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Avian Bornaviruses in North American Gulls

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ABSTRACT: Avian bornaviruses, recently described members of the family *Bornaviridae*, have been isolated from captive parrots and passerines as well as wild waterfowl in which they may cause lethal neurologic disease. We report detection of avian bornavirus RNA in the brains of apparently healthy gulls. We tested 439 gull brain samples from 18 states, primarily in the northeastern US, using a reverse-transcriptase PCR assay with primers designed to detect a conserved region of the bornavirus M gene. Nine birds yielded a PCR product of appropriate size. Sequencing of PCR products indicated that the virus was closely related to aquatic bird bornavirus 1 (ABBV-1). Viral RNA was detected in Herring Gulls (*Larus argentatus*), Ring-billed Gulls (*Larus delawarensis*), and Laughing Gulls (*Leucophaeus atricilla*). Eight of the nine positive birds came from the New York/New Jersey area. One positive Herring Gull came from New Hampshire. Histopathologic examination of one well-preserved brain from a Herring Gull from Union County New Jersey, showed a lymphocytic encephalitis similar to that observed in bornavirus-infected parrots and geese. Bornavirus N protein was confirmed in two Herring Gull brains by immunohistochemistry. Thus ABBV-1 can infect gulls and cause encephalitic brain lesions similar to those observed in other birds.

Key words: Aquatic bird bornavirus, avian bornavirus, gulls, viral encephalitis.

Avian bornaviruses are enveloped, non-segmented negative-strand RNA viruses (order *Mononegavirales*, family *Bornaviridae*, genus *Bornavirus*) best known for causing proventricular dilatation disease (PDD) in captive psittacines (Gray et al. 2010; Hoppes et al. 2013). The most striking feature of PDD is the proventricular dilatation resulting from loss of gut motility and food accumulation; however, lesions can be found throughout the nervous system accounting for other signs such as ataxia and blindness (Hoppes et al. 2010). The etiologic agent of PDD was

identified in 2008 when Kistler and Honkavuori detected avian bornaviruses in tissues from diseased birds (Honkavuori et al. 2008; Kistler et al. 2008). Identification of bornaviral genomic sequences and antigen in the brains of birds with neurologic illness supported these initial findings. Experimental challenge of parrots with *Psittaciform 1 bornavirus* causes PDD-like lesions in parrots of several species (Gray et al. 2010), although not all infected birds develop gross lesions or disease (Mirhosseini et al. 2011).

Delnatte et al. (2011) examined fixed tissues from birds with neurologic disease and detected bornavirus sequences in brain tissue from wild Canada Geese (*Branta canadensis*) and Trumpeter Swans (*Cygnus buccinator*) in eastern Canada. This virus has been named aquatic bird bornavirus 1 (ABBV-1; Kuhn et al. 2014). ABBV-1 infection is common and widespread across the US in several species of wild geese including Canada Geese, Snow Geese (*Chen caerulensis*), and Ross's Geese (*Chen rossii*) as well as in Mute Swans (*Cygnus olor*). Approximately 14% of apparently healthy Canada Goose brains and 22% of Mute Swan brains tested contained ABBV-1 RNA (Payne et al. 2011, 2012; Guo et al. 2012). None of these birds showed obvious signs of disease when culled.

We report the results of a survey of >400 gull brains for bornaviruses and the detection of ABBV-1 in the brains of small numbers of Herring Gulls (*Larus argentatus*), Ring-billed Gulls (*Larus delawarensis*), and Laughing Gulls (*Leucophaeus atricilla*) from northeastern coastal states. Gulls were collected and euthanized by

the Wildlife Services division of the US Department of Agriculture–Animal and Plant Health Inspection Service. Gulls were removed under wildlife damage management (airplane collision avoidance) between April 2008 and August 2013. The heads were chilled or frozen until transported to the Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas. Brain tissue samples were processed as described previously (Guo et al. 2014). Applicable permits were Texas Parks and Wildlife permit, SPR-1111-381 (I.T.), Federal Fish and Wildlife Collecting Permit MB493004A-0 (I.T.), and IBC2014-070 (S.L.P.).

