EVALUTATION OF CANADA GEESE (*Branta canadensis*) AS SENTINELS FOR DETECTION OF TRANSMISSION OF AVIAN INFLUENZA VIRUSES

by

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(Under the Direction of Michael J. Yabsley)

ABSTRACT

Sentinel studies have been used to help understand transmission of numerous wildlife and zoonotic diseases, including avian influenza viruses (AIV). Previous AIV sentinels studies have increased our understanding of the epidemiology of AIV; however these previous studies are not practical for use on a large scale. This study used Canada geese (*Branta canadensis*) as sentinels to detect areas of AIV transmission on regional and local scales. For this evaluation 3,207 serum samples from nine states (Georgia, Massachusetts, Minnesota, Mississippi, New Jersey, North Carolina, Pennsylvania, Washington, and West Virginia) were analyzed with two serological assays: agar gel immunodiffusion and blocking enzyme linked immunosorbent assay. An increasing trend in antibody prevalence was seen as latitude increased. This increasing trend is also seen in virus isolations of dabbling ducks. Furthermore, significant differences were detected between areas <6km apart. These results indicate that Canada geese can be used effectively as sentinels for AIV on both a regional and local scales.

INDEX WORDS: avian influenza virus, *Branta canadensis,* Canada goose, serology, sentinel, blocking ELISA

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DEDICATION

I would like to dedicate this manuscript to my parents Michael and Edith Kistler, my two brothers and sister-in-law Elliot, Jonathan, and Amber Kistler, whom without their support and inquisitiveness I would not be driven to have completed and continue this work.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Avian influenza viruses (AIV) are negative-sense, single-stranded ribonucleic acid influenza A viruses in the family *Orthomyxoviridae.* Although they are able to infect a wide range of mammalian and avian species, these viruses utilize wild birds in the orders Anseriformes (ducks, geese, and swans) and Charadriiformes (gulls, terns, and shorebirds) as natural reservoirs (Olsen et al. 2006, Stallknecht and Shane 1988, Webster et al. 1992). Infections in the natural reservoirs with low pathogenic (LP) AIV is typically subclinical; however, in commercial poultry some strains of H5 and H7 can develop into highly pathogenic AIV (HPAIV) and cause morbidity and mortality in flocks (Lupiani and Reddy 2009, Sims et al. 2003, Swayne and King 2003). Not only are these viruses of concern to the commercial poultry industry where they can cause large economic losses, there is the threat that some of these HPAI can emerge in humans and wildlife causing pandemics (Alexander 2000, Scholtissek et al. 1978, Taubenberger et al. 2005). Such a pandemic was realized most recently in wild birds in Europe and Asia and the emergence of the HPAI H5N1 viruses and the emergence of pandemic H1N1 in North America (Ellis et al. 2004, Neumann et al. 2009).

Traditionally, AIV surveillance has been done using virus isolation in 9-11 day-old specific pathogen-free embryonated chicken eggs (Slemons et al. 1974). This is considered the most sensitive technique for detecting viable virus from wild birds (Woolcock 2008). Recently however, reverse-transcription polymerase chain reaction (RT-PCR) has been used in AIV

surveillance (Ip et al. 2008, Siembieda et al. 2010). In many cases RT-PCR is used as a screening tool and positives samples are further evaluated by virus isolation to detect viable virus (Munster et al. 2007, Siembieda et al., 2010).

In addition to virus isolation, serological assays are useful in understanding the epidemiology of AIV. Historically, only two serological assays have been widely used in AIV antibody detection in wild birds, the agar gel immunodiffusion (AGID) and hemagglutination inhibition (HI) assays (Spackman et al. 2008, Winkler et al. 1972). The AGID assay is commonly used in poultry; however, lacks validation in many wild bird species and has performed poorly in waterfowl (Bahl 1975, Beard 1970, Slemons and Easterday 1972). The HI assay requires serum to be screened through all hemagglutinin 16 subtypes and therefore has not been used in large scale surveillance of wild birds.

Previous studies discovered that experimentally-inoculated Canada geese (*Branta canadensis*) only shed AIV for a short period of time; however, antibodies have been detected in both experimentally- and naturally-exposed geese (Harris et al. unpublished, Pasick et al. 2007). This overarching goal of my research was to evaluate the effectiveness of a serologically-based surveillance system for AIV using Canada geese as sentinels to detect local and regional transmission of AIV. Identification of an effective sentinel species could facilitate virus isolation surveillance studies by focusing efforts to areas of potentially high AIV transmission, could elucidate geographic regions where AIV circulates, and could detect differences in AIV transmission on a local scale that could identify environmental and/or host factors related to the transmission of AIV.

LITERTURE REVIEW

Avian Influenza Virus

Avian influenza viruses are type A influenza viruses in the family *Orthomyxoviridae*. The viral genome consists of eight segments of negative-sense ribonucleic acid, coding for 10 or 11 proteins depending on the virus (Compans et al. 1970, Rott 1992, Suarez 2008, Tumpey et al. 2005**)**. Three of these proteins are found on the surface of the virion: hemagglutinin (HA), neuraminidase (NA), and the membrane ion channel (M2) (Compans et al. 1970, Krug et al. 1973, Rott 1992). The remaining proteins are expressed internally: nucleoprotein (NP), matrix protein (M1), polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2), polymerase acidic protein (PA), nonstructural protein 1 (NS1), nonstructural protein 2 (NS2) (also called the nuclear export protein (NEP)), and in some AIV, the PB1-F2 protein (Chen et al. 2001, O'Neill et al. 1998, Suarez 2008). The two nonstructural proteins are not usually found inside the virion, but are found within the nucleus (NS1) and cytoplasm (NS2) of the host cell (Krug et al. 1973, Tumpey et al. 2005, Webster et al. 1992).

The NP protein is used to distinguish influenza types A, B, and C from each other and shows little antigenic variation within the influenza types (Walls et al. 1986). The matrix protein is also considered to be conserved in influenza A viruses and therefore useful in identification (Schild 1972). Because these two proteins are relatively conserved among influenza A viruses they are used often in assays to detect these viruses in birds and mammals (Fouchier et al. 2000, OIE 2008, Yewdell et al 1985).

Classification of Avian Influenza Viruses

Avian influenza viruses are classified by two surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA) (Webster et al. 1992). To date 16 H subtypes (H1-16) and 9 N subtypes

(N1-9) have been identified (Olsen et al. 2006, Webster et al. 1992). These surface glycoprotein subtypes can be found in any combination, although there is only one H and one N subtype per virus.

