

Antibodies to Avian Influenza Viruses in Canada Geese (*Branta canadensis*): A Potential Surveillance Tool?

Whitney M. Kistler,^{1,2,5} David E. Stallknecht,² Thomas J. Deliberto,³ Seth Swafford,³ Kerri Pedersen,³ Kyle Van Why,³ Paul C. Wolf,³ Jerry A. Hill,³ Darren L. Bruning,³ James C. Cumbee,³ Randall M. Mickley,³ Carl W. Betsill,³ Adam R. Randall,³ Roy D. Berghaus,⁴ and Michael J. Yabsley^{1,2} ¹Daniel B. Warnell School of Forestry and Natural Resources, 180 East Green Street, University of Georgia, Athens, Georgia 30602, USA; ²Southeastern Cooperative Wildlife Disease Study, Department of Population Health, 589 D.W. Brooks Drive, Wildlife Health Building, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602, USA; ³United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, Colorado, USA; ⁴Department of Population Health, College of Veterinary Medicine, 501 D.W. Brooks Drive, University of Georgia, Athens, Georgia 30602, USA; ⁵Corresponding author (email: wkistler@uga.edu)

ABSTRACT: Traditionally, the epidemiology of avian influenza viruses (AIVs) in wild birds has been defined by detection of virus or viral RNA through virus isolation or reverse-transcription polymerase chain reaction. Our goals were to estimate AIV antibody prevalence in Canada geese (*Branta canadensis*) and measure effects of age and location on these estimates. We collected 3,205 samples from nine states during June and July 2008 and 2009: Georgia, Massachusetts, Minnesota, Mississippi, New Jersey, North Carolina, Pennsylvania, Washington, and West Virginia. Serum samples were tested for AIV antibodies with the use of a commercial blocking enzyme-linked immunosorbent assay. Overall, 483 (15%) Canada geese had detectable antibodies to AIV. Significantly higher prevalences were detected in geese collected from northeastern and upper midwestern states compared with southeastern states. This trend is consistent with results from virus isolation studies reporting AIV prevalence in North American dabbling ducks. Within Pennsylvania, significantly higher antibody prevalences were detected in goose flocks sampled in urban locations compared to flocks sampled in rural areas. Antibody prevalence was significantly higher in after-hatch-year geese compared to hatch-year geese. No significant differences in prevalence were detected from 10 locations sampled during both years. Results indicate that Canada geese are frequently exposed to AIVs and, with resident populations, may potentially be useful as sentinels to confirm regional AIV transmission within wild bird populations.

Key words: Avian influenza virus, blocking ELISA, *Branta canadensis* (Canada goose), serology.

Virus isolation and reverse-transcription polymerase chain reaction (RT-PCR) have been the primary tools used to advance our understanding the epidemiology of

avian influenza viruses (AIVs) in wild bird populations (Olsen et al., 2006; Munster et al., 2007); however, both are only effective during the limited time when birds are shedding virus. Recently, a commercial blocking enzyme-linked immunosorbent assay (bELISA) was validated for use in wild birds, and Brown et al. (2009) suggested that serologic testing of wild birds could provide supportive data to advance our current understanding of AIV epidemiology, especially where viral detection is difficult.

Canada geese (*Branta canadensis*) are experimentally susceptible to infection and mount a detectable antibody response, but virus shedding is brief (up to 6 days; Pasick et al., 2007) and reported viral detection prevalence estimates are consistently low (<2%; Winkler et al., 1972; Hinshaw et al., 1986; Harris et al., 2010). In the United States, Canada geese are numerous and exist as resident populations over much of their range (Hestbeck, 1995). They also are found in all 50 states, utilize the same habitats as dabbling ducks (a recognized reservoir for AIVs), and are frequently and easily captured for relocation, banding, and for nuisance removals. Our objectives were 1) to determine regional and local differences in AIV antibody prevalence in resident Canada geese within the United States, and 2) to assess the potential to utilize serologic testing of resident Canada geese as a sentinel system to detect regional or local AIV transmission.

TABLE 1. Prevalence of antibodies to avian influenza in 3,205 Canada geese (*Branta canadensis*) from nine US states as determined by blocking enzyme-linked immunosorbent assay and prevalence of viral RNA detection for 685 Canada geese with the use of a matrix real-time reverse-transcriptase polymerase chain reaction (rRT-PCR).

