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Avian Paramyxovirus Serotype 1 (Newcastle Disease Virus), Avian Influenza Virus, and *Salmonella* spp. in Mute Swans (*Cygnus olor*) in the Great Lakes Region and Atlantic Coast of the United States

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SUMMARY. Since their introduction to the United States in the late 19th century, mute swans (*Cygnus olor*) have become a nuisance species by causing damage to aquatic habitats, acting aggressively toward humans, competing with native waterfowl, and potentially transmitting or serving as a reservoir of infectious diseases to humans and poultry. In an effort to investigate their potential role as a disease reservoir and to establish avian health baselines for pathogens that threaten agricultural species or human health, we collected samples from 858 mute swans and tested them for avian paramyxovirus serotype 1 (APMV-1), avian influenza virus (AIV), and *Salmonella* spp. when possible. Our results indicate that exposure to APMV-1 and AIV is common (60%, $n = 771$, and 45%, $n = 344$, antibody prevalence, respectively) in mute swans, but detection of active viral shedding is less common (8.7%, $n = 414$, and 0.8%, $n = 390$, respectively). *Salmonella* was isolated from three mute swans (0.6%, $n = 459$), and although the serovars identified have been implicated in previous human outbreaks, it does not appear that *Salmonella* is commonly carried by mute swans.

RESUMEN. Paramixovirus aviar serotipo 1 (virus de la enfermedad de Newcastle), virus de influenza aviar y *Salmonella* spp. en cisnes comunes (*Cygnus olor*) de la Región de los Grandes Lagos y en la costa atlántica de los Estados Unidos.

Desde su introducción a los Estados Unidos a finales del siglo 19, los cisnes comunes (*Cygnus olor*) se han convertido en una especie problemática, porque causa daños a los hábitats acuáticos, actuando agresivamente hacia los seres humanos, compite con las aves acuáticas nativas, y potencialmente puede transmitir o servir como reservorio de enfermedades infecciosas para los seres humanos y para las aves comerciales. En un esfuerzo por investigar su posible papel como reservorios de la enfermedad y para establecer líneas base de salud aviar para los patógenos que amenazan a las especies agrícolas o a la salud humana, se recolectaron muestras de 858 cisnes comunes y se analizaron para detectar paramixovirus aviar serotipo 1 (APMV- 1), virus de la influenza aviar (AIV) y *Salmonella* spp. cuando fue posible. Los resultados indican que la exposición a APMV -1 y al virus de influenza aviar es común en cisnes comunes (60%, $n = 771$, y 45%, $n = 344$, para la prevalencia de anticuerpos, respectivamente), pero la detección de la replicación viral activa es menos común (8.7%, $n = 414$, y 0.8%, n = 390, respectivamente). Se aisló *Salmonella* de tres cisnes comunes (0.6%, n = 459), y aunque los serovares identificados han sido implicados en brotes humanos anteriores, no parece que la *Salmonella* sea acarreada comúnmente por los cisnes comunes.

Key words: avian influenza virus, avian paramyxovirus serotype 1, *Cygnus olor*, mute swan, Newcastle disease virus, *Salmonella*

Abbreviations: AIV = avian influenza virus; APMV-1 = avian paramyxovirus serotype 1; bELISA = blocking enzyme-linked immunosorbent assay; BHI = brain heart infusion; CL = confidence limits; HA = hemagglutination assay; HI = hemagglutination inhibition; HPAIV = highly pathogenic avian influenza virus; ICPI = intracerebral pathogenicity index; LPAIV = low pathogenic avian influenza virus; MDT = mean death time; MSU = Michigan State University; NDV = Newcastle disease virus; NVSL = National Veterinary Services Laboratories; PI = percent inhibition; rRT-PCR = real-time reverse transcriptase polymerase chain reaction; SEPRL = Southeast Poultry Research Laboratory; S/N = sample to negative; SPF = specific-pathogen-free; USDA = United States Department of Agriculture; USGS = United States Geological Survey; WS = Wildlife Services

Mute swans (*Cygnus olor*) were first introduced for their aesthetic value to the Atlantic Coast of the United States in the late 19th century. Since then, feral populations of mute swans have become established sporadically across the United States, and annual population surveys indicate an alarming increase in their populations, particularly in areas along the Atlantic Coast and Great Lakes (30,32). As mute swan populations continue to grow, their geographic distribution is also expanding (5). Their complacency toward humans, longevity, and ability to adapt to a wide variety of habitats including rivers, lakes, ponds, and brackish marshes (10) have resulted in a significant increase in their population. Excessive numbers of mute swans can remove bank vegetation, resulting in shoreline erosion and an increase in sedimentation in lakes, ponds, and reservoirs. An individual swan can consume up to eight pounds of submerged aquatic vegetation daily (50), resulting in adverse impacts on wetlands (47) including up to a 95% reduction in biomass of submerged aquatic vegetation (5). Although mute swans eat a considerable amount of vegetation, they also uproot vegetation that they do not eat, leading to the further destruction of plant mass

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(7). This excessive depletion of submerged aquatic vegetation can also lead to competition with native waterfowl for food and resources (4,38).