AI viruses are further classified based on pathogenicity in poultry. There are two pathogenic categories for these viruses, high pathogenic and low pathogenic avian influenza viruses (HPAIV and LPAIV, respectively). Only H5 and H7 viruses have developed into HPAIV and these HPAIV are believed to have been LPAIV that mutated to HPAIV during transmission among domestic birds (Alexander 2000, Horimoto et al. 1995). To confirm if a virus is a HPAIV two tests are used: a chicken challenge test and sequence analysis of the HA cleavage site (OIE 2008). The chicken challenge test can be done two ways: challenging 6-week-old chickens with the isolated virus then calculating the intravenous pathogenicity index (IVPI); or measuring the mortality in 4- to 8-week old chickens. For the virus to be considered highly pathogenic the IVPI index must be >1.2 or a mortality rate $\geq$75% in inoculated chickens. In addition, if there is an insertion of 2 or more basic amino acids (lysine and arginine) in the sequence analysis of the HA cleavage site, the virus is classified as highly pathogenic. Just one of these tests needs to indicate a virus is highly pathogenic for it to be classified as a HPAIV. Contradictory results between the tests have been reported only once in Texas in 2004 (Lee et al. 2005). The pathogenicity of a virus is based on its effects in poultry and does not reflect how the virus will act other species. Some highly pathogenic poultry viruses showed no signs of disease in experimentally inoculated ducks (Alexander et al. 1986).

Transmission of Avian Influenza Viruses

Initially, AIV in wild birds were thought to be primarily respiratory pathogens; however, during surveillance of wild birds it was noted that more isolations came from samples collected from the cloaca than from samples collected from the trachea (Slemons et al. 1974, Webster et al. 1976). This led to experimental infection studies showing that these viruses replicated within the digestive tract of ducks (Webster et al. 1978). In addition, this study showed that AIV were shed in fecal matter and able to remain infective in feces for over 2-weeks. In field studies, researchers found that virus was able to be isolated from feces and water (Hinshaw et al. 1979, Ito et al. 1995). Hence, in a natural environment transmission of AIV is likely by the fecal-oral route from water and soil contaminated with feces. Experimentally, transmission has occurred through direct contact of infected and non-infected birds, through aerosols, and by contact contaminated water (Forrest et al. 2010, Winkler et al. 1972). Transmission by direct contact and aerosols probably do not play an important role in the Anseriformes reservoir; however, they may play a more important role in high density nesting colonies of Charadriiformes, such as those of ring-billed gulls (*Larus delawarensis*) where viruses can be isolated at >20% from 3-week-old chicks (Velarde et al. 2010).

Host Range of Avian Influenza Viruses

Avian influenza virus infections have been detected from more than 100 species of birds in 13 orders (Olsen et al. 2006, Stallknecht and Brown 2008). Several of these viruses have crossed species barriers, infecting and in some cases becoming endemic in humans, pigs, horses, and other mammals (Scholtissek et al. 1978, Webster et al. 1992). This broad host range has led influenza A viruses to be one of the most studied viruses in the world; however, many unknowns regarding AIV in reservoirs and the environment still exist.

Wild birds from two orders (Anseriformes and Charadriiformes) are recognized as the major reservoirs for all subtypes of AI viruses (Deibel et al. 1985, Fouchier et al 2005, Hanson et al 2005, Kawaoka et al. 1988, Kawaoka et al. 1990, Krauss et al 2004, Rohm et al. 1996, Sharp et al. 1993). Within the order Anseriformes, most virus isolates come from species in the family Anatidae, genus *Anas,* particularly mallards (*Anas platyrhynchos*) (Olsen et al. 2006, Stallknecht and Shane 1988, Stallknecht and Brown 2007). Among Charadriiformes, most virus isolations have been from members of the family Scolopacidae with most from ruddy turnstones (*Arenaria interprets*) (Kawaoka et al. 1988, Stallknecht 2003). Although, in the Laridae family high virus isolation rates (>20%) have been detected in nesting colonies of ring-billed gulls (Velarde et al. 2010).

Although wild birds in Anseriformes and Charadriiformes serve as reservoirs for all known subtypes of AIV, these subtypes are not equally distributed among these reservoirs. In the family Anatidae, particularly in the genus *Anas,* the predominant AIV subtypes isolated include H3, H4, and H6, with sporadic detections of most other subtypes (Halvorson et al. 1985, Hinshaw et al. 1985, Hinshaw and Webster 1980, Krauss et al. 2004, Munster et al. 2007, Sharp et al. 1997). Although, in some locations and years other subtypes have been isolated more frequently (Hanson et al. 2003, Hanson et al. 2005, Wilcox et al. unpublished). Interestingly, H16 has not been isolated from Anatidae and H13 has rarely been isolated from these birds (Fouchier et al. 2005, Munster et al. 2007). In Charadriiformes, H3, H11, H13, and H16 have been the most frequently isolated subtypes (Graves 1992, Kawoaka et al. 1988, Krauss et al. 2004, Munster et al. 2007).

Spatial and Temporal Variation

Along with subtype differences between Anseriformes and Charadriiformes, location and seasonality of viral transmission is unique to both orders. Among the Anseriformes (primarily mallard ducks), prevalence peaks in the northern United States and Canada when waterfowl are in high density staging flocks preparing for fall migration (Halverson et al. 1985, Hinshaw et al. 1978, Sharp et al. 1993, Slemons et al. 1991, Wilcox et al. unpublished). Prevalence decreases sharply in October and November as birds migrate south and prevalence remains low while birds are on the wintering grounds (Kocan et al. 1980, Smitka et al. 1981, Stallknecht et al. 1990). It is believed that the late summer and early fall spike in prevalence is due to the influx of large numbers of susceptible hatch-year birds at these pre-migration staging areas. Supporting this hypothesis are studies that detected significantly higher virus isolation rates in hatch-year birds during fall sampling (Alfonso et al. 1995, Hanson et al. 2003, Sharp et al. 1993, Wilcox et al. unpublished). This peak in viral shedding is seen in northern areas of North America every year during late summer and early fall; however, prevalence of some subtypes has been shown to be a two to four year cycle in Alberta, Canada (Krauss et al. 2004, Sharp et al. 1993). In addition, increased viral shedding has been seen in dabbling ducks in late winter in Texas (Hanson et al. 2005).