State	Number of sample locations		2008		2009		Total		rRT-PCR (2008–2009)	
	2008	2009	<i>n</i>	Positive (%)	<i>n</i>	Positive (%)	<i>n</i>	Positive (%)	<i>n</i>	Positive (%)
Georgia	1	10	56	6 (13)	301	20 (6)	357	26 (7)	0	0 (0)
Massachusetts	4	0	19	6 (32)	0	0	19	6 (32)	34	0 (0)
Minnesota	4	7	83	19 (23)	143	55 (38)	226	74 (33)	0	0 (0)
Mississippi	4	7	112	1 (0.9)	128	0	240	1 (0.4)	213	0 (0)
New Jersey	9	25	163	25 (15)	537	123 (23)	700	148 (21)	100	6 (6)
North Carolina	4	4	115	1 (0.9)	129	3 (2)	244	4 (2)	244 ^a	0 (0)
Pennsylvania	4	29	132	33 (25)	694	140 (20)	826	174 (21)	0	0 (0)
Washington	4	5	144	10 (7)	245	26 (11)	389	36 (9)	14	0 (0)
West Virginia	4	5	124	10 (8)	80	5 (6.3)	204	15 (7)	80	0 (0)
Total	38	92	948	111 (12)	2,257	372 (16)	3,205	483 (15)	685	6 (0.9)

^a Same geese tested for rRT-PCR and serologic testing.

In June and July 2008 and 2009, we collected serum from 3,205 Canada geese from multiple locations in nine states (Table 1) during banding and nuisance removal. In addition, we collected and preserved combined cloacal/oropharyngeal swabs during both years as previously reported (Swayne et al., 2008). Serum samples were tested for AIV antibodies with a bELISA (IDEXX Laboratories, Westbrook, Maine, USA). Cloacal and oropharyngeal swabs were tested at the National Animal Health Laboratory Network with the use of a real-time RT-PCR targeting the matrix gene (Spackman et al., 2002). All work was approved by the University of Georgia Animal Care and Use Protocol A2010 06-101.

To understand local variation better, we sampled 10 locations in 2008 and 2009. In five of the locations the geese were euthanized and in the other five locations the geese were released back on location ($n=5$ southeastern Pennsylvania and $n=5$ southern New Jersey). In addition, we categorized Pennsylvania locations sampled in 2009 into urban ($n=15$) and rural ($n=13$) categories with the use of ArcMap v10 (ESRI, Redlands, California, USA) and a Pennsylvania

Department of Transportation urban boundaries map, which bases urban and rural categories on population numbers from the US Census Bureau. We used 2009 Pennsylvania locations for this analysis because of the number of locations ($n=28$), and we were able to sample locations across the entire state.

We used population-averaged generalized estimating equations (GEE) logistic regression to compare differences in antibody prevalence estimates on a regional scale (by latitude, Table 2), between years (Table 2), and on a local scale with the use of the 10 locations sampled in both years and urban vs. rural data from Pennsylvania 2009 (Table 3). We used population-averaged GEE because it accounts for clustering of sample locations. (Hanley et al., 2003) and significance was based on 95% confidence intervals for the odds ratio. In addition, we used a χ^2 test for independence to compare antibody prevalence in after-hatch-year geese and hatch-year geese.

We detected antibodies in 483 (15%) of 3,205 Canada geese by the bELISA and the AIV matrix gene in six (0.9%) of 685 cloacal/oropharyngeal swabs (Table 1).

TABLE 2. Avian influenza antibody prevalence in Canada geese determined by blocking enzyme-linked immunosorbent assay compared by latitude and year with the use of generalized estimating equations (GEE) logistic regression model.

Variable	<i>n</i>	Positive (%)	Odds ratio (95% confidence interval) ^a
Latitude (degrees)			
44–48.9	615	110 (18)	6.3 (2.8–13.8)
39–43.9	1,564	328 (21)	7.1 (3.6–14)
34–38.9	570	26 (5)	1.4 (0.6–3.4)
29–33.9	456	19 (4.2)	Referent ^b
Year			
2008	948	111 (12)	Referent
2009	2,257	372 (16)	1.4 (1.0–2.0)

^a Based on a multivariable GEE logistic model adjusted for clustering of samples by location.

^b Referent is the comparison group.

We found higher antibody prevalence in after-hatch-year geese ($n=2,391$) compared with hatch-year geese ($n=518$; 17% and 1.9% respectively, $\chi^2=78.7$, $P<0.001$).

When we analyzed the data on a regional scale, geese sampled at higher latitudes had significantly higher antibody prevalence than those sampled at the lowest latitude, but we detected no significant differences between 2008 and 2009 (Table 2). When we analyzed the data on a local scale we found no differences among the 10 locations sampled in both years, regardless if the birds were released or euthanized (Table 3). We did, however, detect higher antibody prevalence in urban sample locations than rural locations sampled in Pennsylvania in 2009 (Table 3).