Mute swans exhibit territorial behavior toward other birds, which has contributed to declines in native bird numbers (49). In Michigan specifically, mute swans outcompete the state-threatened trumpeter swan (*Cygnus buccinator*) in nesting areas and have negatively impacted the trumpeter swan recovery program (33). In addition to killing trumpeter swans while defending their territory, mute swans begin nesting earlier in the year, which may interfere with successful trumpeter swan nesting (22).

Mute swans also present a hazard to human health and safety because of their aggressive behavior toward humans during the nesting season. A territorial mute swan is credited with the drowning of a kayaker in Illinois on April 12, 2012. In addition to physical attack, there is also potential for fecal contamination of water sources by mute swans in areas with high human recreational use. Although some studies have examined specific cases of disease in mute swans, we sought to establish baselines for agricultural and/or zoonotic pathogens of interest on a much larger geographic and quantitative scale than has been conducted previously to more fully assess the role of mute swans as vectors of disease.

Avian paramyxovirus serotype 1. One of the viruses identified for examination was avian paramyxovirus serotype 1 (APMV-1), also referred to as Newcastle disease virus (NDV), because it infects and causes disease in both wild and domestic birds (29). The virus can cause mortality or be asymptomatic depending on the virulence of the strain and the resistance of the host that is infected (1). Most of the 200 bird species that have been reported as infected do not exhibit any clinical signs; however, a virulent NDV infection in unvaccinated poultry may result in up to 100% mortality. In waterfowl, virulence varies with the strain of APMV-1 and the susceptibility of the host species and their immunity; however, mortality caused by virulent viruses is typically lower than poultry (25). Virulent NDV infection in species such as cormorants (13,28), and feral pigeons (26) has resulted in large-scale mortality events and spillover into other species such as pelicans (52) and gulls (15). The primary concern in the United States is the introduction of virulent NDV into poultry facilities, which often results in mandatory destruction of the entire flock to contain the outbreak. This makes NDV one of the most economically important diseases for poultry production (2). Very little is known about the incidence of APMV-1 in wild birds except that nearly all species are susceptible (23), and a large diversity and wide distribution of low-pathogenicity viruses across different geographic regions exist in waterfowl and shorebirds (25). More recently, evidence has been presented that suggests the potential for intercontinental spread of APMV-1 by wild birds (39). APMV-1 antibodies have been reported in mute swans previously (16); however, relatively small sample sizes and limited geographic distribution were examined.

Avian influenza virus. Wild birds are the natural reservoir of avian influenza virus (AIV), and highly pathogenic AIV (HPAIV) specifically is of concern because it can cause morbidity and mortality in humans, wild birds, and domestic birds. Although HPAIV is not endemic in North America, the virus has resulted in large-scale die-offs in poultry (21) and certain species of wild birds (9,48), in addition to human mortality (11) in other countries. In Europe, mute swans have been identified as important vectors of AIV (21). Not only are mute swans highly susceptible to HPAIV, but their size and color render them easy to detect when mortality occurs (21), suggesting that they are good epidemiologic sentinels (48). There is concern that low pathogenic avian influenza, specifically H5 and H7 subtypes, may become highly pathogenic after introduction into poultry (34). Thus, characterization of the subtypes carried by mute swans as well as the apparent prevalence of both AIV virus and antibodies are important for better understanding the epidemiology of the virus in this species. Since exposure history affects transmission dynamics, determination of the prevalence of low pathogenic avian influenza virus (LPAIV) in mute swans may provide insight into the percentage of mute swans that would be afforded immunity to HPAIV if it were introduced into the United States. It also might be used to clarify whether AIV is, or could become, endemic in the resident mute swan populations in the United States, thus serving as a regular source of infection for other birds.

Salmonella. Salmonellae are enteric bacteria that can cause disease in animals and humans. Most serotypes are not host specific (14), leading to abundant transmission opportunities both within and between species. Chickens, ducks, and geese are known hosts of *Salmonella* spp., and the likelihood of human contact with infected domestic birds is typically higher compared to wild bird species. *Salmonella* has been documented previously in mute swans (12,44), and as mute swan populations continue to increase in urban and recreational areas of the United States, the potential for fecal contamination and subsequent transmission to humans is elevated.