We tested brain tissues of 439 gulls from 18 states (Table 1). Represented species included Herring Gulls, Ring-billed Gulls, Laughing Gulls, Great Black-backed Gulls (*Larus marinus*), Mew Gulls (*Larus canus*), and Bonaparte's Gulls (*Chroicocephalus philadelphia*).

RNA was isolated from brain tissue samples using the Qiagen RNeasy Mini kit (Qiagen, Valencia, California, USA) based on the manufacturer's instructions and first strand cDNA was generated using the Applied Biosystems® High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Grand Island, New York, USA) as previously described (Guo et al. 2014). Primers targeting a 350-nucleotide region of the bornavirus M gene, PCR conditions, and sequencing of PCR products were also described by Guo et al. (2014). Tissue samples from two not-previously-frozen, PCR-positive brains were placed in 10% buffered formalin, embedded in paraffin, sectioned at 4 µm, stained with H&E and examined by bright-field microscopy. Immunohistochemistry (IHC) was performed to confirm the presence of viral protein according to the method of Wunschmann et al. (2011) using a polyclonal antibody raised against recombinant parrot bornavirus N protein. Antibody specificity was previously verified

using brain sections from a psittacine bird with confirmed parrot bornavirus infection (positive control) and brain sections of psittacines with diagnoses other than PDD (negative controls) (Wunschmann et al. 2011).

Of 439 gull brains sampled, nine (2.1%) were positive by reverse-transcriptase PCR. While overall prevalence of bornavirus infection in gulls was low, 8/9 positive samples were collected from two coastal counties in New Jersey and New York. The prevalences of bornavirus RNA in gull samples from sites in New Jersey and New York were 5.7% and 10.4%, respectively. All positive New York and New Jersey samples came from Newark, Liberty airport (EWR) in Union County or from John F. Kennedy airport (JFK) in Queens County; gulls collected at other locations in these states were negative. Thus the positive gulls came from locations <30 km apart. At JFK, 1/17 Herring Gull samples was positive and 3/14 Laughing Gulls were positive. Five Ring-billed Gulls taken from this site were negative. At EWR, 1/7 Ring-billed Gulls was positive, 3/3 Herring Gulls were positive, and neither of two laughing gulls was positive. These results suggest localized bornavirus infection in gulls associated with the major airports in the New York Harbor region. When results were analyzed by species we found no positive Great Black-backed, Mew, or Bonaparte's gulls. In the case of both Mew and Bonaparte's gulls, all samples came from Alaska, and only two Bonaparte's Gulls were tested. Only one of the Great Black-backed gulls came from northern New Jersey. One of 156 Ring-billed Gulls was positive but it was from EWR. Four of 116 Herring Gulls were positive (3.4%) but all positive birds were from JFK except for the single New Hampshire bird. Four of 95 laughing gulls (4%) were positive but all were from JFK. It appears therefore that any species differences reflect sampling location rather than obvious differences in susceptibility. There was no

TABLE 1. Brain samples from gulls collected in the USA for wildlife damage management were tested by PCR for avian bornavirus RNA. Numbers of tested birds are provided by state. Numbers of PCR-positive birds are shown in parentheses. Dashes indicate none collected.

State	Great Black-backed Gull (<i>Larus marinus</i>)	Herring Gull (<i>Larus argentatus</i>)	Ring-billed Gull (<i>Larus delawarensis</i>)	Laughing Gull (<i>Leucophaeus atricilla</i>)	Mew Gull (<i>Larus canus</i>)
Maine	—	5	5	—	—
Vermont	—	—	6	—	—
New Hampshire	8	24 (1)	—	—	—
Massachusetts	6	10	5	—	—
Rhode Island	—	14	6	—	—
Connecticut	—	—	7	—	—
New York	—	17 (1)	7	24 (3)	—
New Jersey	2	4 (3)	17 (1)	29	—
Pennsylvania	5	1	45	12	—
North Carolina	—	5	15	4	—
Louisiana	—	—	5	8	—
Florida	—	4	—	12	—
Texas	—	1	1	6	—
Ohio	—	9	17	—	—
Illinois	—	12	14	—	—
Wisconsin	—	5	—	—	—
Minnesota	—	—	6	—	—
Arkansas	—	5	—	—	51
Total	21	116 (4)	156 (1)	95 (4)	51

obvious seasonality in bornavirus prevalence. Differences that did occur reflect sampling times. Positive samples were taken in March (3), June (2), and July (4). Seven positive samples were taken in 2011 and two in 2013. No gulls were sampled in 2012.

