DOI: 10.7589/2016-07-160

Journal of Wildlife Diseases, 53(3), 2017, pp. 607–611

Aquatic Bird Bornavirus-Associated Disease in Free-Living Canada Geese (*Branta canadensis*) in the Northeastern USA

Maureen Murray,[1] Jianhua Guo,[2] Ian Tizard,[2,6] Samuel Jennings,[1] H. L. Shivaprasad,[3] Susan Payne,[2] Julie C. Ellis,[1] Arnaud J. Van Wettere,[4] and Kathleen M. O'Brien[5] [1]Cummings School of Veterinary Medicine, Tufts University, 200 Westboro Road, North Grafton, Massachusetts 01536, USA; [2]College of Veterinary Medicine, Texas A&M University, 4467 TAMU, College Station, Texas 77843, USA; [3]California Animal Health and Food Safety Laboratory System, University of California, Davis, 18830 Road 112, Tulare, California 93274, USA; [4]School of Veterinary Medicine and Utah Veterinary Diagnostic Laboratory, Utah State University, 950 E 1400 N, Logan, Utah 84341, USA; [5]US Fish and Wildlife Service, Rachel Carson National Wildlife Refuge, 321 Port Road, Wells, Maine 04090, USA; Corresponding author (email: itizard@cvm.tamu.edu)

ABSTRACT: During the winter of 2013–14, 22 Canada geese (*Branta canadensis*) were admitted to the Wildlife Clinic at the Cummings School of Veterinary Medicine at Tufts University with nonspecific neurologic abnormalities and emaciation. Five of these geese, along with three geese that were submitted dead, were evaluated via histopathology, immunohistochemistry, and reverse transcription PCR (RT-PCR) for bornaviruses. Histopathologically, six of the eight birds had lymphoplasmacytic encephalitis. One bird, which also had encephalitis, had a dilated esophagus. Lead poisoning, West Nile virus, avian influenza, and avian paramyxovirus infection were excluded from the diagnosis. Brain tissue from all eight geese was positive for bornaviral N-antigen on immunohistochemistry. Frozen brain tissue from five birds was available for bornavirus RT-PCR. Three of the five birds were positive for the bornavirus M gene. Formalin-fixed paraffin-embedded brain tissue was evaluated on the remaining three geese via RT-PCR, with one of these geese testing positive. A bornavirus was subsequently cultured in duck embryo fibroblasts from the brain of one Canada Goose. This virus genome was sequenced, and the virus was identified as aquatic bird bornavirus 1. We were unable to identify any unusual features of this genome that would account for its apparent pathogenicity, given that subclinical infection with bornavirus in waterfowl is common in North America.

Key words: Aquatic bird bornavirus, *Branta canadensis*, encephalitis, neurologic disease.

Since the identification of bornaviruses as the cause of psittacine proventricular dilatation disease in 2008, these viruses have been discovered in multiple species (Payne et al. 2012). While bornaviral infection can cause gastrointestinal dysfunction and/or neurologic abnormalities, bornaviruses have also been identified in asymptomatic birds, including free-living Canada geese (*Branta canadensis*) in North America (Payne et al. 2011; Delnatte et al. 2013). The correlation between exposure, infection, and development of disease is not understood (Delnatte et al. 2014).

This report describes a cluster of cases of neurologic disease in free-living Canada geese in the northeastern US, the association of disease with bornavirus infection, and the genomic characterization of the bornavirus isolated from the brain of an affected goose. From September 2013 through April 2014, 22 cases of neurologic disease accompanied by emaciation were observed in Canada geese admitted to the Cummings School of Veterinary Medicine at Tufts University in North Grafton, Massachusetts, US. Diagnostics to investigate the presence of bornaviruses were performed on five geese that were admitted alive to Tufts Wildlife Clinic (TWC) and three additional geese that were presented dead for necropsy through the Northeast Wildlife Disease Cooperative.