Objectives. Mute swans are actively managed in the Great Lakes region and along the Atlantic Coast because their growing population numbers have led to a significant amount of damage to aquatic habitat, competition with native waterfowl, and human conflicts. The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service, Wildlife Services (WS), works to mitigate damage to property, agriculture, and natural resources caused by mute swans in conjunction with state wildlife agencies. As a part of this management effort, we opportunistically sampled mute swans for various pathogens including APMV-1, AIV, and *Salmonella* spp. with the objective of establishing baseline avian health data and evaluating the potential role mute swan populations may play in the epidemiology of a variety of diseases of concern in regard to agricultural species or human health.

MATERIALS AND METHODS

Sample collection. Mute swans were sampled in Michigan, New Jersey, Rhode Island, New York, Indiana, Wisconsin, and Massachusetts, USA, from March 2011 through September 2012. The number of sampling locations and time of year that samples were collected varied by state. Mute swans were lethally removed by WS following the American Veterinary Medical Association Guidelines on Euthanasia (6). All samples were collected *post mortem* within 2 hr of death.

Approximately 10 ml of blood was collected from each swan by making a small cut in the jugular vein and then lowering the head below the body to allow blood to flow into the collection tube. The tube was labeled with a unique barcode and then transferred to a cooler with ice packs. Blood was centrifuged at 1500 rpm for 15 min, and then $\geq$1 ml of serum was transferred to a 2 ml cryogenic vial. Serum was stored refrigerated at 4 C until shipping within 3 days of collection.

Cloacal and oropharyngeal swabs were collected from each bird using sterile polyester-tipped swabs (Puritan®, Puritan Medical Products Company, Guilford, ME). Swabs from individual birds were combined in a single cryogenic vial with a unique barcode containing 3 ml of brain heart infusion broth (BHI; Becton Dickinson and Co., Sparks, MD) and were immediately transferred to a cooler with ice packs. Swabs were left in the sample vial after collection and were stored frozen at -80 C until testing. The BHI medium was prepared and distributed by the USDA National Veterinary Services Laboratories (NVSL) in Ames, Iowa.

An additional cloacal swab was collected using the BBL CultureSwab collection and transport system (Becton Dickinson) that contained Amies Liquid Medium, Liquid Stuart Medium, and Cary-Blair Transport Medium. It was labeled with a bird-specific barcode and kept cool until shipping within 3 days of collection.

Collection site information including county, state, and global positioning system coordinates in WGS84 was recorded on a standardized data sheet, which also included bird-specific information and individually unique barcodes.

Testing: APMV-1. *Serology*. A blocking ELISA (bELISA) (Svanova Biotech AB, Uppsala, Sweden, or ID VET Montpellier, France) was used by Michigan State University (MSU) to detect the presence of APMV-1 antibodies in 771 samples according to the manufacturer's instructions and using multiple positive and negative controls. Percent inhibition (PI) values greater than 40% were considered positive, whereas PI values less than 30% were considered negative. PI values between 30% and 40% were considered undetermined, and the samples were retested once.

Virus detection and isolation. Swab samples were forwarded to the USDA's Southeast Poultry Research Laboratory (SEPRL) in Athens, GA, for APMV-1 testing. The matrix real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assay, which targets the M gene, was used to screen the 414 samples (54). Since this assay does not detect 70% of class I low-virulence APMV-1s, the multiplex rRT-PCR assay, which targets the L gene, was run as well (27). RNA extraction was calculated as previously described by Diel *et al.* (15).

Swab medium from each APMV-1 PCR positive sample was inoculated into three 9-to-11-day-old specific-pathogen-free (SPF) embryonating chicken eggs using standard methods as previously described by Senne (40). All isolates were identified, subtyped, and characterized according to standard procedures at the SEPRL.

Intracerebral pathogenicity index (ICPI) assay. One-day-old SPF chicks were inoculated intracerebrally with 50 µl of a 1:10 dilution of viral infected allantoic fluid, monitored daily for 8 days, and scored as 0 if normal, 1 if sick, or 2 if dead. The ICPI value was calculated as the mean score per bird per observation (3).

Hemagglutination assay. The hemagglutination assay (HA) was completed using microtiter methods. The HA of allantoic fluids harvested from inoculated embryonating eggs was used to identify APMV-1–positive embryos. Known APMV-1 positive serum was used for the micro-beta hemagglutination inhibition (HI) assay to that confirm HA activity was due to APMV-1.

