BioOne RESEARCH EVOLVED

Identification of Avian Paramyxovirus Serotype-1 in Wild Birds in the USA

Author(s): Kerri Pedersen , David R. Marks , Claudio L. Afonso , Scott R. Stopak , Dawn Williams-Coplin , Kiril M. Dimitrov , Patti J. Miller , and Thomas J. DeLiberto

Source: Journal of Wildlife Diseases, 52(3):657-662.
Published By: Wildlife Disease Association
DOI: http://dx.doi.org/10.7589/2015-10-278
URL: http://www.bioone.org/doi/full/10.7589/2015-10-278

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

DOI: 10.7589/2015-10-278

Journal of Wildlife Diseases, 52(3), 2016, pp. 657–662
© Wildlife Disease Association 2016

Identification of Avian Paramyxovirus Serotype-1 in Wild Birds in the USA

Kerri Pedersen,[1,6] David R. Marks,[2] Claudio L. Afonso,[3] Scott R. Stopak,[4] Dawn Williams-Coplin,[3] Kiril M. Dimitrov,[3] Patti J. Miller,[3] and Thomas J. DeLiberto[5] [1]US Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, 4101 LaPorte Avenue, Fort Collins, Colorado 80521, USA; [2]US Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, 2803 Jolly Road, Suite 100, Okemos, Michigan 48864, USA; [3]US Department of Agriculture, Agricultural Research Service, Southeast Poultry Research Laboratory, 934 College Station Road, Athens, Georgia 30605, USA; [4]Animal and Plant Health Inspection Service, Wildlife Services, US Department of Agriculture, 9134 Blackeagle Drive, Boise, Idaho 83709, USA; [5]US Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, Colorado 80521, USA; [6]Corresponding author (email: Kerri.Pedersen@aphis.usda.gov)

ABSTRACT: In the US, sampling for avian paramyxovirus serotype-1 (APMV-1) is generally conducted when morbidity or mortality events occur involving certain families of wild birds known to be affected by the virus, such as cormorants (Family Phalacrocoracidae), pigeons, doves (Family Columbidae), or pelicans (Family Pelecanidae). To quantify the prevalence of APMV-1 in apparently healthy wild birds and to determine its geographic distribution, we collected swab and serum samples from >3,500 wild birds, representing eight orders from 1 January 2013 to 30 September 2013. Antibody prevalence was highest in wild birds of Order Suliformes (44.9%), followed by Pelecaniformes (24.4%), Anseriformes (22.7%), and Columbiformes (11.7%), with a relatively high occurrence of virulent viruses in Columbiformes (100% of virulent viruses isolated). As expected, viral shedding was comparatively much lower, and positives were only identified in Orders Accipitriformes (1.4%), Columbiformes (1.0%), Anseriformes (0.8%), and Charadriiformes (0.4%). We also demonstrate circulating virulent APMV-1 viruses of genotype VI in apparently healthy Rock Pigeons (*Columba livia*) from March through September in three states.

Key words: Avian paramxyovirus serotype-1, Newcastle disease, Newcastle disease virus, pigeon paramyxovirus serotype-1, wild birds.

Newcastle disease, caused by virulent strains of avian paramyxovirus serotype-1 (APMV-1), is a disease of economic concern for commercial and backyard poultry producers due to high mortality (up to 100%), impact of outbreaks, and trade restrictions associated with infection (Aldous and Alexander 2001). Although infection in wild birds can cause disease (Leighton and Heckert 2007), mortality due to virulent APMV-1 is typically undetected in wild birds compared to poultry (Kim et al. 2007). However, virulent APMV-1 infection in certain species such as cormorants (Family Phalacrocoracidae; Kuiken 1999) and pigeons (Family Columbidae; Kim et al. 2008a) has resulted in large-scale mortality events and occasional spillover into other species, such as pelicans (Family Pelecanidae; USGS 1999), gulls (Family Laridae; Diel et al. 2012), and poultry (Irvine et al. 2011). In addition to virulent forms, a large diversity and wide distribution of low-pathogenicity viruses across different geographic regions exist in waterfowl (Anseriformes) and shorebirds (Charadriiformes; Kim et al. 2007). These low-virulence viruses occasionally spill over into poultry, causing minor respiratory problems (Hamid et al. 1990).

Large-scale efforts to actively identify the species involved in transmission of APMV-1 in the US have been limited to specific species or orders (Kim et al. 2007; Coffee et al. 2010; Pedersen et al. 2014). We evaluated viral shedding, exposure (antibody presence), and host distribution of APMV-1 in a diversity of apparently healthy wild birds across the US.