The five live geese admitted to TWC were found within the greater Boston area. All geese were after hatching year birds. Each displayed ataxia, emaciation, and weakness. White blood cell counts were not elevated. No abnormalities of the gastrointestinal tract were noted clinically or on radiographs. Blood lead levels were evaluated in all birds and were not considered clinically significant ($<$20 μg/dL), with the exception of one goose (bird 5), which had a lead level of 59.5 μg/dL. This goose was treated with chelation therapy (calcium ethylenediaminetetraacetic acid, 30

TABLE 1. Clinical and diagnostic findings in Canada geese (*Branta canadensis*) evaluated for aquatic bird bornavirus-1 infection. All birds brought to the clinic alive (numbers 1–5) showed ataxia and weakness; one bird (number 8) had been observed to have ataxia while still alive in the field. The results for the laboratory diagnostics were from examination of brain tissue using histopathology (HP) for lymphoplasmacytic meningoencephalitis, immunohistochemistry (IHC), and reverse transcription-PCR (RT-PCR). NA = not applicable.

Bird no.	Clinical findings				Diagnostic tests		
	Days in clinic	Outcome	Body mass (kg)				
			Admission	Postmortem	HP	IHC	RT-PCR
1	25	Euthanasia	2.6	4.0	Moderate	Severe	Positive[a]
2	13	Euthanasia	2.1	2.4	Severe	Moderate	Negative[a]
3	9	Died	2.3	2.5	Severe	Moderate	Negative[a]
4[b]	5	Euthanasia	2.3	2.5	Mild	Severe	Positive
5[c]	7	Euthanasia	3.0	3.2	Mild	Mild	Negative[d]
6[e]	NA	NA	NA	5.7	NA	Moderate	Negative
7[e]	NA	NA	NA	4.0	NA	Moderate	Positive
8[e]	NA	NA	NA	3.5	Mild	Severe	Positive

[a] RT-PCR performed on formalin-fixed paraffin-embedded tissue (rather than frozen).

[b] Dilated esophagus on gross postmortem examination.

[c] Elevated blood lead (59.5 μg/dL); no improvement in clinical signs following treatment and decrease of blood lead to <20 μg/dL.

[d] PCR positive in kidney.

[e] Freeze/thaw artifacts present.

mg/kg subcutaneously, twice daily, and dimercaptosuccinic acid, 30 mg/kg orally, twice daily) with no clinical improvement. All birds were treated with meloxicam (0.5 mg/kg orally twice daily) and general supportive care. Birds were housed in the clinic for 5 to 25 d. While all geese had excellent appetites, increased in weight, and gained strength, their ataxia did not improve. One goose died, and the remaining four were humanely euthanatized.

The three geese submitted for necropsy were found in Wells, Maine. A member of the public reported two dead Canada geese to the Rachel Carson National Wildlife Refuge. These geese were not observed antemortem, so the potential presence of clinical signs of disease is unknown. During collection of the two dead geese, another Canada Goose was observed stumbling and falling over at an adjacent marsh. This goose died a few days later and was collected. All three geese were brought to the Cummings School for necropsy.

Samples of brain, heart, spleen, and kidney were pooled and tested by reverse transcription PCR (RT-PCR) for the presence of West Nile virus (Bhatnagar et al. 2007). The RT-PCR for avian influenza virus and avian paramyxovirus matrix genes in tissue samples were performed as described by Spackman and Suarez (2008) and Wise et al. (2004). The PCR of tissue pools were negative for West Nile virus, avian influenza, and avian paramyxovirus in all birds.

The cDNA synthesis, RT-PCR screening, cloning, and sequencing were performed as described by Guo et al. (2014). The genome sequence obtained was submitted to GenBank under accession no. KP972428.