Mean death time (MDT). Nine-to-11-day-old SPF embryonating chicken eggs were inoculated with viral-infected allantoic fluid as described previously (3). The viral-infected allantoic fluids were collected from the inoculated eggs either after the death of the embryo or after 6 days postinoculation and were titrated by HA using the Spearmann-Karber method to calculate the EID_{50} (24). MDT over 90 hr was expected for low-virulence APMV-1.

Amplification and sequencing. Extracted RNA from viral-infected fluid was extracted and then converted to cDNA by reverse transcription and amplified using PCR. This larger amount of cDNA was used to sequence the virus. Amplification of the RNA and sequencing of the complete coding region of the fusion gene was performed according to methods previously described by Kim *et al.* (27).

Phylogenetic analysis. A 374 base pair and full fusion region of the F gene was used to construct the phylogenetic trees and to phylogenetically classify the mute swan NDV isolates ($n = 2$) because the highest percentage nucleotide corresponded to a partial GenBank sequence. This was conducted according to methods previously described by Diel *et al.* (15). The evolutionary history was inferred by using the maximum likelihood method based on the general time reversible model (15). The tree with the highest log likelihood (−10,190.6466) is shown (Fig. 1). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood method, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (4 categories; +G, parameter = 1.7362). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 45.0285% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 117 nucleotide sequences because this was the number of nucleotides of the most closely related sequence available in GenBank. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1662 positions in the final data set. Evolutionary analyses were conducted in MEGA5 (46).

Accession numbers. The sequences of the F gene of the APMV-1 isolates characterized in this study are available in GenBank under accession numbers KF444680 and KF444681.

AIV. *Serology*. All serum samples were tested for the presence of AIV antibodies using a multispecies bELISA (IDEXX Laboratories, Westbrook, ME) according to the manufacturer's instructions and using multiple positive and negative controls. Sample-to-negative (S/N) ratios <0.50 were considered positive, whereas those ≥ 0.50 were considered negative.

Virus detection and isolation. Swab samples were thawed at 37 C and homogenized by vortexing. RNA extractions were performed using the QIAamp viral RNA mini kit (Qiagen Sciences, Germantown, MD) using 140 µl of sample material, according to the manufacturer's instructions. The 390 samples were initially screened by rRT-PCR for the AIV matrix gene at MSU following protocols described by Spackman *et al.* (43). Matrix-positive samples were further tested with H5- and H7-specific assays (41,43). Virus isolation was conducted on all matrix positive samples at the NVSL by inoculating a suspension of each specimen into embryos of SPF chicken eggs (45).

***Salmonella* detection.** Samples were submitted to MSU for *Salmonella* culture and serogrouping. Specimens were selectively enriched in tetrathionate brilliant green broth with 36 µl in 9 ml of iodine (per sample) and incubated at 42 C. At 24 hr, 100 µl of culture was transferred to 10 ml of Rappaport-Vissiliadis broth and incubated for 1824 hr at 37 C. Cultures were then inoculated onto two agar plates selective for *Salmonella* growth (brilliant green agar with novobiocin and xylose-lysine-tergitol-4 agar) and incubated for 18–24 hr at 37 C. If nonlactose fermenting colonies were observed, five morphologically distinct colonies with typical characteristics of *Salmonella enterica* organisms were selected from either of the two agar plates, inoculated onto triple sugar iron agar slant and lysine iron agar slant, and incubated at 37 C for 18–24 hr to identify reactions typical of *S. enterica*. If reactions were typical of *S. enterica*, no further testing was necessary. Presumptive positive isolates were confirmed as necessary with Vitek Legacy GNI+ cards (BioMerieux, Durham, NC). All positives were identified by standard methods. Each isolate was serotyped by slide agglutination with antisera specific for *Salmonella* O groups and tube agglutination for flagellar (H) antigens. Serotyping of isolates based on the Kauffman-White classification scheme was performed at MSU by standard methods. Isolates where serotyping was incomplete were forwarded to the Michigan Department of Community Health for further testing.

Data analysis. Mean seroprevalence and 95% confidence limits were determined using an exact binomial distribution for prevalence of both pathogens. Co-infection relationships between individuals that were actively infected with both AIV and APMV-1 were determined using Fisher's exact test.