Wild birds were opportunistically sampled by the US Department of Agriculture's Animal and Plant Health Inspection Service–Wildlife Services during removal of wild birds from airports and other locations that posed a threat to human health and safety. In addition, wild birds were trapped with mist nets, rocket nets, and swim-in traps specifically for sampling and were subsequently released. Sam-

ples were collected 1 January 2013 to 30 September 2013 in 44 states (Supplementary Material Table S1).

Cloacal and oropharyngeal swabs were collected from wild birds using sterile Dacron-tipped swabs (Puritan Medical Products Company LLC, Guilford, Maine, USA). Both swabs were placed in a single cryogenic vial containing 3 mL of brain-heart infusion broth (Becton Dickinson and Company, Sparks, Maryland, USA). The vials were immediately placed in a cooler with ice packs, then stored refrigerated, and shipped with ice packs within 3 d of collection.

When possible, blood was collected from swabbed birds via the jugular, brachial, or metatarsal vein, and via postmortem intracardiac puncture from euthanized birds. After centrifugation, $\geq$1 mL of serum was transferred to a 2-mL cryogenic vial. Sera samples were stored refrigerated and shipped within 3 d of collection.

Swabs were tested at the Michigan State University Genomics Laboratory (East Lansing, Michigan, USA) using multiplex real-time reverse transcription PCR (rRT-PCR), to identify infection with class I and class II APMV-1 (Kim et al. 2008b). If negative, testing was considered complete. Positive samples were forwarded to the US Department of Agriculture's Agricultural Research Services Southeast Poultry Research Laboratory (Athens, Georgia, USA) for confirmatory testing (Pedersen et al. 2014). Briefly, swab medium (0.1 mL) from each sample was inoculated into five 9-to-11-d-old specific-pathogen-free embryonating chicken eggs using standard methods (Senne 2008). Virulence of isolates was assessed by requiring an intracerebral pathogenicity index of $\geq$0.7 in 1-d-old chickens or multiple basic amino acid residues at the C-terminus of the fusion protein cleavage sites (residues 113–116) and a phenylalanine residue at position 117 (OIE 2012).

Extracted RNA was converted to cDNA and amplified using one-tube reverse-transcriptase PCR (SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase, Life Technologies, Carlsbad, California, USA). The PCR reactions were subjected to electrophoresis in 1% agarose gels, and DNA bands were excised and purified using the QuickClean II Gel Extraction Kit (GenScript, Piscataway, New Jersey, USA). The APMV-1 isolates were sequenced (Miller et al. 2015) and deposited in GenBank (KP780870–KP780878).

A blocking enzyme-linked immunosorbent assay (ID VET, Montpellier, France) was used to detect antibodies against APMV-1 using multiple positive and negative controls according to the manufacturer's instructions. Percent inhibition values >40% were considered positive, <30% were considered negative, and values 30–40% were considered undetermined and were retested once.

The majority (68%) of the 3,789 wild birds sampled were after-hatch-year (AHY) birds, 14.5% were hatch year (HY), and the remainder were of undetermined (UND) age. Viral shedding was slightly higher in HY birds (1.3%; 95% confidence limits [CL] 0.62–2.6) compared to AHY birds (0.58%; 95% CL: 0.35–0.96) or UND birds (0.3%; 95% CL: 0.08–1.1). Antibody prevalence was highest in AHY (19.2%; 95% CL: 17.69–20.81), followed by UND (17.4%; 95% CL: 14.61–20.7), and HY (9.9%; 95% CL: 7.65–12.78).

Although recorded for each bird, sex was undetermined for 74.4%. Viral shedding was similar for females (1.4%; 95% CL: 0.63–2.98) and males (1.1%; 95% CL: 0.53–2.48) where sex was identified (n=963). Antibody prevalence was also similar in females (21.9%; 95% CL: 18.17–26.05) and males (19.8%; 95% CL: 16.58–23.48).

We tested 3,779 swabs for APMV-1, and 3,578 sera samples for antibody to APMV-1. The majority (3,568) of these were paired samples from the same birds. Antibody prevalence was highest in wild birds of Order Suliformes (44.9%), followed by Pelecaniformes (24.4%), Anseriformes (22.7%), and Columbiformes (11.7%; Table 1). We isolated virus from 24 samples that were positive on the initial rRT-PCR (Table 2).