In contrast, there is only a single location in North America, Delaware Bay, USA, where AIV are consistently isolated from Charadriiformes (primarily ruddy turnstones) and these prevalence rates tend to be higher in birds using Delaware Bay as a stopover during the spring migration (Kawoaka et al. 1988, Krauss et al. 2004, Hanson et al. 2008). In Charadriiformes outside of Delaware Bay, viral shedding is usually detected in low amounts (Hanson et al. 2008, Munster et al. 2007); however, in nesting colonies of ring-billed gulls, on Lake Erie and Lake

Ontario Canada, viral shedding has been detected at increased levels (> 20%) (Velarde et al. 2010).

Avian Influenza and Canada Geese

Numerous previous studies have included Canada geese in surveillance efforts for AIV (Tables 1 and 2). These studies indicate that Canada geese are naturally- and experimentally-susceptible to infection with AIV (Nettles et al. 1985, Pasick et al. 2007, Rosenberger et al. 1974) and have a lower number of virus isolations when compared to other members of the Anatidae family (i.e. genus *Anas*) collected at the same time and locations (Hinshaw et al. 1985, Ip et al. 2008). Most importantly, studies indicate that naturally exposed geese mount a detectable antibody response (Easterday et al. 1968). Experimental inoculations of Canada geese with AIV showed similar results with experimintally inoculated birds seroconverting while shedding low levels of detectable virus when compared to natural AIV reservoirs (Costa et al. 2010, Homme and Easterday 1970, Pasick et al. 2007, Winkler et al. 1972). Collectively, these results indicate that Canada geese are susceptible to infection with LPAI viruses, survive the infection, and seroconvert, but because they have only minimal viral shedding, they are not likely important reservoirs or amplifying hosts for AIV.

Table 1.1. Summary of serological studies examining Canada geese for antibodies to avian influenza viruses.

Location	SerologicAssay	n	Positive	Prevalence	Reference
Missouri, USA	HI*	4	3	75%	Easterday et al. 1968
Wisconsin, USA	HI*	3	2	66%	
Michigan, USA	HI*	5	3	60%	
Illinois, USA	HI*	55	1	1.8%	
Michigan, USA	HI*	131	5	3.8%	
Missouri, USA	HI*	143	1	0.7%	
New York, USA	HI*	100	2	2%	Winkler et al. 1972
Wisconsin, USA	HI*	972	57	5.9%	
	AGP ɾ	1,359#	8&	0.6%	
Minnesota, USA	HI*and AGP ɾ	65	0	0	Bahl et al. 1977
Pennsylvania, USA	HI* and AGPɾ	261	90	34.5%	Nettles et al. 1985
Maryland and North Carolina, USA	HI and EI	28	4	14%	Graves 1993
Canada	ELISA	24	10	42%	Pasick et al. 2007
GA, WV, MN	AGPɾ	336	4	1.2%	Harris et al. in press
Total		2,127	185	8.7%	

* HI, Hemagglutination inhibition

^ EI, elution inhibition

ɾ AGP, ager gel precipitin assay

Samples taken collectively from all previous listed locations

& Three samples were not positive for HI assay

Table 1.2. Summary of avian influenza virus isolation studies in Canada geese.

Location	**n**	**Positive**	**Prevalence (%)**	**Reference**
Maryland, USA	52	1	1.9%	Rosenberger et al. 1974
Minnesota, USA	65	0	0	Bahl et al. 1977
Quebec and Ontario, Canada	7	4	57.1	Boudreault et al. 1980
Michigan, USA	11	0	0	Smitka et al. 1981
New York, USA	275	0	0	Deibel et al. 1985
Alberta, Canada and New York, USA	277	0	0	Hinshaw et al. 1985
Pennsylvania and Maryland, USA	1,504	2	0.31%	Nettles et al. 1985
Ohio, USA	315	0	0	Slemons et al. 1991
Maryland, USA	348	0	0	Graves 1993
Pennsylvania, USA	5	0	0	Alfonso et al. 1995
Alaska, USA	663	4	0.60%	Ito et al. 1995
Alaska, USA	249	4	1.61%	Ip et al. 2008
Germany	97	1	1.03%	Pannwitz et al. 2009
Georgia, USA Minnesota, USA West Virginia, USA	1,668	0	0	Harris et al. in press
Total	5,536	22	0.4%	

Sentinels in Avian Influenza

Sentinel species are valuable tools in studying the epidemiology of numerous wildlife and zoonotic diseases. In studying AIV in wild birds, sentinels have been used in aiding the understanding of transmission of these viruses. Mallard ducks (*Anas platyrhynchos*) were used to attempt detection of AIV transmission on waterfowl wintering grounds in Oklahoma, mallard ducks and domestic turkeys (*Meleagris gallopavo*) were used to detect onset and intraspecies AIV transmission in Minnesota, and mallard ducks were used to detect transmission of H5 and H7 AIV, including HPAI H5N1, in Europe (Globig et al., 2009, Halverson et al., 1985, Kocan et al., 1980). These studies helped show seasonality of transmission and provided an effective model for detection of transmission of AIV of potential economic and human health importance. However, they relied on the release of captive-reared live birds that are naïve and susceptible to infection with AIV. Furthermore, they required repeated sampling at short intervals to ensure detection of viral shedding, making these studies practical only on local scales.

Avian Influenza Serologic Assays

Historically, two serologic assays, the agar gel immunodiffusion (AGID) and hemagglutination inhibition (HI) assays, have been the most widely used for AIV antibody detection in wild birds (Bahl et al. 1977, Nettles et al. 1985, Winkler et al. 1972). The AGID assay, considered the gold standard in poultry, has not been validated in most wild bird species and has poor sensitivity in waterfowl species (Bahl 1975, Brown et al. 2009, Beard 1970, Slemons and Easterday 1972). The HI assay requires screening against all 16 hemagglutinin subtypes before presence or absence of antibodies can be determined. These reasons make large scale AIV serologic studies in wild birds impractical.

