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Subtype-specific influenza A virus antibodies in Canada geese (*Branta canadensis*)

Whitney M. Kistler [a,b,*], David E. Stallknecht [a], Thomas J. DeLiberto [c], Kyle Van Why [c], Michael J. Yabsley [a,b]

[a] Southeastern Cooperative Wildlife Disease Study, Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, United States
[b] Daniel B. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602, United States
[c] United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Fort Collins, CO 80521, United States

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ABSTRACT

Historically, surveillance for influenza A viruses (IAVs) in wild birds has relied on viral detection assays. This was largely due to poor performance of serological assays in wild birds; however, recently developed commercial serological assays have improved the ability to detect IAV antibodies in wild birds. Serological surveillance for IAV antibodies in Canada geese (*Branta canadensis*) has shown that, despite a low prevalence of virus isolations, Canada geese are frequently exposed to IAVs and that exposure increases with latitude, which follows virus isolation prevalence patterns observed in dabbling ducks. The objectives of this study were to further evaluate IAV antibodies in Canada geese using a subtype-specific serological assay to determine if Canada geese are exposed to subtypes that commonly circulate in dabbling ducks. We collected serum samples from Canada geese in Minnesota, New Jersey, Pennsylvania, and Wisconsin and tested for antibodies to IAVs using a blocking ELISA. Positive samples were further tested by hemagglutination inhibition for 10 hemagglutinin IAV subtypes (H1–H10). Overall, we detected antibodies to NP in 24% (714/2919) of geese. Antibodies to H3, H4, H5, and H6 subtypes predominated, with H5 being detected most frequently. A decrease in H5 HI antibody prevalence and titers was observed from 2009 to 2012. We also detected similar exposure pattern in Canada geese from New Jersey, Minnesota, Washington and Wisconsin. Based on the published literature, H3, H4, and H6 viruses are the most commonly reported IAVs from dabbling ducks. These results indicate that Canada geese also are frequently exposed to viruses of the same HA subtypes; however, the high prevalence of antibodies to H5 viruses was not expected as H5 IAVs are generally not well represented in reported isolates from ducks.

1. Introduction

Wild birds in the orders Anseriformes and Charadriiformes are considered the natural reservoirs for influenza A viruses (IAVs) (Olsen et al., 2006) and historical surveillance for these viruses in wild birds has relied on viral detection by either virus isolation or RT-PCR (Hinshaw et al., 1985; Wallensten et al., 2007). However,

* Corresponding author at: Southeastern Cooperative Wildlife Disease Study, Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, United States.
Tel.: +1 806 885 0238.
E-mail address: whitney.kistler@gmail.com (W.M. Kistler).

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serological assays have been developed recently that have a high sensitivity at detecting antibodies to IAVs, thus these assays can be used to improve surveillance approaches (Brown et al., 2009; Lebarbenchon et al., 2012). The duration of detectable antibodies can be >1 year in naturally infected ducks (Tolf et al., 2013), and with repeated infections, they may persist for the life of the bird. In contrast, viral shedding is of short duration, often <10 days (Costa et al., 2011). The long duration of antibodies allows for sampling during times when birds are more easily captured (e.g. summer molting) or in species where information about their role in the maintenance of IAVs is limited. Serology has been recently used to supplement virus isolation data and advance our current understanding of IAVs in Canada geese (*Branta canadensis*) (Kistler et al., 2012).

Traditionally, Canada geese have not been implicated in an important role in the epidemiology of IAVs. Although Canada geese have a near ubiquitous distribution in the United States (US) and share aquatic habitats with known IAVs reservoir species (Hestbeck, 1995), IAV isolations from Canada geese are rare (Harris et al., 2010). This perceived low prevalence of viral isolation is likely due to brief and infrequent viral shedding patterns reported in experimentally infected Canada geese (Berhane et al., 2014; Pasick et al., 2007) and sample timing which often occurred during a 3–4-week flight-less molting period during June and early July (Harris et al., 2010). Using serologic testing, Canada geese were found to be frequently exposed to IAVs and the prevalence of antibodies increased with latitude (Kistler et al., 2012). This increase in antibody prevalence in geese followed a similar trend of virus shedding data in dabbling ducks (Hinshaw et al., 1985; Stallknecht et al., 1990).