The seven class II (virulent) viruses we identified were classified as pigeon paramyxovirus serotype-1 (PPMV-1), or genotype VI,

TABLE 1. Number of wild bird samples with antibody to or viral shedding of avian paramyxovirus serotype-1 (APMV-1) by species that were collected 1 January–30 September 2013 across the USA.

Order Common name (*Scientific name*)	APMV-1 (virus) (positive/*n*)	APMV-1 (antibody) (positive/*n*)
Accipitriformes	1/72	6/73
Red-tailed Hawk (*Buteo jamaicensis*)	1/34	3/36
Turkey Vulture (*Cathartes aura*)	0/25	3/25
Anseriformes	13/1,654	369/1,624
American Black Duck (*Anas rubripes*)	0/2	1/2
American Green-winged Teal (*Anas crecca*)	2/26	6/26
American Wigeon (*Anas americana*)	0/6	1/5
Blue-winged Teal (*Anas discors*)	0/33	7/32
Canada Goose (*Branta canadensis*)	0/936	201/917
Gadwall (*Anas strepera*)	1/1	1/1
Greater Scaup (*Aythya marila*)	0/1	1/1
Greater White-fronted Goose (*Anser albifrons*)	0/13	7/13
Lesser Scaup (*Aythya affins*)	0/3	1/3
Lesser Snow Goose (*Chen caerulescens*)	0/20	13/19
Mallard (*Anas platyrhynchos*)	2/297	64/289
Muscovy Duck (*Cairina moschata*)	0/7	1/7
Mute Swan (*Cygnus olor*)	2/238	57/236
Northern Pintail (*Anas acuta*)	0/11	2/11
Northern Shoveler (*Anas clypeata*)	6/20	3/20
Wood Duck (*Aix sponsa*)	0/31	3/31
Charadriiformes	2/511	8/464
California Gull (*Larus californicus*)	0/15	1/15
Laughing Gull (*Larus atricilla*)	0/157	2/138
Ring-billed Gull (*Larus delawarensis*)	2/125	5/118
Columbiformes	8/823	92/783
Eurasian Collared Dove (*Streptopelia decaocto*)	0/12	2/11
Rock Pigeon (*Columba livia*)	7/654	90/649
Zebra Dove (*Geopelia striata*)	1/42	0/35
Gruiformes	0/56	2/53
American Coot (*Fulica americana*)	0/34	1/34
Hawaiian Coot (*Fulica alai*)	0/11	1/8
Passeriformes	0/245	3/229
American Crow (*Corvus brachyrhynchos*)	0/20	2/20
European Starling (*Sturnus vulgaris*)	0/142	1/134
Pelecaniformes	0/49	12/49
American White Pelican (*Pelecanus erythrorhynchos*)	0/5	4/5
Glossy Ibis (*Plegadis falcinellus*)	0/2	1/2
Great Egret (*Ardea alba*)	0/6	1/6
White Ibis (*Eudocimus albus*)	0/14	6/14
Suliformes	0/369	136/303
Double-crested Cormorant (*Phalacrocorax auritus*)	0/369	136/303

[a] Orders with <20 birds sampled were excluded from the analysis because there were no positives and the sample size was too low to make any conclusions.

from Rock Pigeons (*Columba livia*; Table 2), and they were phylogenetically similar to other paramyxoviruses isolated from pigeons in the US. PPMV-1 is a genetic variant of APMV-1 adapted to pigeons and doves that contains a virulent cleavage site and can have variable pathogenicity in chickens (Aldous et al. 2004). Although PPMV-1 is considered

TABLE 2. Species and collection sites in the USA for wild birds that were positive by real-time reverse transcription PCR for avian paramyxovirus serotype-1 (APMV-1) and corresponding antibody results for samples collected 1 January–30 September 2013.