Recently, more studies have begun using enzyme linked immunosorbent assays (ELISA) in screening wild bird serum for antibodies to AIV (De Marco et al. 2004, Pasick et al. 2007, Sullivan et al. 2009). Use of these ELISAs has shown increased sensitivity for AIV antibody detection compared to the AGID assay (Sullivan et al. 2009). However, many of these ELISAs are not commercially available making quality control and use among different laboratories difficult. Three commercially available ELISAs have been tested in wild birds. Two of these assays Ingezim Influenza A^{R}, (Ingenasa, Spain) and influenza A antibody competition^{R} (Idvet, France) have not been thoroughly validated for use with wild bird species (Pérez-Ramírez 2010). Although both have been used in surveillance of wild birds they have only been used to analyze 13 samples from experimentally inoculated birds outside of traditionally understood AIV reservoir species. The third ELISA flockcheck AI multiS-screen antibody test kit^{R} (IDEXX, USA) has been been very sensitive 100% (95% CI: 96.5, 100.0) and specific 86% (95% CI: 75.6, 87.4) in detecting antibodies in numerous experimentally infected wild bird species from 10 taxonomic orders including Anseriformes and Charadriiformes (Brown et al. 2009). Furthermore, this assay has been proven in large scale wild bird AIV surveillance and has outperformed the AGID assay in both experimentally- and naturally-exposed birds (Brorn et al. 2009, Brown et al. in press).

Natural History of Canada Geese

There are seven subspecies of Canada geese in North America: *Branta canadensis canadensis, maxima, interior, moffitti, parvipes, occidentalis,* and *fulva* (Banks et al. 2003). *Branta canadensis maxima*, *moffitti,* and *interior* are considered to compose the majority of the breeding populations of Canada geese in the continental United States (Atlantic flyway 1999, Gabig 2000). In the Central and Pacific Flyways the Rocky Mountain population (*B. c. moffitti*)

are not considered a nuisance in most areas and undergo longer migrations than other geese that breed within the continental United States; however, portions of the Pacific population (*B. c. moffitti*) are beginning to migrate less (Saake et al. 2001). Because of the lower population sizes and migration of many geese nesting in the continental U. S. portions of the Pacific Flyway, this flyway has not participated in liberalized population reduction methods implemented in the U. S. (Hogan 2006). The Central, Mississippi, and Atlantic Flyways have much larger problems with nuisance geese with breeding populations of these birds reaching around one million birds in each of these flyways.

In the Central Flyway there are three management populations of Canada geese: Hi-Line, Great Plains, and Western Prairie populations made up of *B. c. maxima, moffitti,* and *interior* (Gabig 2000). In the Mississippi and Atlantic Flyways there are seven management populations of Canada geese (Hindman et al. 2004, Leafloor et al. 2004, Sheaffer et al. 2005). Four are found in the Atlantic Flyway: the Atlantic, North Atlantic, Southern James Bay, and the Atlantic Flyway Resident populations. The Southern James Bay population is also found in the Mississippi Flyway in addition to the Mississippi Valley population, Eastern Prairie population, and the Mississippi Flyway Resident population. The Atlantic, North Atlantic, Southern James Bay, Mississippi Valley, and the Eastern Prairie populations are subarctic-nesting, experiencing long migrations from wintering grounds to breeding grounds located in northern Canada. These migratory populations consist of mainly two subspecies of Canada geese. *Branta canadensis interior* are the most abundant subspecies in the Atlantic, Southern James Bay, Mississippi Valley, and Eastern Prairie populations, while *B. c. canadensis* is the most common subspecies in North Atlantic populations. The Atlantic and Mississippi Flyway Resident populations can experience short migrations, but breed below 48° north latitude in the Atlantic Flyway and 50°

north latitude in the Mississippi Flyway (Atlantic Flyway 1999, Holevinski et al. 2006, Rusch et al. 1996, Zenner et al. 1996). *Branta canadensis maxima* is reported as the predominant subspecies in both resident populations; however, ***B. c. moffitti, interior,*** and ***canadensis*** can also be found among these populations (Atlantic Flyway 1999, Orr et al. 1998, Zenner et al. 1996). Evaluating all these populations from both the Atlantic and Mississippi Flyways for proper management has been difficult. One of the main issues is if all 7 populations should be managed separately or as one large population for each flyway (Rusch et al. 1996).

Starting in 1936, a midwinter survey (MWS) was conducted annually on wintering grounds for Canada geese (Reeves et al. 1968, Rusch et al. 1996). For the first 20 years the MWS was not standardized; however, that changed in 1955 when state and federal agencies took the place of volunteers and infrequent aerial surveys (Moser and Caswell 2004). The MWS for Canada goose populations was ended in the Mississippi Flyway in 1969 and replaced with a mid-December survey specifically for goose populations in this flyway (Leafloor et al. 2004). The MWS and mid-December survey lasted as the main population estimations for Canada geese until 1991 (Hindman et al. 2004, Leafloor et al. 2004). In 1991, spring breeding ground surveys for Canada geese were enabled to help better manage each individual population of Canada geese (Schneider et al. 1994, Trost et al. 1990). The MWS and mid-December survey were inadequate at separating each population from each other. The growing numbers in resident populations was masking the decline in migratory populations detected in breeding ground surveys used for some migratory populations beginning in the 1970s (Atlantic Flyway 1999, Malecki et al. 1980). The change to spring breeding ground surveys has helped the Canada goose reach the populations levels seen today with geese being found nearly ubiquitously

throughout the United States and Canada (Atlantic Flyway 1999, Leafloor et al. 2004). However, this was not always the situation.

During the 1950s and 1960s Canada goose populations in the southern portions of both the Atlantic and Mississippi Flyways began to decline (Orr et al. 1998, Trost and Malecki 1985). This decline was attributed to 3 main causes: addition of Canada goose refuges in the northern part of the Atlantic and Mississippi Flyways (Addy and Heyland 1968, Hankla and Rudolph 1967), over-harvesting of Canada goose populations in their southern range (Hankla and Rudolph 1967, Raveling 1978, Trost and Malecki 1985), and large numbers of resident populations in northern areas of the Atlantic and Mississippi Flyways attracting migrant geese and causing them to stay in these areas longer (Gates et al. 2001). This decline brought about many reintroduction programs to National Wildlife Refuges in the southern portions of both flyways beginning in 1956 (Hankla 1968). Over 12 years > 20,000 Canada geese were tranlocated. The reintroductions, however, were largely unsuccessful in restoring and extending wintering ranges of the Canada goose. The discovery of the giant Canada goose (*Branta canadensis maxima*), in 1962 in Rochester Minnesota, led to reintroductions from private collections and the Rochester, Minnesota flock to restore this goose to historical distributions (Hanson 1965). These introductions were much more successful then the first attempts and states within the Atlantic and Mississippi flyways began to reintroduce Canada geese to increase or restore their winter flocks (Atlantic Flyway 1999, Zenner et al. 1996). The success of these reintroductions has led to the establishment of resident Canada goose populations becoming the largest management populations in the Atlantic and Mississippi Flyways (Hogan 2006).