Three of the five geese that had been hospitalized at TWC had poor body conditions with reduced muscle mass and adipose tissue at postmortem examination (Table 1). The other two geese were in good body condition at necropsy due to weight gain during hospitalization. Of the three birds that were presented dead, one (bird 8) had moderately reduced body condition, while the other two geese were in good body condition (Table 1). The two well-conditioned birds (birds 6 and 7) were those that had been found dead without observed antemortem

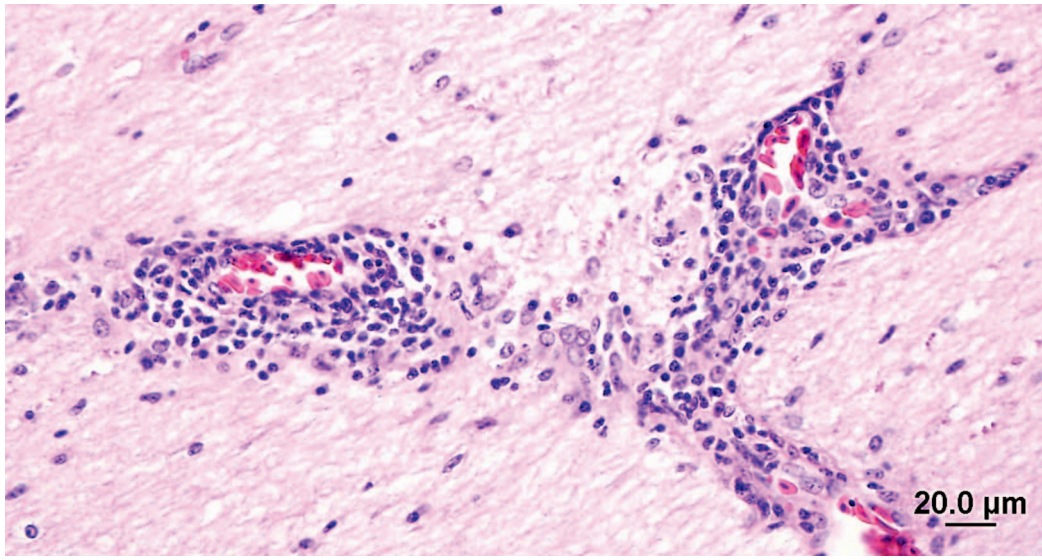

FIGURE 1. A brain section from a bornaviral infected Canada Goose (*Branta canadensis*) showing significant perivascular cuffing. This pathology is characteristic of many viral encephalitides including bornavirus infection. H&E stain.

signs of disease. Bird 4 had a dilated intrathoracic esophagus measuring up to 2.5 cm in diameter. No other bird showed significant gross lesions.

On histopathologic examination, six of the eight geese had varying degrees of lymphoplasmacytic meningoencephalitis characterized by mild to severe perivascular cuffing and gliosis randomly scattered throughout the brain (Fig. 1 and Table 1). The two birds without encephalitis (birds 6 and 7) were those that had not been observed antemortem. Four geese had mild lymphoplasmacytic myocarditis (birds 1, 2, 4, and 6). All birds had mild lymphoplasmacytic inflammation in the liver (typically portal infiltrates with mild extension into hepatic lobules) and the renal interstitium, except bird 4, which lacked renal inflammation. Four birds had mild to severe lymphocytic ganglioneuritis. The affected ganglia included ganglia near the heart in bird 1, periadrenal ganglia in birds 2 and 8, and ganglia within the esophageal wall in bird 4.

Immunohistochemistry was performed as described by Wünschmann et al. (2011) using a polyclonal cross-reacting antibody to nucleoprotein from parrot bornavirus genotype 4. Immunohistochemistry showed detectable avian bornaviral N antigen mostly in the nuclei and cytoplasm of glial cells and occasionally in the neurons of the brains in all eight geese. Neuronal processes and ependymal cells also stained for bornaviral antigen (Fig. 2). The detectable level of infection ranged from severe in birds 1, 4,

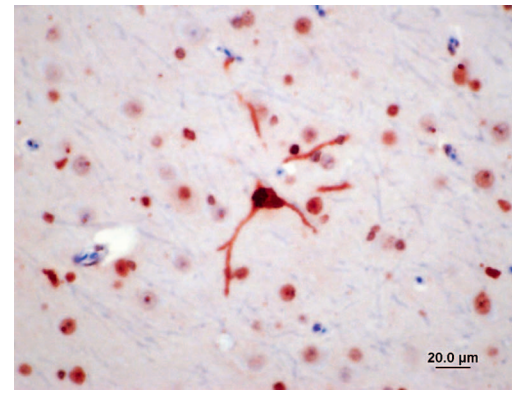

FIGURE 2. A Canada Goose (*Branta canadensis*) brain section stained using immunohistochemistry against bornaviral N antigen. The viral antigen is present not only in the nucleus and cytoplasm of a neuron and glial cells, but also within the neuronal processes. Nuclear staining is characteristic of this RNA virus. H&E stain.

