetic characterization of H5N1 influenza A viruses isolated from zoo tigers in Thailand. Virology 344:480–491. 2006.

2. Bailey, C. F. Experimental infection of raccoon, skunk, and thirteen-lined ground squirrel with avian derived influenza A viruses. M.S. Thesis. University of Minnesota, Minneapolis, Minnesota. 37 pp. 1983.

3. Callan, R. J., G. Early, H. Kida, and V. S. Hinshaw. The appearance of H3 influenza viruses in seals. J. Gen. Virol. 76:199–203. 1995.

4. Choi, Y. K., J. H. Lee, G. Erickson, S. M. Goyal, H. S. Joo, R. G. Webster, and R. J. Webby. H3N2 influenza virus transmission from swine to turkeys, United States. Emerging Infect. Dis. 10:2156–2160. 2004.

5. Clark, L., and J. Hall. Avian influenza in wild birds: status as reservoirs, and risk to humans and agriculture. Ornithol. Monograph. 60:3–29. 2006.

6. Feare, C. J. The role of wild birds in the spread of HPAI H5N1. Avian Dis. 50:440–447. 2007.

7. Gallan, R. J., G. Early, H. Kida, and V. S. Hinshaw. The appearance of H3 influenza viruses in seals. J. Gen. Virol. 76:199–203. 1995.

8. Geraci, J. R., D. J. St. Aubin, I. K. Barker, R. G. Webster, V. S. Hinshaw, W. J. Bean, H. L. Ruhnke, J. H. Prescott, G. Early, A. S. Barker, S. Madoff, and R. T. Schooley. Mass mortality of harbor seals: pneumonia associated with influenza A virus. Science New Series, 215, no. 4536: 1129–1131. 1982.

9. Hall, J. S., K. T. Bentler, G. Landolt, S. A. Elmore, R. B. Minnis, T. A. Campbell, S. C. Barras, J. J. Root, J. Pilon, K. Pabilonia, C. Driscoll, D. Slate, H. Sullivan, and R. G. McLean. Influenza infection in wild raccoons. Emerging Infect. Dis. 14:12. 2008. Available from http://www.cdc.gov/EID/content/14/12/1842.htm.

10. Henzler, D. J., D. C. Kradel, S. Davison, Z. F. Ziegler, D. Singletary, P. Debok, A. E. Castro, H. Lu, R. Eckroade, D. Swayne, W. Lagoda, B. Schmucker, and A. Nesselrodt. Epidemiology, production losses, and

control measures associated with an outbreak of avian influenza subtype H7N2 in Pennsylvania (1996–98). Avian Dis. 47:1022–1036. 2003.

11. Hinshaw, V. S., W. J. Bean, J. Geraci, P. Fiorelli, B. Early, and R. G. Webster. Characterization of two influenza A viruses from a pilot whale. J. Virol. 58:655–656. 1986.

12. Hinshaw, V. S., R. G. Webster, B. C. Easterday, and W. J. Bean Jr. Replication of avian influenza viruses in mammals. Infect. Immun. 34:354–361. 1981.

13. Karasin, A. I., I. H. Brown, S. Carman, and C. W. Olsen. Isolation and characterization of H4N6 avian influenza viruses from pigs with pneumonia in Canada. J. Virol. 74:9322–9327. 2000.

14. Keawcharoen, J., K. Oraveerakul, T. Kuiken, R. A. M. Fouchier, A. Amonsin, S. Payungporn, S. Noppornpanth, S. Wattanodorn, A. Theamboonlers, R. Tantilertcharoen, R. Pattanarangsan, N. Arya, P. Ratanakorn, A. D. M. E. Osterhaus, and Y. Poovorawan. Avian influenza H5N1 in tigers and leopards. Emerging Infect. Dis. 10:2189–2191. 2004.

15. Lvov, D. K., V. M. Zdanov, A. A. Sazonov, N. A. Braude, E. A. Vladimirteeva, L. V. Agafonova, E. I. Skljanskaja, N. V. Kaverin, V. I. Reznik, T. V. Pysina, A. M. Oserovic, A. A. Berzin, I. A. Mjasnikova, R. Y. Podcernjeva, S. M. Klimenko, V. P. Andrejev, and M. A. Yakhno. Comparison of influenza viruses isolated from man and from whales. Bull. W.H.O. 56:923–930. 1978.

16. Sanderson, G. C. Raccoon. In: Wild furbearer management and conservation in North America. Novak, M., J. A. Baker, M. E. Obbard, and B. Malloch, eds. Ontario Trappers Association, North Bay, Ontario, Canada. pp. 487–499. 1987.

17. Shortridge, K. F., N. N. Zhou, Y. Guan, P. Gao, T. Ito, Y. Kawaoka, S. Kodihalli, S. Krauss, D. Markwell, K. G. Murti, M. Norwood, D. Senne, L. Sims, A. Takada, and R. Webster. Characterization of avian influenza H5N1 influenza viruses from poultry in Hong Kong. Virology 252:331–342. 1998.

18. Slemons, R. D., M. C. Shieldcastle, L. D. Heyman, K. E. Bednarik, and D. A. Senne. Type A influenza viruses in waterfowl in Ohio and implications for domestic turkeys. Avian Dis. 35:165–173. 1991.

19. Songserm, T., A. Amonsin, R. Jam-on, N. Sae-Heng, N. Meemak, N. Pariyothron, S. Payungporn, S. Payungporn, A. Theamboonlers, and Y. Poovorawan. Avian influenza H5N1 in naturally infected domestic cat. Emerging Infect. Dis. 12:681–683. 2006.

20. Stallknecht, D. E. Ecology and epidemiology of avian influenza viruses in wild bird populations: waterfowl, shorebirds, pelicans, cormorants, etc. In: Avian Dis. Special Issue, Fourth International Symposium on Avian Influenza. 1997 Proceedings 47:61–69. 2003.

21. Swayne, D. E., D. A. Senne, and C. W. Beard. Avian influenza. In: Laboratory manual for the isolation and identification of avian pathogens. Swayne, D. E., F. R. Glisson, M. W. Jackwood, J. E. Pearson, and W. M. Reed, eds. Kennett Square, PA: American Association of Avian Pathologists, pp. 150–155. 1998.

22. Thanawongnuwech, R., A. Amonsin, R. Tantilertcharoen, S. Damrongwatanapokin, A. Theamboonlers, S. Payungporn, K. Nanthaporn-phiphat, S. Ratanamungklanon, E. Tunak, T. Songserm, V. Vivatthanava-nich, T. Lekdumrongsak, S. Kesdangsakinwut, S. Tunhikorn, and Y. Poovorawan. Probable tiger-to-tiger transmission of avian influenza H5N1. Emerging Infect. Dis. 11:699–701. 2005.

23. Widjaja, L., S. L. Krauss, R. J. Webby, T. Xie, and R. S. Webster. Matrix gene of influenza A viruses isolated from wild aquatic birds: ecology and emergence of influenza A viruses. J. Virol. 78:8771–8779. 2004.

ACKNOWLEDGMENT

We would like to thank Dr. Janice Pederson at the National Veterinary Services Laboratory for providing diagnostic testing and subtyping of samples.