Because of this significant increase in population numbers and the ability of Canada geese to reside in a wide variety of habitats, conflicts have increased substantially, especially in

urban areas (Conover and Chasko 1985, Powell et al. 2004). Several state and federal government agencies have developed management plans to help control the populations or reduce human wildlife conflicts (Atlantic Flyway 1999, Hogan 2006, United States Fish and Wildlife Service 2009, Zenner et al. 1996). Many of these plans involve lethal removal of nuisance geese, relocation programs, and special hunting seasons (Heusmann 1999, Holevinski et al. 2006, United States Fish and Wildlife Service 2009). Modeling results suggests an increased hunter harvests in combination with euthanasia in urban areas are needed to reduce these populations to manageable levels (Coluccy et al. 2004, Hogan 2006).

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CHAPTER 2

EVALUTATION OF CANADA GEESE (*Branta canadensis*) AS SENTINELS FOR DETECTION OF TRANSMISSION OF AVIAN INFLUENZA VIRUSES

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ABSTRACT

Sentinel studies have been used to help understand transmission of numerous wildlife and zoonotic diseases, including avian influenza viruses (AIV). Previous sentinel studies, utilizing release of captive-raised ducks, have been used to detect areas of AIV transmission; however, they are impractical for use on a large scale. The current study was conducted to evaluate the effectiveness of resident Canada geese (*Branta canadensis*) as sentinels of AIV on regional and local scales. For this evaluation 3,207 samples were collected during June and July in 2008 and 2009 from nine states: Georgia, Massachusetts, Minnesota, Mississippi, New Jersey, North Carolina, Pennsylvania, Washington, and West Virginia. Serum samples were tested for AIV antibodies using an agar gel immundiffiusion assay (AGID) and/or a commercial blocking enzyme linked immunosorbant assay (bELISA). Overall, 482 (15%) Canada geese had antibodies to AIV. Of those samples tested by both assays, 40 (4.2%) were positive by AGID and 111 (12%) were positive by bELISA with an overall agreement of 91%. Significantly higher prevalence rates were detected in geese collected from northeastern and upper midwestern states (e.g., Minnesota, Pennsylvania, and Massachusetts) compared with southeastern states (e.g., Mississippi, North Carolina, and Georgia). This trend of significantly higher antibody prevalence has also been reported in studies utilizing AIV isolations from dabbling ducks. Within Pennsylvania, significantly higher antibody prevalence rates were detected in goose flocks sampled in urban locations compared to flocks sampled in rural areas. For geese that were aged, after-hatch-year geese had significantly higher antibody prevalence rates than hatch-year geese. For 10 locations that were sampled during 2008 and 2009, no difference in prevalence was noted between years or outcome of goose flock (euthanasia or released back on location). Interestingly, we found that two locations in both New Jersey and Washington as close as 5.8km

had significant differences in prevalence suggesting that an unknown local factor had a major influence on the likelihood of exposure of those geese to AIV. The results from this study indicate that Canada geese can be used as effective sentinels for detecting regional and local transmission of AIV.

Key words: avian influenza virus, Canada goose, serology, sentinel, blocking ELISA

INTRODUCTION

Sentinels species are an important tool in helping to understand the epidemiology of numerous wildlife and zoonotic diseases. Previous research has demonstrated the effectiveness of a good sentinel species in detecting the location and onset of transmission of numerous pathogens. In some instances, certain wildlife species may be highly susceptible to developing severe disease and are thus easily recognized due to mortality. For example, mortality of American crows (*Corvus brachyrhynchos*) can be used as an early warning for local West Nile virus transmission because their mortality occurs before the onset of human or equine cases (Edison et al., 2001). In other cases, wildlife may be commonly exposed or infected with pathogens which can be exploited to determine areas of transmission of a particular pathogen. In New Zealand, for example, feral pigs (*Sus scrofa*) can be used as alternative hosts for the detection of *Mycobacterium bovis* in areas with low populations of the primary reservoir, the brushtail possum (*Trichosurus vulpecula*) (Nugent et al. 2002). In the United States, white-tailed deer (*Odocoileus virginianus*) have been used to determine areas where humans are at risk of exposure to *Ehrlichia chaffeensis*, a tick-borne zoonotic pathogen that utilizes deer as reservoirs (Yabsley et al., 2003).

Wildlife may be superior to humans and domestic animals as sentinels for certain pathogens because wildlife species may have limited movement which helps identify areas of

exposure, are often free of drug exposure or vaccination, have frequent exposure to the environment, and are infested with large numbers of ectoparasites. Avian influenza viruses utilize a wide range of avian hosts as reservoirs, primarily members of the Anseriformes (ducks, geese, and swans) and Charadriiformes (gulls, terns, and shorebirds). Birds become infected by exposure to virus that is present in the environment. An effective sentinel species for AIVs should fit the following criteria: 1) be susceptible to infection with AIVs, 2) the resulting infection should result in a detectable antibody response, 3) ideally would not play a large role in spreading and maintaining AIVs within the flock 4) must be easily sampled and have a nearly ubiquitous distribution that places them in contact with known AIV reservoirs, and 5) are non-migratory to indicate they are not moving hundreds of miles.

Based on previous research, Canada geese (*Branta canadensis*) fit all of these criteria and should make effective sentinels for AIV. Experimental and field studies of Canada geese indicate they are susceptible to infection with AIV and they mount a detectable antibody response (Winkler et al., 1972, Pasick et al., 2007). Only a low prevalence of virus shedding (often <2%) has been detected in wild Canada geese which is significantly lower than shedding prevalence rates reported in dabbling ducks (Anatini) sampled at the same time and locations (Hinshaw et al., 1985, Ip et al., 2008; Harris et al., in press). Additionally, experimental infections of Canada geese with low pathogenic AIV results in only brief periods of viral shedding (up to 3 days) compared to experimentally infected mallard ducks (*Anas platyrhynchos*) which can shed up to 21 days (Pasick et al., 2007, Costa et al. 2010). In the United States, Canada goose numbers have reached over 3.3 million birds, they can be found in all 50 states, and they utilize aquatic habitats that place them into contact with other aquatic birds and their habitats, including dabbling ducks (Zenner et al., 1996, Atlantic Flyway Council, 1999, Gabig, 2000, Hogan, 2006).