TABLE 2. The sequence comparison of the aquatic bird bornavirus (ABBV-1) isolate from Canada Goose (*Branta canadensis*) bird 8 (isolate CG-N14-89) and the previously published genome of ABBV-1 (isolate CG-062; Payne et al. 2011). The viruses are almost identical.

Virus gene	Nucleotide sequence		Amino acid sequence	
	Identity (%)	Length	Identity (%)	Length
N	99	1116	99	371
X	100	264	100	87
P	99	609	100	202
M	99	429	100	142
G	99	1500	98	499
L	99	6430	99	1711

and 8, moderate in birds 2, 3, 6, and 7, to mild in bird 5 (Table 1).

Frozen brain and kidney tissue was available from five geese (birds 4–8). The brain and kidney tissues from birds 4, 7, and 8 were strongly-RT-PCR positive indicating the presence of avian bornavirus RNA in these tissues (Table 1). Bird 5 was positive in kidney only. Bird 6 was negative in both tissues. For birds 1–3, RT-PCR was performed on formalin-fixed paraffin-embedded brain tissue. Bird 1 was positive; birds 2 and 3 were negative (Table 1).

Virus isolation was performed as described by Guo et al. (2014). The RT-PCR products from cultured virus at passage 2 was cloned into pCRTM4-TOPO vector and its genome sequenced. The genome of this bornavirus was organized in a manner identical to that observed in other aquatic bird bornaviruses (ABBV; Table 2). Nucleotide and amino acid sequence identities ranged from 99% to 100% for all viral proteins when compared to previously published ABBV-1 sequences (Payne et al. 2011). Phylogenetic analysis based on the complete gene sequences indicated that this virus was essentially identical to ABBV-1.

Surveys of Canada geese for ABBV conducted in Canada and the northeastern US suggest that the prevalence of infection is between 7.4% and 52% (Payne et al. 2011; Delnatte et al. 2013). In all cases the virus has been characterized as ABBV-1 (Kuhn et al. 2015). Aquatic bird bornavirus-1 does not inevitably cause disease; the majority of ABBV infected birds are clinically normal. Aquatic bird bornavirus is not innocuous, however. The two birds described in the first report of signs resembling proventricular dilatation disease in Canada geese showed gross lesions of emaciation and dilatation of the proventriculus and nonsuppurative encephalitis on histopathology (Daoust et al. 1991). Tissues from both birds subsequently tested positive for ABBV-1 (Delnatte et al. 2013). While upper gastrointestinal tract impaction is characteristic of bornavirus-associated disease, neurologic signs in the absence of impaction were present in the majority of cases in Canada geese reported by Delnatte et al. (2013) and were the predominant signs observed in this disease cluster of cases of neurologic disease in northeastern US.

Although three birds with consistent clinical signs, histopathologic lesions, and positive immunohistochemistry finding were RT-PCR negative in brain tissue, one was RT-PCR positive in kidney, and two were birds in which RT-PCR was performed on formalin-fixed paraffin-embedded rather than frozen brain tissue. It was not unexpected that RT-PCR might fail to pick up the virus in formalin-fixed tissues, especially if the amount of virus was low. We suggest that six of the eight examined birds were affected by bornavirus-mediated disease and that the remaining two were likely unaffected carriers. The cause of death in the latter two birds, which did not have lymphoplasmacytic meningoencephalitis on histopathologic examination, was undetermined, although both showed degenerative tubular changes in the kidneys, suspicious for a toxic etiology.