Our work is the first large-scale study to show that Canada geese are frequently exposed to and develop antibodies to AIVs; however, consistent with previous studies (Winkler et al., 1972; Hinshaw et al., 1986), we detected a low prevalence of viral infection in geese. The increase in antibody prevalence with latitude follows the similar trends of virus isolations seen consistently in North American dabbling ducks (Hinshaw et al., 1985; Stallknecht et al., 1990), suggesting a common source. These regional trends were consistent between years, as evidenced by a failure to detect differences in antibody prevalence at 10 locations sampled in both years. Although we were unable to detect significant differences between 2008 and 2009, our results indicate that there are small fluctuations in antibody prevalence

TABLE 3. Avian influenza antibody prevalence in Canada geese as determined by blocking enzyme-linked immunosorbent assay comparing 10 locations sampled in 2008 and 2009 and the 2009 Pennsylvania locations divided into urban and rural sample locations.

Variable	Number of sample locations	<i>n</i>	Positive (%)	Odds ratio (95% confidence interval)
Euthanized	5	302	82 (27)	1.2 (0.9–1.6)
Released	5	278	53 (19)	Referent
2008	10	304	65 (21)	Referent
2009	10	276	70 (25)	1.2 (0.2–2.5)
Pennsylvania urban ^a	15	422	107	3.7 (1.7–6.0)
Pennsylvania rural ^a	13	239	27	Referent

^a GPS data were not available for one location.

between years, and our sample size may not have been sufficient to detect significant differences.

On a local (within state) scale, differences in antibody prevalence were detected with higher prevalence estimates from geese sampled in urban compared to rural areas. Only data from Pennsylvania were used for this analysis because sample sizes from other states were not sufficient. However, a relatively high (compared to other southeastern states) antibody prevalence was observed in 8 of 10 sites in Georgia that were within the Atlanta metropolitan area. These observed prevalence differences may reflect a true difference in transmission or an artifact, such as increased survival of geese in urban areas (Balkcom, 2010). With regard to the latter, however, the duration of the detectable immune response in naturally infected Canada geese is unknown. The detection of antibodies in hatch-year birds sampled in June and July was unexpected, especially in southern locations where geese have been consistently negative by virus isolation (Harris et al., 2010). These positive results could have resulted from a very low level of transmission during late spring and early summer or passive transfer of antibodies as described in gulls and geese (Bönner et al., 2004; Velarde et al., 2010). The local variation in antibody prevalence we detected should be analyzed with caution because some flocks undergo molt migrations (Dieter and Anderson, 2009) and other movements (Dunton and Combs, 2010) that can affect exposure in geese. In addition, differences in local habitat, such as water temperature and macroinvertebrate community, may play a role in exposure to AIVs at local levels (Nazir et al., 2011; Abbas et al., 2012).

Our regional AIV antibody prevalence estimates from Canada geese reflect a distribution that is consistent with AIV isolation trends in North American ducks. We believe that Canada geese could be

used as an inexpensive serologic sentinel system to monitor regional trends in AIV transmission. Major disadvantages of this approach include a lack of subtype or virus-specific (e.g., H5N1) data and the acquisition of isolates for characterization. For this reason, we believed that such a system should be further evaluated as a supplement to guide traditional virus-detection-based surveillance approaches, but not as a replacement. On a local scale, the utility of this system is questionable. We were able to detect differences at the state level and smaller; however, the detection of this variation deserves additional attention. If the local differences we detected are related to differences in transmission potential across the landscape, understanding these differences could provide a better understanding of the risk to both domestic animals and humans.