Finally, large numbers of Canada geese are banded or handled during relocation or nuisance operations annually which provides relatively easy access to birds for sampling.

The overall goal of the current study was to evaluate a serologic surveillance system for regional and local transmission of AIV in resident Canada geese from numerous populations throughout the United States. Specific objectives to achieve this goal were to 1) serologically test Canada geese from numerous locations in the United States for exposure to AIVs; 2) determine if there were any age relationships to serologic status; 3) determine if there were differences in antibody prevalence between urban and rural sites; and 4) evaluate the effects of latitude on antibody prevalence.

MATERIALS AND METHODS

Sample collection and processing

Non-migratory Canada geese were collected from nine states (Georgia, Massachusetts, Minnesota, Mississippi, New Jersey, North Carolina, Pennsylvania, Washington, and West Virginia) in June and July 2008 and 2009. Birds were captured for a variety of reasons including nuisance birds that were being euthanized or relocated, banding, or other research projects. Birds were sampled during this period because they are easy to capture during their molting period. Blood samples were collected via the medial metatarsal vein of geese that were released (FAO, 2007) and via cardiocentesis on geese that were euthanized. All procedures were done under approved permits from the United States Department of Agriculture.

Whole blood samples (up to 3mL) were collected into Vacutainer® serum separator tubes (Becton Dickinson, Franklin Lanes, NJ, USA), allowed to clot, stored at 4 C, and centrifuged within 24 hrs. Serum was removed, transferred to individual screw cap tubes (Starsedt Inc., Newton, North Carolina, USA), and stored at -20 C until serological testing.

Serological testing

Serological testing was done using two assays, the agar gel immundiffiusion assay (AGID) (National Veterinary Services Laboratories, U.S. Department of Agriculture Animal and Plant Health Inspection Service, Ames, Iowa, USA) and a blocking enzyme linked immunosorbant assay (bELISA) (FlockCheck AI MultiS-Screen Antibody Test Kit, IDEXX Laboratories, Westbrook, Maine, USA). The 947 samples collected in 2008 were analyzed with both assays while the 2,260 samples collected in 2009 were only analyzed using the bELISA assay. Both assays were performed following protocols in Brown et al. (in press) with the following exception: only 100 serum samples were tested in duplicate with the bELISA. The remaining 3,107 serum samples were tested only once.

Data analysis

For the 947 serum samples tested with the bELISA and AGID assays, kappa statistics and 95% confidence intervals (CI) were calculated to estimate the agreement between the two assays. These samples were further analyzed with McNemar's χ^2 test to determine if there was a significant difference between the proportions of positive results detected with the two assays. The coefficient of variation was calculated to compare the variability in S/N values among the 100 samples run in duplicate with the bELISA assay. Chi square analysis was used to compare serologic results between the 4 latitude groups, age classes, and years. Additionally, data from virus isolation studies (Webster et al., 1976, Kocan et al., 1980, Deibel et al., 1985, Stallknecht et al., 1990, Slemons et al., 1991, Alfonso et al., 1995, Hanson et al., 2003, and Ferro et al., 2008) in dabbling ducks were divided into the same 4 latitude groups and analyzed using a Chi square test for comparison. The dabbling duck studies used in this analysis were selected because the

sampling locations could be placed accurately into one of the 4 latitude groups and analysis was restricted to the Anatini tribe were possible. For the latitude analysis, samples from the state of Washington were not included because it was the only state included from the Pacific Flyway. To compare prevalence rates between urban and rural sites, samples collected from Pennsylvania in 2009 were categorized using ArcView v9.3 (ESRI, Redlands, California, USA) and land use data from http://www.pasda.psu.edu. These sampled were analyzed using a logistic regression model with generalized estimating equations (GEE) to account for clustering of locations. Pennsylvania was chosen for this analysis because there were sufficient numbers of sampling locations of these two categories spread across a wide range of the state. Ten locations (three each in NJ and PA, two in WV, and one each from MN and MS) were sampled during both years of the study. These flocks were divided into two groups, five were nuisance flocks that were removed from the location and euthanized and five flocks that were captured for banding and geese were released at the same location. In 2009, new Canada geese had moved into the locations previously utilized by the euthanized flocks the year before so geese were removed and sampled again. At the locations where flocks were released, many of the same birds remained the second year and were resampled. These samples were analyzed with by GEE to determine if exposure levels changed between years and whether euthanizing or releasing geese at the location had an effect on prevalence rates. Additionally, differences in prevalence rates at two sites in New Jersey and Washington that were very close (5.8km or closer) were analyzed with Fisher's exact test. Data analyses were done using SAS v9.1 (SAS Institute Inc., Cary, North Carolina, USA) and Stata v11 (StataCorp, College Station, Texas, USA).

RESULTS

Overall, 482 (15%) of 3,207 Canada geese had antibodies to AIV with the bELISA assay (Table 1). Of the 947 samples tested with both assays, significantly more geese were positive with the bELISA assay compared to the AGID assay (12% and 4.2% respectively, McNemar's $\chi^2 = P < 0.001$) and the overall agreement was 91% with a κ of 0.36 (CI 0.26, 0.46), indicating a moderate to fair agreement (Landis and Koch 1977). Thirty of the samples tested positive with both assays, nine were AGID positive bELISA negative, 81 were bELISA positive AGID negative, and 827 were negative with both assays. Of the 100 serum samples tested in duplicate with the bELISA, 23 were positive for both samples and the mean coefficient of variation was 9.9% (SD=7.4%) with a total agreement of 95%.

Significant differences were noted by years, state, and region. On a regional scale, AIV antibody prevalence significantly increased with latitude which also corresponds with published virus isolation data from dabbling ducks (Table 2). The northern states of Minnesota and Massachusetts had significantly higher antibody prevalence rates compared with all other states and the southern states of Mississippi and North Carolina had significantly lower prevalence rates than all the other states (Table 1). Among the southern states, Georgia had significantly higher antibody prevalence than the two other southeastern states ($p < 0.001$ and $p=0.003$, respectively). Between years 2009 had a significantly higher antibody prevalence than 2008 ($\chi^2=12.5$, $p < 0.001$). New Jersey and Minnesota had a significantly higher antibody prevalence rates in 2009 compared to 2008 ($\chi^2=4.3$, $p=0.4$ and $\chi^2=5.7$, $p=0.2$, respectively) and Georgia had a significantly higher antibody prevalence in 2008 compared to 2009 ($\chi^2=6.1$, $p=0.01$).