Results from these previous studies suggests that serological surveillance of IAVs in Canada geese may provide an inexpensive sentinel system to monitor or supplement surveillance efforts to understand spatial and annual trends in IAV transmission in waterfowl populations. However, subtype-specific serological data are needed to understand if antibodies detected in Canada geese are representative of the predominant subtypes detected in waterfowl, especially dabbling ducks. Based on virus isolation results from dabbling ducks, hemagglutinin subtypes H3, H4, and H6 are most commonly reported during peak IAV transmission in late summer and early fall (Wilcox et al., 2011). The objectives of this study were to determine long term trends in IAVs antibodies to the nucleoprotein (NP) and to detect subtype-specific antibodies in Canada geese.

2. Materials and methods

In June and July 2010–2012, we collected blood samples ($n = 2225$) from Canada geese from 116 locations (Fig. 1) in Pennsylvania during banding and nuisance removal programs. Blood samples were collected from the medial metatarsal vein from geese being released and by cardiocentesis from birds that were euthanized. Blood samples were placed in Vacutainer® serum separator tubes (BD, Franklin Lakes, NJ, USA) and placed on wet ice in the field. After transport to a laboratory (<1 day) blood samples were centrifuged (15 min at 1200 × *g*) and serum was removed and stored at −20 C until testing.

We first screened serum samples for presence of antibodies to the IAV NP using a commercial blocking enzyme-linked immunosorbent assay (bELISA; IDEXX Laboratories, Westbrook, ME, USA). Samples that had antibodies to the IAV NP were then screened by a hemagglutination inhibition (HI) assay using antigen from the Southeastern Cooperative Wildlife Disease Study (University of Georgia, Athens, GA, USA; Table 1) and positive control serum from specific pathogen-free chickens (National Veterinary Service Laboratories, United States Department of Agriculture, Ames, IA, USA). Canada goose serum was first treated with 10% chicken red blood cells (1:1 dilution), incubated at room temperature for 1 h, and then centrifuged for 10 min at 800 × *g*. The supernatant was then removed and used for the HI assays. The HI assays for all subtypes were conducted as previously described (Pedersen, 2008) using 4 HA/25 μl and a positive cut-off titer of ≥32.

We also included Canada goose samples collected in 2009 during a previous study (Kistler et al., 2012). These

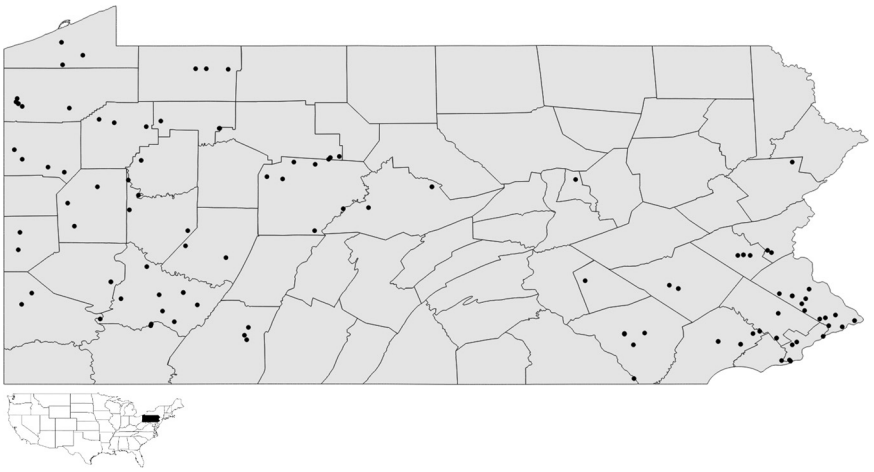

Fig. 1. Sample location distribution in Pennsylvania 2009–2012.