RESULTS

From March 2011 to September 2012, we collected samples from 858 mute swans in Indiana, Massachusetts, Michigan, New Jersey, New York, Rhode Island, and Wisconsin (Table 1). The majority of the samples (85%) were collected from after-hatch-year birds. A total of 771 mute swans were submitted for APMV-1 antibody testing, 414 for APMV-1 viral detection, 344 for AIV antibody testing, 390 for AIV virus detection, and 459 samples for *Salmonella* testing (Table 1). There were 527 mute swans that were tested for

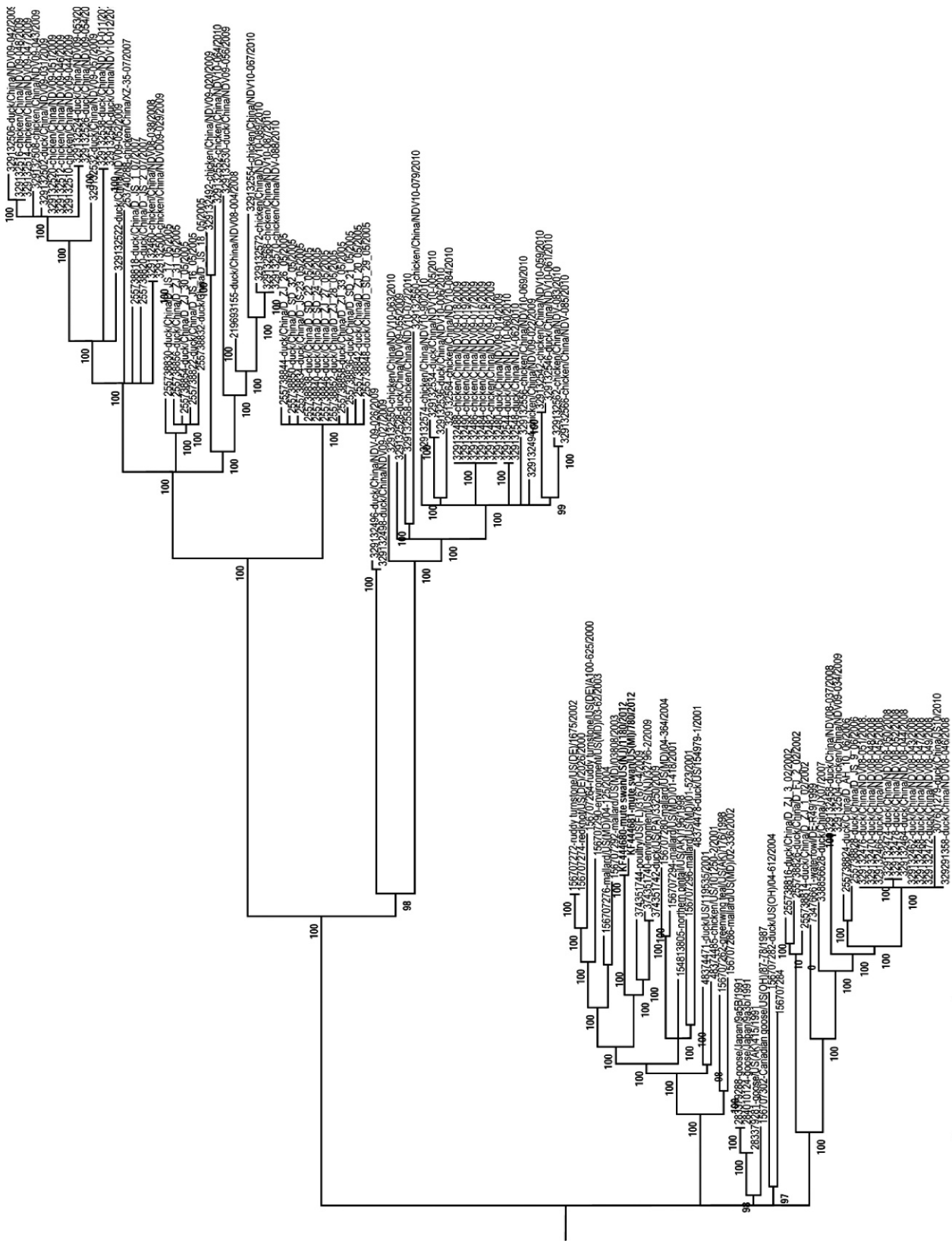

Fig. 1. Molecular phylogenetic analysis of the isolated APMV-1 viruses. The full fusion coding region was compared to identical region from GenBank isolates, and a phylogenetic analysis was conducted to determine the relationship among the isolates and most closely related viruses. The evolutionary history was inferred by using the maximum likelihood method based on the general time reversible mode.

Table 1. Apparent prevalence of avian influenza virus and Newcastle disease virus in mute swans (*Cygnus olor*) in selected states in the United States as detected by rRT-PCR (swabs) and ELISA (serum).