Age was significantly associated with antibody prevalence with after-hatch-year geese (n=2,391) having significantly higher prevalence rates compared with hatch-year geese (n=518) (17% and 1.9% respectively, $p < 0.0001$). Geese sampled from urban sites in Pennsylvania during the 2009 sampling season had a significantly higher antibody prevalence compared with rural sites (OR 3.2 CI (1.7, 6.0)). For the 10 sites that were sampled in 2008 and resampled in 2009, no differences in antibody prevalence were noted between the two years (OR 1.2 CI (0.9, 1.6)). Furthermore, no differences in antibody prevalence were noted between geese that were euthanized or those that were released back onto the same location from which they were captured (OR 1.2 CI (0.2, 2.5)). Interestingly, we observed significant local variation in antibody prevalence between two sites in both New Jersey and Washington (Table 3). Both pairs of locations were sampled in 2009 and one of the pairs was 4.8km away from each other on Lake Washington, Washington and the other pair located was located on separate water bodies 5.8km apart in southern New Jersey.

DISCUSSION

Results from the current study support two previous studies that indicated the bELISA assay was a more sensitive assay than the AGID assay (Brown et al., 2009a, Brown et al., in press). Using birds experimentally-infected with both high and low pathogenic AIV, the diagnostic sensitivity and specificity of the bELISA assay was 86% (95% CI: 75.6, 87.4) and 100% (95% CI: 96.5, 100.0), respectively (Brown et al. 2009a). Additionally, the bELISA assay was effective in a large scale surveillance study of wild bird serum from 10 taxonomic orders (Brown, in press). Although the sensitivity and specificity were not able to be calculated in the study, antibody prevalence was highest in orders of birds that utilize aquatic habitats. We also

observed an increase in antibody prevalence with latitude, a result that corresponds with previous studies of virus isolations from dabbling ducks with higher prevalence of viral shedding from ducks sampled from staging areas in Canada and the northern United States (U.S.) and lower prevalence of viral shedding from ducks sampled during migration and on wintering grounds (Stallknecht and Brown, 2008). Collectively, these data indicate that Canada geese are effective sentinels for detecting regional transmission of AIV in the U.S.

The finding of higher antibody prevalence in after-hatch-year birds compared to hatch-year birds was expected. Canada geese in this study were sampled in June and July, before peak viral shedding in dabbling ducks (typically from August to September) (Hinshaw et al., 1985); thus, opportunities for hatch-year birds to be exposed this early in the transmission season would be rare. Although some dabbling ducks may have been shedding early in the season, it is also possible that the 10 hatch-year Canada geese (that had antibodies to AIV) were exposed to virus shed from alterative hosts such as Charadriiformes or the detected antibody response may have been the result of maternal antibodies. In Charadriiformes viral shedding is usually highest in spring at migration stop-over locations or in nesting colonies (Krauss et al., 2004, Velarde et al., 2010). On rare occasions, in some locations, high prevalence of viral shedding (>10%) has been reported in dabbling ducks during early spring (Hanson et al., 2005).

Interestingly, in Pennsylvania, we detected significantly higher antibody prevalence in Canada geese sampled in urban areas compared with rural areas. The remaining states that had sufficient prevalence for this type of analysis did not have sufficient numbers of flocks collected in the different land-use types to permit additional testing; however, among southeastern states, Georgia had a relatively high prevalence and eight of our ten collections sites within Georgia

occurred inside the city limits of Atlanta, a large metropolitan city. Currently, it is unknown if urban populations are more likely to be exposed to AIV in other areas. This observed higher prevalence in urban sites in Pennsylvania and Georgia could be attributed to long-term duration (>1 year) of detectable antibodies in geese combined with an increased survival rate of geese urban areas (Balkcom, 2010). To date, studies on AIV antibody persistence in naturally-infected Canada geese have not been conducted.

We failed to detect a significant difference in antibody prevalence at locations sampled in both years regardless of the flocks being released back onto the sampling location or euthanized and a new flock moving into the area. This would suggest that Canada goose flocks that utilize these areas were exposed to similar levels of AIV during both years of the study. We did, however, detect a significant increase in antibody prevalence in 2009 overall and in New Jersey and Minnesota. In Georgia the prevalence was significantly higher in 2008. The increase seen among the different years could be related to increased exposure within these states during those years or due to sampling different locations within the states that had and increased risk of exposure than those sampled in the lower prevalence year. There were 300 more samples collected from 13 more locations from New Jersey in 2009 and in Minnesota most of the 2009 samples were collected around a large urban area in the cities of Minneapolis and St. Paul. In Georgia in 2008, only one location was sampled within the city limits of Savannah and although most of locations sampled in 2009 were in Atlanta there were a few (n=2) rural locations with a low prevalence rate (2.8%).

At two of our study areas, we detected significant differences in antibody prevalence between closely sampled locations suggesting that local factors were causing variability in geese

exposure to AIV and this sentinel system was able to detect these differences. The two flocks sampling in Washington were captured at the same lake (Lake Washington) where they both co-mingle with several thousand dabbling ducks (D.L. Bruning, personal communication). Because both flocks co-mingled with the dabbling ducks, the large difference in exposure between the two goose flocks was surprising. However, it was not known how the ducks and geese interacted in late summer and early fall when AIV shedding by the ducks would be highest. The other pair of locations in southern New Jersey was from two separate water bodies. The location with the higher antibody prevalence was at a landfill and the other location was a community park. At both locations there were a small number ($n<10$) of dabbling ducks present during sampling. In addition, environmental factors, particularly water, are known to play an important role in the transmission of AIV (Roche et al., 2009) and differences in temperature, pH, and salinity all affect the persistence of AIV in water (Brown et al., 2009b). Therefore, environmental factors related to transmission could play an important role in the exposure of Canada geese in this study.