GenBank accession no.	Common name	State	Virus isolated	Class	Genotype[a]	Cleavage site	Virulence	ICPI[b]	APMV-1 antibody
	Ring-billed Gull	New Jersey	No						Not tested
KP780875	Rock Pigeon	Michigan	Yes	II	VI a	$_{112}$RRQRR↓F$_{117}$	High	1.13	Negative
KP780876	Rock Pigeon	Michigan	Yes	II	VI a	$_{112}$RRQRR↓F$_{117}$	High	0.88	Negative
	Red-tailed Hawk	Michigan	No						Negative
	Mute Swan	Michigan	No						Positive
KP780870	Rock Pigeon	Maryland	Yes	II	VI a	$_{112}$RRQRR↓F$_{117}$	High	0.73	Negative
KP780877	Mallard	Idaho	Yes	I	NA	$_{112}$ERQER↓L$_{117}$	Low	0.48	Positive
	Mute Swan	New Jersey	No						Negative
KP780871	Rock Pigeon	Pennsylvania	Yes	II	VI a	$_{112}$RRQRR↓F$_{117}$	High	0.79	Positive
KP780872	Rock Pigeon	Pennsylvania	Yes	II	VI a	$_{112}$RRQRR↓F$_{117}$	High	1.15	Positive
KP780873	Rock Pigeon	Pennsylvania	Yes	II	VI a	$_{112}$RRQRR↓F$_{117}$	High	0.66	Negative
KP780874	Rock Pigeon	Pennsylvania	Yes	II	VI a	$_{112}$RRQRR↓F$_{117}$	High	1.20	Positive
KP780878	American Green-winged Teal	Alaska	Yes	I	NA	$_{112}$ERQER↓L$_{117}$	Low	0.56	Negative
	Zebra Dove	Hawaii	No						Negative
	Ring-billed Gull	Minnesota	No						Not tested
	Northern Shoveler	Oregon	No						Negative
	Northern Shoveler	Oregon	No						Negative
	Northern Shoveler	Oregon	No						Negative
	American Green-winged Teal	Oregon	No						Negative
	Northern Shoveler	Oregon	No						Negative
	Gadwall	Oregon	No						Positive
	Northern Shoveler	Oregon	No						Negative
	Northern Shoveler	Oregon	No						Negative
	Mallard	Oregon	No						Negative

[a] NA = not applicable.
[b] ICPI = intracerebral pathogenicity index.

endemic in pigeons and doves in the US, it is a concern to poultry (Kommers et al. 2001) because spillover can result in severe economic consequences.

Class I viruses are common to wild birds, are typically of low virulence, and have been identified in many species, such as Mute Swans (*Cygnus olor*; Pedersen et al. 2014), Northern Pintails (*Anas acuta*; Ruenphet et al. 2011), and other waterfowl and shorebirds (Kim et al. 2007). The two class I viruses we identified were in an apparently healthy Mallard (*Anas platyrhynchos*) and American Green-winged Teal (*Anas crecca*; Table 2).

Antibody prevalence of APMV-1 was much higher than virus prevalence (Table 1), which is not surprising because virus is shed for a limited, but variable time, depending on the

species infected (Kuiken et al. 1998), while antibodies persist much longer and vary depending on many factors related to the host (Staszewski et al. 2007) and the amount of antigen to which they are exposed (Broggi et al. 2013). There was very little concordance between the 24 rRT-PCR-positive samples and the serology results (Table 2). This is most likely because antibodies had not yet developed, had dropped below detectable levels, or had developed as a result of exposure to other APMVs. In addition, because none of our samples was collected in the fall (October through December), when viral transmission might be facilitated by the concentration of birds preceding and during migration (Vickers and Hanson 1982), antibody prevalence and viral shedding prevalence were likely skewed.

Our results suggest that wild birds are commonly exposed to APMV-1, but active viral shedding in apparently healthy birds is relatively uncommon, and consequently the risk to poultry appears low. Further studies focusing on pigeons are warranted because of their greater risk of contact with poultry (Gilchrist 2005), and because all virulent viruses we identified originated from Rock Pigeons.

We thank the wildlife biologists and technicians who collected samples for this study. We also thank the Michigan State University Genomics laboratory for conducting rRT-PCR for the samples, and Sarah Bevins for assistance with statistical analysis. Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2015-10-278.

LITERATURE CITED

Aldous EW, Alexander DJ. 2001. Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1). *Avian Pathol* 30:117–128.

Aldous EW, Fuller CM, Mynn JK, Alexander DJ. 2004. A molecular epidemiological investigation of isolates of the variant avian paramyxovirus type 1 virus (PPMV-1) responsible for the 1978 to present panzootic in pigeons. *Avian Pathol* 33:258–269.

Broggi J, García O, Miranda F, Pagès A, Soriguer RC, Figuerola J. 2013. Immune response to Newcastle disease virus vaccination in a wild passerine. *J Wildl Dis* 49:1004–1008.

Coffee LL, Hanson BA, Luttrell MP, Swayne DE, Senne DA, Goekjian VH, Niles LJ, Stallknecht DE. 2010. Avian paramyxoviruses in shorebirds and gulls. *J Wildl Dis* 46:481–487.