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LITERATURE CITED

- ABBAS, M. D., J. NAZIR, P. STUMPF, AND R. E. MARSCHANG. 2012. Role of water fleas (*Daphnia magna*) in the accumulation of avian influenza viruses from the surrounding water. *Intervirology*. doi: 10.1159/000334691.
- BALCOM, G. D. 2010. Demographic parameters of rural and urban adult resident Canada geese in Georgia. *Journal of Wildlife Management* 74: 120–123.
- BÖNNER, B. M., W. LUTZ, S. JÄGER, T. REDMANN, B. REINHARDT, U. REICHEL, V. KRAJEWSKI, R. WEISS, J. WISSING, W. KNICKMEIER, W. H. GERLICH, U. C. WEND, AND E. F. KALETA. 2004. Do Canada geese (*Branta canadensis* Linnaeus, 1758) carry infectious agents for birds and man? *European Journal of Wildlife Research* 50: 78–84.
- BROWN, J. D., D. E. STALLKNECHT, R. D. BERGHAUS, M. P. LUTTRELL, K. VELEK, W. KISTLER, T. P. COSTA, M. J. YABSLEY, AND D. SWAYNE. 2009. Evaluation of a commercial blocking enzyme-linked immunosorbent assay to detect avian influenza virus antibodies in multiple experimentally infected avian species. *Clinical and Vaccine Immunology* 16: 824–829.
- DIETER, C. D., AND B. J. ANDERSON. 2009. Molt migration by giant Canada geese in eastern South Dakota. *Human–Wildlife Conflicts* 3: 260–270.
- DUNTON, E. M., AND D. L. COMBS. 2010. Movements, habitat selection, associations, and survival of giant Canada Goose broods in central Tennessee. *Human–Wildlife Interactions* 4: 192–201.
- HANLEY, J. A., A. NEGASSA, M. E. D. EDWARDES, AND J. E. FORRESTER. 2003. Statistical analysis of correlated data using generalized estimating equations: An orientation. *American Journal of Epidemiology* 157: 364–375.
- HARRIS, M. T., J. D. BROWN, V. H. GOEKJIAN, M. P. LUTTRELL, R. L. POULSON, B. R. WILCOX, D. E. SWAYNE, AND D. E. STALLKNECHT. 2010. Canada geese and the epidemiology of avian influenza viruses. *Journal of Wildlife Diseases* 46: 981–987.
- HESTBECK, J. B. 1995. Population study and management of Atlantic flyway Canada geese. *Journal of Applied Statistics* 22: 877–890.
- HINSHAW, V. S., J. M. WOOD, R. G. WEBSTER, R. DEIBEL, AND B. TURNER. 1985. Circulating influenza and paramyxoviruses in waterfowl from two different areas of North America. *Bulletin of the World Health Organization* 63: 711–719.
- , V. F. NETTLES, L. F. SCHORR, J. M. WOOD, AND R. G. WEBSTER. 1986. Influenza virus surveillance in waterfowl in Pennsylvania after the H5N2 avian outbreak. *Avian Diseases* 30: 207–212.
- MUNSTER, V. J., C. BAAS, P. LEXMOND, J. WALDENSTRÖM, A. WALLENSTEN, T. FRANSSON, G. F. RIMMELZWAAN, W. E. P. BEYER, M. SCHUTTEN, B. OLSEN, A. D. M. E. OSTERHAUS, AND R. A. M. FOUCHIER. 2007. Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. *PLoS Pathogens* 3: e61.
- NAZIR, J., R. HAUMACHER, A. C. IKE, AND R. E. MARSCHANG. 2011. Persistence of avian influenza viruses in lake sediment, duck feces, and duck meat. *Applied and Environmental Microbiology* 77: 4981–4985.
- OLSEN, B., V. J. MUNSTER, A. WALLENSTEN, J. WALDENSTRÖM, A. D. M. E. OSTERHAUS, AND R. A. M. FOUCHIER. 2006. Global patterns of influenza A virus in wild birds. *Science* 312: 384–388.
- PASICK, J., Y. BERHANE, C. EMBURY-HYATT, J. COPPS, H. KEHLER, K. HANDEL, S. BABIUK, K. HOOPER-MCGREY, Y. LI, Q. M. LE, AND S. L. PHUONG. 2007. Susceptibility of Canada geese (*Branta canadensis*) to highly pathogenic avian influenza virus (H5N1). *Emerging Infectious Diseases* 13: 1821–1827.
- SPACKMAN, E., D. A. SENNE, T. J. MYERS, L. L. BULAGA, L. P. GARBER, M. L. PERDUE, K. LOHMAN, L. T. DAUM, AND D. L. SUAREZ. 2002. Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the Avian H5 and H7 hemagglutinin subtypes. *Journal of Clinical Microbiology* 40: 3256–3260.
- STALLKNECHT, D. E., S. M. SHANE, P. J. ZWANK, D. A. SENNE, AND M. T. KEARNEY. 1990. Avian influenza viruses from migratory and resident ducks of coastal Louisiana. *Avian Diseases* 34: 398–405.
- SWAYNE, D. E., D. A. SENNE, AND D. L. SUAREZ. 2008. Influenza, 5th edition. *In* A laboratory manual for the isolation, identification, and characterization of avian pathogens, L. Dufour-Zavala, D. E. Swayne, J. R. Glisson, J. E. Pearson, W. M. Reed, M. W. Jackwood and P. R. Woolcock (eds.). American Association of Avian Pathologists, Kennett Square, Pennsylvania. pp. 128–134.
- VELARDE, R., S. E. CALVIN, D. OJKIC, I. K. BARKER, AND É. NAGY. 2010. Avian influenza virus H13 circulating in ring-billed gulls (*Larus delawarensis*) in southern Ontario, Canada. *Avian Diseases* 54: 411–419.
- WINKLER, W. G., D. O. TRAINER, AND B. C. EASTERDAY. 1972. Influenza in Canada geese. *Bulletin of the World Health Organization* 47: 507–513.

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