			Avian influenza (%, 95% CL)		Newcastle disease virus (%, 95% CL)	
State (*n*)	County	Season[A] (*n*)	Swabs	Serum	Swabs	Serum
All (858)			0.8 (0.15–2.2)	45.1 (36.7–50.5)	8.7 (6.2–11.8)	59.9 (56.4–63.4)
Indiana (7)	Steuben	Spring (7)	0[B]	66.6 (22.3–95.7)	0	50 (11.8–88.2)
Massachusetts (12)	Worchester	Winter (12)	16.7 (2.01–48.4)	N/A[C]	16.6 (20.1–48.4)	N/A
Michigan (461)	All	All	0	54.1 (46.8–61.3)	7.6 (4.4–12.0)	52.4 (47.6–57.2)
	Allegan	Spring (2)	0	100 (15.8–100)	0	100 (15.8–100)
	Alpena	Summer (20)	0	30 (6.6–65.2)	0	38.9 (17.3–64.3)
	Arenac	Spring (16)	0	76.9 (46.1–94.9)	0	62.5 (35.4–84.8)
	Barry	Summer (7)	0	75 (19.4–99.3)	0	N/A
	Bay	Spring (45)	0	76.7 (61.3–88.2)	17.4 (4.9–38.7)	57.8 (42.1–72.3)
	Calhoun	Spring (10)	0	50 (18.7–81.2)	0	20 (2.5–55.6)
		Summer (6)	N/A	N/A	N/A	100 (47.8–100)
	Charlevoix	Summer (10)	0	90 (55.4–99.7)	0	70 (34.7–93.3)
	Chippewa	Spring (3)	N/A	N/A	N/A	0
	Gratiot	Spring (9)	0	22.2 (2.8–60.0)	0	55.5 (21.2–86.3)
	Hillsdale	Spring (20)	0	53.3 (26.5–78.7)	0	63.2 (38.3–83.7)
	Iosco	Winter (7)	N/A	85.7 (42.1–99.6)	N/A	57.1 (18.4–90.1)
	Lake	Spring (2)	N/A	N/A	N/A	N/A
	Livingston	Spring (10)	0	66.6 (29.9–92.5)	N/A	66.7 (29.9–92.5)
	Mackinac	Spring (19)	0	83.3 (58.5–96.4)	0	61.1 (35.7–82.7)
		Summer (10)	N/A	N/A	N/A	100 (69.1–100)
	Macomb	Spring (5)	N/A	100 (47.8–100)	N/A	60 (14.6–94.7)
	Manistee	Spring (3)	N/A	100 (29.2–100)	N/A	100 (29.2–100)
	Monroe	Spring (95)	0	60 (48.8–70.4)	21.4 (8.2–40.9)	44.6 (34.1–55.2)
		Summer (10)	N/A	N/A	N/A	42.8 (9.8–81.5)
	Montcalm	Spring (10)	0	22.2 (2.8–60.0)	10 (0.2–44.5)	33.3 (7.4–70.0)
	Muskegon	Spring (10)	0	50 (18.7–81.2)	0	30 (6.6–65.2)
	Newaygo	Spring (15)	N/A	71.4 (41.8–91.6)	N/A	40 (16.3–67.7)
		Summer (4)	N/A	N/A	N/A	50 (6.7–93.2)
	Saginaw	Spring (18)	0	76.4 (50.1–93.1)	N/A	52.9 (27.8–77.0)
	St. Clair	Spring (40)	0	83.8 (67.9–93.8)	18.1 (2.2–51.7)	59 (42.0–74.4)
		Winter (10)	0	70 (34.7–93.3)	0	50 (18.7–81.2)
	Tuscola	Spring (25)	0	70.5 (44.0–89.6)	27.2 (6.0–60.9)	43.5 (23.1–65.5)
	Van Buren	Spring (10)	0	44.4 (13.6–78.7)	0	11.1 (0.2–48.2)
	Wayne	Winter (10)	0	70 (34.7–93.3)	0	70 (34.7–93.3)
New Jersey (210)	All	Summer (210)	0	18.4 (10.9–28.1)	3.0 (0.6–8.6)	57.6 (50.1–64.8)
	Atlantic	Summer (56)	0	14.7 (4.95–31.0)	5.1 (0.6–17.3)	31.3 (18.6–46.2)
	Cape May	Summer (27)	N/A	N/A	N/A	85.7 (63.6–96.9)
	Hudson	Summer (47)	0	12.5 (0.3–52.6)	0	83.7 (69.2–93.1)
	Salem	Summer (55)	0	24 (9.3–45.1)	3.8 (0–19.6)	61.5 (47.0–74.6)
	Sussex	Summer (25)	0	20 (5.7–43.6)	0	10 (8.6–49.1)
New York (28)	All	Spring (28)	0	72.7 (39.0–94.0)	16.6 (3.6–41.4)	83.3 (62.6–95.3)
	Monroe	Spring (10)	N/A	N/A	66.6 (9.4–99.1)	77.8 (39.9–97.1)
	Oneida	Spring (2)	0	50 (1.2–98.7)	0	100 (15.8–100)
	Orleans	Spring (4)	0	100 (39.7–100)	0	100 (39.7–100)
	Wayne	Spring (12)	0	60 (14.6–94.7)	11.1 (0.2–48.2)	77.8 (39.9–97.1)
Rhode Island (129)	All	All (129)	2.0 (0.05–10.6)	47.8 (32.9–62.3)	19.0 (9.9–31.4)	87.3 (79.9–92.7)
	Bristol	Spring (3)	0	0	33.3 (0.8–90.5)	100 (2.5–100)
	Kent	Summer (20)	5.0 (0.1–24.8)	75 (50.8–91.3)	5 (0.1–24.8)	65 (40.7–84.6)
	Newport	Summer (7)	0	0	50 (1.2–98.7)	100 (59.0–100)
	Providence	Summer (27)	0	50 (11.8–88.1)	14.3 (0.3–57.8)	100 (85.7–100)
	Washington	Summer (72)	0	22.2 (6.4–47.6)	26.9 (11.5–47.7)	87.9 (77.5–94.6)
Wisconsin (11)	All	All (11)	0	N/A	11.1 (0.2–48.2)	50 (1.2–98.7)
	Door	Spring (5)	0	N/A	20 (0.5–71.6)	N/A
	Fond du Lake	Winter (2)	0	N/A	0	N/A
	Rock	Summer (2)	N/A	N/A	N/A	50 (1.2–98.7)
	Winnebago	Spring (2)	0	N/A	0	N/A