Brain samples from two bornavirus-positive gulls from EWR had not been frozen and as a result, were in unusually good condition on arrival at the laboratory. They also yielded large amounts of PCR product and were therefore examined by histopathology and IHC. Lesions consistent with bornavirus-mediated encephalitis were apparent in one bird (Fig. 1). Using IHC and an antiserum directed against bornavirus N antigen, both birds showed mild positive staining in the brain and one also had faint staining in the optic nerve (Fig. 2). Sequence analysis of PCR products from eight of the positive gulls showed that all were infected with viruses closely related ABBV-1 based on partial M sequences.

We are not surprised to find bornavirus-positive gulls at airport sites close to New York City because a previous study of Canada Geese in this area had shown that 50% were positive for bornavirus (Payne et al. 2011). Both the major airports surveyed are located near extensive wetlands where waterfowl and gulls are abundant. Given that avian bornaviruses

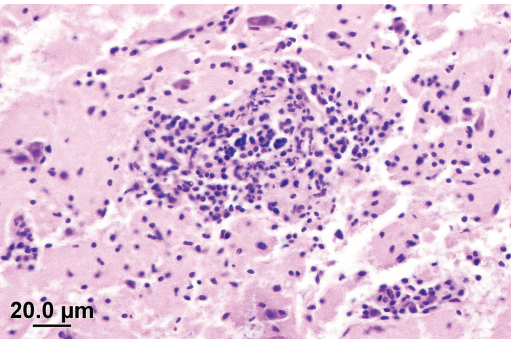


FIGURE 1. Histologic section of the cerebrum of a Herring Gull (*Larus argentatus*) showing a lymphoplasmacytic perivascular infiltration typical of bornavirus infection. H&E stain.

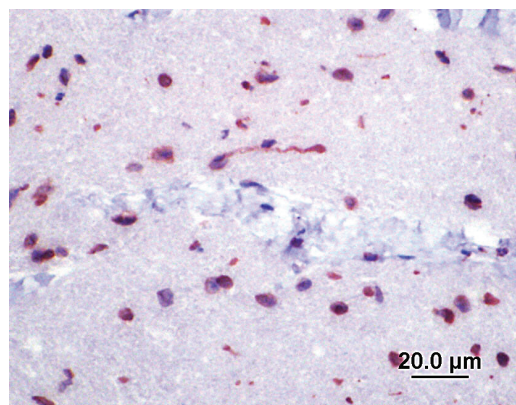


FIGURE 2. Section of optic nerve from a Herring Gull (*Larus argentatus*) showing positive immunohistochemical staining for bornavirus nucleoprotein.

are probably spread by the fecal-oral route (Hoppes et al. 2010), there is a high probability that gulls encounter goose droppings at resting/loafing sites. Both Canada Geese and gulls interact extensively with humans who feed them in urban parks and ponds and this may also increase the likelihood of gulls being exposed to goose and swan feces. Our findings suggest that gulls may, under certain circumstances, be potential bornavirus carriers, especially in an endemic area around New York harbor. We did not find additional positive birds in other sites, suggesting that bornavirus infection in gulls may be unique to the New York City area. The proximity of the two major airports to the wetlands at Jamaica Bay and Hackensack marshes may be of relevance to this finding and needs further examination.

Daoust et al. (1991) and Delnatte et al. (2013) described neurologic disease and PDD-like lesions in the brains of bornavirus-infected Canada Geese. None of the positive birds in our survey was obviously diseased but the severity of the histopathologic lesions and positive IHC in the best-preserved samples suggest that ABBV-1 may be pathogenic for gulls.

We suggest that gulls likely acquire bornavirus infection via fecal-oral spread

from infected Canada Goose or Mute Swan flocks. It is not known if gull-gull transmission can occur, or if this virus can cause significant morbidity or mortality in these species.

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