One paradox associated with avian bornavirus infections is the unpredictability of disease. Virus infection is relatively common, but overt disease is much less so. The conditions contributing to the development of clinical disease are unclear. This outbreak occurred during an unusually severe winter. Extremely cold temperatures reduced the area of open water available for wintering

geese resulting in populations of higher density than normal and reduced food availability. It is possible that this combination of crowding and starvation might have contributed to the development of disease. However, the following winter, 2014–15, was also severe, with record-breaking amounts of snow in Massachusetts. Although a large number of emaciated Canada geese (40 birds from December 2014 through March 2015) were admitted to TWC as a result of deep snow cover preventing grazing, only one goose showed clinical signs consistent with neurologic disease and tested positive for ABBV-1 via RT-PCR on brain tissue.

We were unable to identify any unusual features of the viral genome that would account for its apparent pathogenicity given that subclinical infection with bornavirus in waterfowl is common in North America. Although the factors resulting in clinical disease associated with ABBV-1 in Canada geese are unclear, the cases presented in this report suggest that infection can result in morbidity and mortality in Canada geese.

We thank Ian Lipkin for providing the anti-PaBV used for immunohistochemistry.

LITERATURE CITED

Bhatnagar J, Guarner J, Paddock CD, Shieh WJ, Lanciotti RS, Marfin AA, Campbell GL, Zaki SR. 2007. Detection of West Nile virus in formalin-fixed, paraffin-embedded human tissues by RT-PCR: A useful adjunct to conventional tissue-based diagnostic methods. *J Clin Virol* 38:106–111.

Daoust PY, Julian RJ, Yason CV, Artsob H. 1991. Proventricular impaction associated with nonsuppurative encephalomyelitis and ganglioneuritis in two Canada geese. *J Wildl Dis* 27:513–517.

Delnatte P, Nagy E, Ojkic D, Leishman D, Crawshaw G, Elias K, Smith DA. 2014. Avian bornavirus in free-ranging waterfowl: Prevalence of antibodies and cloacal shedding of viral RNA. *J Wildl Dis* 50:512–523.

Delnatte P, Ojkic D, DeLay J, Campbell D, Crawshaw G, Smith DA. 2013. Pathology and diagnosis of avian bornavirus infection in wild Canada geese (*Branta canadensis*), trumpeter swans (*Cygnus buccinator*) and mute swans (*Cygnus olor*) in Canada: A retrospective study. *Avian Pathol* 42:114–128.

Guo J, Payne S, Zhang S, Turner D, Tizard I, Suchodolski P. 2014. Avian bornaviruses: Diagnosis, isolation, and genotyping. *Curr Protoc Microbiol* 34:15I.1.1–15I.1.33.

Kuhn JH, Dürrwald R, Bào Y, Briese T, Carbone K, Clawson AN, deRisi JL, Garten W, Jahrling PB, Kolodziejek J, et al. 2015. Taxonomic reorganization of the family *Bornaviridae*. *Arch Virol* 160:621–632.

Payne S, Covaleda L, Jianhua G, Swafford S, Baroch J, Ferro PJ, Lupiani B, Heatley J, Tizard I. 2011. Detection and characterization of a distinct bornavirus lineage from healthy Canada geese (*Branta canadensis*). *J Virol* 85:12053–12056.

Payne SL, Delnatte P, Guo J, Heatley JJ, Tizard I, Smith DA. 2012. Birds and bornaviruses. *Anim Health Res Rev* 13:145–156.

Spackman E, Suarez DL. 2008. Type A influenza virus detection and quantitation by real-time RT-PCR. *Methods Mol Biol* 436:19–26.

Wise MG, Suarez DL, Seal BS, Pedersen JC, Senne DA, King DJ, Kapczynski DR, Spackman E. 2004. Development of a real-time reverse-transcription PCR for detection of Newcastle disease virus RNA in clinical samples. *J Clin Microbiol* 42:329–338.

Wünschmann A, Honkavuori K, Briese T, Lipkin WI, Shivers J, Armein AG. 2011. Antigen tissue distribution of avian bornavirus (ABV) in psittacine birds with natural spontaneous proventricular dilatation disease and ABV genotype 1 infection. *J Vet Diagn Invest* 23:716–726.

Submitted for publication 8 July 2016.
Accepted 13 December 2016.