Other studies have utilized free-ranging birds as sentinels for AIV transmission. Pinioned mallard ducks are often used and a few examples include attempting detection of AIV transmission on waterfowl wintering grounds in Oklahoma, determining seasonality of AIV transmission in Minnesota, and in Europe, have been used as an early detection system for H5 and H7 viruses including highly pathogenic H5N1 virus transmission (Kocan et al., 1980, Halverson et al., 1985, Globig et al., 2009). All of these studies relied on releasing captive-reared flightless birds that inter-mingled with wild bird reservoirs (Anseriformes and Chradriiformes). During these studies, ducks had to be sampled repeatedly to maximize detection of viral shedding. These studies are impractical on larger scales because of the need

for susceptible captive-raised birds for release, birds must be recaptured for repeatedly sampling, and special equipment for RT-PCR and virus isolation. In contrast, a serologic based system can be useful because antibodies persist longer in birds than viral shedding, Canada geese are easy to capture during molting times, and testing is cheaper compared to other diagnostics such as virus isolation and RT-PCR. However, serologic based testing has limitation including the lack of a viral isolate for full characterization, limited information on specific subtypes of AIV that the birds have been infected with previously, and although we targeted resident flocks of Canada geese which do not undergo long migrations, these birds do move around on a local scale so exposure may not occur at the exact location where birds are sampled (Zenner et al., 1996, Luukkonen et al., 2008). Nevertheless, we believe that Canada geese can make useful sentinels for AIV transmission on a regional, and possibly more local, scale.

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Table 2.1. Prevalence of antibodies to avian influenza in 3,207 Canada geese (*Branta canadensis*) from nine states as determined with the bELISA assay.

State	2008		2009		Total	
	n	Positive (%)	N	Positive (%)	n	Positive (%)
Georgia[c]	56	7 (13)*	301	18 (6.0)	357	25 (7.0)
Massachusetts[a]	19	6 (32)	NS	NS	19	6 (32)
Minnesota[a]	83	19 (23)	143	55 (38)*	226	74 (33)
Mississippi[d]	112	1 (0.9)	128	0	240	1 (0.4)
New Jersey[b]	163	25 (15)	537	123 (23)*	700	148 (21)
North Carolina[d]	115	1 (0.9)	129	3 (2.3)	244	4 (1.6)
Pennsylvania[b]	132	33 (25)	694	142 (20)	826	174 (21)
Washington[c]	144	10 (6.9)	245	26 (11)	389	36 (9.3)
West Virginia[c]	123	9 (7.3)	83	5 (6.0)	206	14 (6.8)
Total	947	111 (12)	2,260	372 (16)*	3,207	482 (15)

a – p < 0.001 b, c, d
b – p < 0.001 c, d
c – p < 0.01 d
***significant**
NS- not sampled

Table 2.2. Results from Canada goose antibody prevalence determined with the bELISA assay and virus isolations in dabbling ducks from previous studies categorized by latitude.

Latitude	Virus Isolation %[a]	Serology %
44-48.9°	12.9	32.5
39-43.9°	11.9	21.4
34-38.9°	1.4	4.5
29-33.9°	1.5	4.6
χ^2 for trend	231.8 ($p < 0.0001$)	152 ($p < 0.0001$)

a – data from Webster et al., 1976, Kocan et al., 1980, Deibel et al., 1985, Stallknecht et al., 1990, Slemons et al., 1991, Alfonso et al., 1995, Hanson et al., 2003, and Ferro et al., 2008.

Table 2.3. Results from locations < 5.8km apart sampled in 2009.

Location	Result (%)	Distance apart km	p-value
Lake Washington, WA	13/38 (34)	4.8	0.007
Lake Washington, WA	1/19 (5)		
Cumberland County Landfill, NJ	25/40 (63)	5.8	0.02
South Vineland Park, NJ	1/12 (8)		

CHAPTER 3

CONCLUSIONS

Sentinel studies have been used to help understand transmission of numerous wildlife and zoonotic diseases, including avian influenza viruses (AIV). Previous sentinel studies, utilizing release of captive-raised ducks, have been used to detect areas of AIV transmission; however, they are impractical for use on a large scale. The current study was conducted to evaluate the effectiveness of resident Canada geese (*Branta canadensis*) as sentinels of AIV on regional and local scales. Canada geese were chosen because they are susceptible to infection with AIV, mount a detectable antibody response as a result of infection, do not shed large amounts of virus and therefore are not likely to maintain AIV within the flock, are easy to sample because they experience a yearly molt resulting in a flightless period, and are they are ubiquitously distributed utilizing aquatic habitats putting them in contact with known AIV reservoir species.

For this evaluation 3,207 samples were collected during June and July in 2008 and 2009 from nine states: Georgia, Massachusetts, Minnesota, Mississippi, New Jersey, North Carolina, Pennsylvania, Washington, and West Virginia. Serum samples were tested for AIV antibodies using an agar gel immundiffiusion assay (AGID) and/or a commercial blocking enzyme linked immunosorbant assay (bELISA). Overall, 482 (15%) Canada geese had antibodies to AIV. Of those samples tested by both assays, 40 (4.2%) were positive by AGID and 111 (12%) were positive by bELISA with an overall agreement of 91%. Significantly higher prevalence rates were detected in geese collected from northeastern and upper midwestern states (e.g., Minnesota, Pennsylvania, and Massachusetts) compared with southeastern states (e.g., Mississippi, North Carolina and Georgia). Between years 2009 had significantly higher antibody prevalence than

2008 overall and within New Jersey and Minnesota. In Georgia the opposite was seen 2008 having significantly higher antibody prevalence than 2009. This could be the result of increased risk of AIV exposure in these states in those years or could be attributed to sampling in new locations within these states that either had higher or lower risk of exposure compared to other locations. Within Pennsylvania, significantly higher antibody prevalence rates were detected in goose flocks sampled in urban locations compared to flocks sampled in rural areas. For geese that were aged, after-hatch-year geese had significantly higher antibody prevalence rates compared with hatch-year geese. For 10 locations that were sampled during 2008 and 2009, no difference in prevalence was noted between years or outcome of goose flock (euthanasia or released back on location). Interestingly, we found that two locations in both New Jersey and Washington as close as 5.8km had significant differences in prevalence.

Increased sensitivity of the bELISA over the AGID assay supports research previously done comparing the two assays. Seeing an increase in antibody prevalence with latitude was expected due to the increased shedding seen in dabbling ducks in northern latitudes, indicating that we were able to detect increased risk of exposure in areas there should have been an increased risk. The increased risk of exposure in urban areas in Pennsylvania was an expected result because of the increased survival rate of Canada geese in urban areas. The increased survival rate means that geese that use urban areas have a longer time to be exposed to AIV. Not seeing a difference among any of the flocks at the 10 locations sampled at the same year indicates that flocks using these areas are exposed to AIV at the same rate as previous flocks and years. The significant differences in locations sampled >6km apart indicate that there are some unknown environmental, flock history, or host interaction factors that influence exposure to AIV

on a local scale. Taken together we feel these results indicate that Canada geese are effective sentinels for AIV.