[A]Spring is defined as March through May, summer is defined as June through August, and winter is defined as December through February. No samples were collected from September through November.

[B]Samples were submitted, but none of them tested positive.

[C]No samples were submitted for testing.

antibodies to both APMV-1 and AIV. The apparent prevalence of active APMV-1 infection based on rRT-PCR was 8.7% ($n = 414$; 95% confidence limits [CL]: 6.2–11.8) and the seroprevalence was 59.9% ($n = 771$; 95% CL: 56.4–63.4). Of 36 APMV-1 virus-positive samples, 25 were seropositive, 6 were seronegative, and 5 were not tested due to insufficient serum. The apparent prevalence of active AIV infection based on rRT-PCR was 0.8% ($n = 390$; 95% CL: 0.15–2.2), and the seroprevalence was 45.1% ($n = 344$; 95% CL: 36.7–50.5). Serum was available for testing only 1 of the 3 AIV virus positive, samples and it was also antibody positive. No AIV viruses were isolated, and none of the samples were positive by rRT-PCR for the H5 or H7 subtype.

Two low-virulence APMV-1 viruses were isolated, and these were subjected to phylogenetic analysis to determine the evolutionary distances to other APMV-1 strains (Fig. 1). Both viruses were class I; however, one of the mute swans was collected in Atlantic County, NJ, in August, and the other was collected in Tuscola County, MI, in April. The nearest related isolates are the viruses G/2009/US/OR/374351754 (partial sequence not shown), G/2001/US/48374471, G/2001/US/48374485, G/2009/US/FL/374351744, G/2009/US/PA/374351742, and G/2009/US/NJ/374351740 (Fig. 1). The ICPI for both isolates was 0.00, and MDT was >168 hr.

In 2011 a total of 459 samples were tested for *Salmonella,* and three of them tested positive. Two of the culture positive samples were collected in New Jersey, and one was collected in Rhode Island. The sample from Rhode Island was identified to the species level as *S. enterica* serovar Typhimurium. Strain typing revealed this isolate was negative for the antibiotic-resistant *Salmonella* Typhimurium DT104. One of the positive samples from New Jersey was identified as *Salmonella enterica* serovar Braenderup, but the other positive sample was identified only as Group B. Further identification was not possible because the serotype combination is unnamed. *Salmonella* testing was discontinued in 2012 because so few positives were identified in 2011.

DISCUSSION

Serologic evidence of NDV has been detected previously in swans (16); however, this previous detection was in aviculturist flocks while our samples were collected from wild birds. Our serosurvey indicated nearly 60% of mute swans were antibody positive for APMV-1, suggesting that they may be an important reservoir host for APMV-1 or help maintain the virus in the environment. Consequently, they may serve as a risk to poultry that come into contact with them, especially domestic fowl and turkeys that utilize common water sources. Poultry workers and backyard poultry owners whose recreational activities overlap with areas utilized by mute swans also may serve as mechanical vectors of APMV-1 to their flock.