Diel DG, Miller PJ, Wolf PC, Mickley RM, Musante AR, Emanueli DC, Shively KJ, Pedersen K, Afonso CL. 2012. Characterization of Newcastle disease viruses isolated from cormorant and gull species in the United States in 2010. *Avian Dis* 56:128–133.

Gilchrist P. 2005. Involvement of free-flying wild birds in the spread of the viruses of avian influenza, Newcastle disease and infectious bursal disease from poultry products to commercial poultry. *World Poultry Sci J* 61:198–214.

Hamid H, Campbell RSF, Lamichhane C. 1990. The pathology of infection of chickens with the lentogenic V4 strain of Newcastle disease virus. *Avian Pathol* 19: 687–696.

Irvine RM, Aldous E, Fuller C, Alexander D, Brown IH, Davis C. 2011. Newcastle disease: A continuing threat to UK poultry. *Vet Rec* 168:673.

Kim LM, King DJ, Curry PE, Suarez DL, Swayne DE, Stallknecht DE, Slemons RD, Pedersen JC, Senne DA, Winker K, et al. 2007. Phylogenetic diversity among low-virulence Newcastle disease viruses from waterfowl and shorebirds and comparison of genotype distributions to those of poultry-origin isolates. *J Virol* 81:12641–12653.

Kim LM, King DJ, Guzman H, Tesh RB, da Rosa APAT, Bueno R Jr, Dennett JA, Afonso CL. 2008a. Biological and phylogenetic characterization of pigeon paramyxovirus serotype 1 circulating in wild North American pigeons and doves. *J Clin Microbiol* 46:3303–3310.

Kim LM, Suarez DL, Afonso CL. 2008b. Detection of a broad range of class I and II Newcastle disease viruses using a multiplex real-time reverse transcription polymerase chain reaction assay. *J Vet Diagn Invest* 20:414–425.

Kommers GD, King DJ, Seal BS, Brown CC. 2001. Virulence of pigeon-origin Newcastle disease virus isolates for domestic chickens. *Avian Dis* 45:906–921.

Kuiken T. 1999. Review of Newcastle disease in cormorants. *Waterbirds* 22:333–347.

Kuiken T, Heckert RA, Riva J, Leighton FA, Wobeser G. 1998. Excretion of pathogenic Newcastle disease virus by Double-crested Cormorants (*Phalacrocorax auritus*) in absence of mortality or clinical signs of disease. *Avian Pathol* 27:541–546.

Leighton FA, Heckert RA. 2007. Newcastle disease and related avian paramyxoviruses. In: *Infectious diseases*

of wild birds, Thomas NJ, Hunter DB, Atkinson CT, editors. Blackwell Publishing, Ames, Iowa, pp. 3–16.

Miller PJ, Haddas R, Simanov L, Lublin A, Rehmani SF, Wajid A, Bibi T, Khan TA, Yaqub T, Setiyaningsih S, et al. 2015. Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features. *Infect Genet Evol* 29:216–229.

Office International des Epizooties (OIE). 2012. Newcastle disease. In: *Manual of diagnostic tests and vaccines for terrestrial animals*. World Organization for Animal Health, Paris, France, pp. 555–573.

Pedersen K, Marks DR, Arsnoe DM, Afonso CL, Bevins SN, Miller PJ, Randall AR, DeLiberto TJ. 2014. 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. *Avian Dis* 58: 129–136.

Ruenphet S, Jahangir A, Shoham D, Morikawa K, Miyoshi Y, Hanawa E, Okamura M, Nakamura M, Takehara K. 2011. Surveillance and characterization of Newcastle disease viruses isolated from Northern Pintail (*Anas acuta*) in Japan During 2006–09. *Avian Dis* 55: 230–235.

Senne DA. 2008. Virus propagation in embryonating eggs. In: *A laboratory manual for the isolation, identification, and characterization of avian pathogens*, Dufour-Zavala L, editor. American Association of Avian Pathologists, Jacksonville, Florida, pp. 204–208.

Staszewski V, Gasparini J, McCoy KD, Tveraa T, Boulinier T. 2007. Evidence of an interannual effect of maternal immunization on the immune response of juveniles in a long-lived colonial bird. *J Anim Ecol* 76: 1215–1223.

US Geological Survey (USGS). 1999. Newcastle disease. In: *Field manual of wildlife diseases: General field procedures and diseases of birds*. USGS, Washington, DC, pp. 175–179.

Vickers ML, Hanson RP. 1982. Newcastle disease virus in waterfowl in Wisconsin. *J Wildl Dis* 18:149–158.

Submitted for publication 14 October 2015.
Accepted 15 January 2016.