Our data suggest that mute swans in New York and Rhode Island are more likely to be exposed to APMV-1. However, the time of year that samples were collected varied by state, making it difficult to identify any definitive patterns of exposure, and the mechanism for maintenance of APMV-1 in the environment is not fully understood (20).

Age-related immunity is one explanation for our detection of serological evidence of AIV and APMV-1 as significant since most of our samples were collected from after-hatch-year birds, allowing adequate time for exposure and development of antibodies to the viruses. Since sample collection was not distributed evenly across space and time, we are unable to determine whether co-infections are more likely in certain areas.

Our results not only indicate that mute swans are exposed to APMV-1 regularly, but also suggest that active viral infection is not uncommon since almost 9% of all swans tested were PCR positive, and the majority of samples collected (98.6%) were from apparently healthy birds. Although 9% were APMV-1 positive, we were able to isolate only two viruses. Interestingly, the two full fusion sequences (1662 base pairs) were very similar to each other (99.99% identity) despite the fact the mute swans were collected in Michigan and New Jersey, thus suggesting that this particular lineage of virus may be more adapted to replicate in swans, or that the virus was recently transported between the two locations by some unknown means (e.g., migrating waterfowl). Since mute swans are nonmigratory birds (37), it is unlikely that the virus moved between locations via mute swans. However, the distance they travel and the amount of commingling between subpopulations is unknown.

The most closely related relatives to our isolates were viruses isolated from wild birds, except for one isolate from a chicken and the other from an unspecified species of poultry (Fig. 1). The identification of sequences so closely related to isolates of poultry origin suggests that there may be spillover among wild birds and poultry, although it is difficult to determine which direction. Also, the viruses may have spilled over more than once based on the different dates of isolation of the poultry isolates (2001 and 2009). Spillover from poultry to wild bird species has been documented (17), and our study suggests that either mute swans may have become infected as a result of spillover from other species or they may represent a reservoir of the virus. It appears that there is very little geographic or evolutionary divergence not only because the mute swan samples we were able to isolate and sequence were from Michigan and New Jersey, but also because the isolates in GenBank with geographic data that were most closely related to our mute swan isolates originated from Oregon, Florida, Pennsylvania, and New Jersey from 2001 to 2009.

Large-scale surveillance efforts to identify AIVs in wild birds in the United States have indicated that mute swans are carriers of LPAIV (35). Specifically, of 2797 samples collected from mute swans, 0.4% were positive for H5N1, H5N2, or H7N3 (USDA-WS, unpublished data), all of which are subtypes associated with disease in humans or poultry (11,36,42), and was higher than the 0.2% of all subtypes from all species found in the 283,434 samples tested nationwide (USDA-WS, unpublished data). Results from our study suggest it is not uncommon for mute swans to be exposed to AIV (45.1% antibody prevalence); however, active viral shedding was less common (0.8%; 95% CL: 0.15–2.2). Also, two of the three AIV virus–positive samples in our study were collected from mute swans from the same location and day during a morbidity/mortality event of unknown cause. This artificially inflated the apparent prevalence, suggesting that active viral infection in mute swans is relatively uncommon in the areas we sampled. Mute swans are nonmigratory in the United States and consequently are not subject to the same physiological demands placed on migratory birds (53), which could explain the lower prevalence of active viral infection. However, if the virus became established as endemic in a resident population such as mute swans, this would be disconcerting due to the implications for direct and indirect transmission to other birds or humans.

We detected two serovars of *Salmonella,* both of which are in the top 20 most frequently reported serovars associated with human cases in the United States (8). Detection of *Salmonella* Typhimurium was of particular interest because it has been identified as a significant cause of food poisoning and enteric fever in humans (31) and is responsible for the majority of salmonellosis in cattle (14). This strain was DT104 negative, which is not surprising since it is unlikely that mute swans would be carrying an antibiotic-resistant

strain of *Salmonella*. *Salmonella* Braenderup is typically an uncommon serotype in the United States; however, it has been documented in human cases that were traced back to consumption of infected chickens, eggs, and tomatoes (19). Even though the third *Salmonella* positive sample could not be identified beyond Group B, this finding is still significant because some of the *Salmonella* serotypes most pathogenic to humans are included in Group B, including *Salmonella* Typhimurium and *Salmonella* Paratyphi (18).

Our results suggest that as mute swan populations continue to expand their geographic distribution, the potential for disease transmission will also increase since AIV and APMV-1 antibodies were common and active viral infections for both diseases were detected. Since the poultry industry in the United States is worth more than $38 billion (51), and these viruses can infect poultry, biosecurity measures to prevent contact in conjunction with additional studies to identify other pathogens of interest are recommended.

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