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Newcastle Disease Virus in Double-crested Cormorants in Alabama, Florida, and Mississippi

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ABSTRACT: In order to understand the epidemiology of Newcastle disease (ND) outbreaks in double-crested cormorants (*Phalacrocorax auritus*), a study was conducted on wintering migratory cormorants (*P. a. auritus*) in Alabama and Mississippi (USA) and non-migratory cormorants (*P. a. floridanus*) that breed in Florida (USA). Antibodies against ND virus were detected by the hemagglutination-inhibition method in sera from 86 of 183 (47%) migratory cormorants over-wintering in eight roosting sites in Alabama and Mississippi between November, 1997 and April, 1999. Titers ranged from 5 to 40. Antibody prevalences in sera collected from females in early winter (November and December) (26%) and late winter (February and March) (56%) were significantly different ($P = 0.0007$). None of 45 serum samples from 1- to 7-wk-old nestlings from 11 colonies in Florida during the 1997–98 and 1998–99 breeding seasons was positive. However, antibodies were detected in yolk samples from 98 of 126 (78%) eggs collected in these same colonies. Titers ranged from 4 to 256. The prevalence of antibodies in eggs collected from fresh-water colonies (63% prevalence, $n = 30$) and salt-water colonies (82% prevalence, $n = 96$) was significantly different ($P = 0.041$). ND virus was not isolated from tissues of 18 cormorants and cloacal and tracheal swabs from 202 cormorants collected in Alabama and Mississippi; virus was also not isolated from cloacal and tracheal swabs from 51 nestlings from Florida.

Key words: Double-crested cormorants, eggs, Newcastle disease virus, *Phalacrocorax auritus*, serology, serum.

Epizootics of velogenic Newcastle disease (ND) have occurred during the last decade with increasing frequency in breeding populations of the double-crested cormorant (*Phalacrocorax auritus auritus*) in Canada and north central United States. Some of these epizootics have been large; for example, at least 5,000 cormorants died during 1990 (Wobeser et al., 1993) and 20,000 in 1992 (Glaser et al., 1999). Almost all of the dead cormorants were juveniles. In an epizootic that occurred during the summer of 1995 in Saskatchewan (Canada), 64% of the juveniles at risk were estimated to have died (Kuiken et al., 1998c). Another large epizootic occurred in central Saskatchewan in August of 1997 (Kuiken et al., 1998a). The populations of this subspecies are migratory and over-winter in the Gulf Coast region between Texas (USA) and Florida (Dolbeer, 1991). The source of ND virus to which these populations have been exposed has not been determined. It has been suggested that these birds may have been exposed on their wintering grounds (Wobeser et al., 1993), although epizootics have not been documented for the non-migratory Florida subspecies (*P. auritus floridanus*). During the winter the potential exists for interaction between the two subspecies, allowing for possible transfer of the virus (Dolbeer, 1991). The objective of the present study was to examine populations of double-crested cormorants in Florida and other Gulf Coast states for ND virus and antibodies to ND virus to determine their epizootiological roles in this disease.

Serum samples were obtained from 183 cormorants at eight winter roosts in Alabama and Mississippi during the winter months between November 1997 and April 1999 (Fig. 1). The exact ages of these birds were not determined; they could have been hatched as early as the summer immediately prior to sample collections. Because these birds were collected considerably inland from the coastal range of *P. auritus floridanus*, they were presumed to

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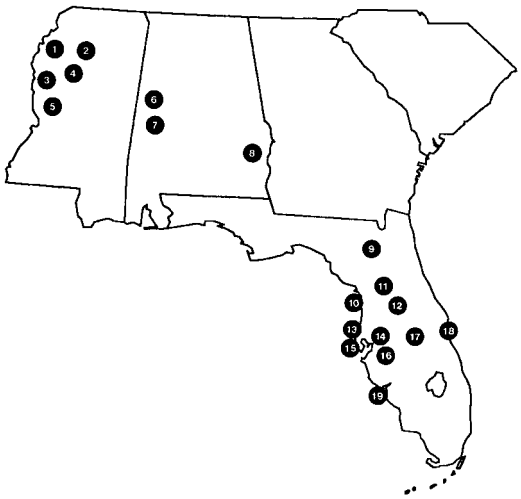


FIGURE 1. Collection sites for samples from double-crested cormorants in Mississippi, Alabama, and Florida (USA). (1) = Lake Whittington, Mississippi (33°26′N, 90°51′W), (2) = Malmaison Wildlife Management Area, Mississippi (33°40′N, 90°05′W), (3) = Lake Washington, Mississippi (33°05′N, 91°00′W), (4) = Little Mossy Lake, Mississippi (33°21′N, 90°26′W), (5) = Lake Chotard, Mississippi (32°34′N, 91°01′W), (6) = Limestone Creek, Alabama (32°52′N, 87°50′W), (7) = Lubbub Creek, Alabama (33°06′N, 88°10′W), (8) = Walter F. George Reservoir, Alabama (31°40′N, 84°59′W), (9) = Orange Lake, Florida (29°26′N, 82°12′W), (10) = Sandy Hook Key in Crystal River State Buffer Preserve, Florida (28°53′N, 82°41′W), (11) = Lake Griffin, Florida (28°50′N, 81°53′W), (12) = Gourd Neck Key in Lake Apopka, Florida (28°34′N, 81°41′W), (13) = Anclote Keys, Florida (28°10.5′N, 82°52′W), (14) = Medard Park Island, Florida (27°55′N, 82°09′W), (15) = I25 Marker Spoil Island, Florida (27°58′N, 82°49′W), (16) = Unnamed Phosphate Mine Pond, Florida (27°46′N, 82°11′W), (17) = Rabbit Island in Lake Kissimmee, Florida (27°56′N, 81°15′W), (18) = Banana River Aquatic Preserve, Florida (28°17′N, 80°39′W), and (19) = Tarpon Bay Keys, Florida (26°27′N, 82°04.5′W).

belong to the migratory subspecies, *P. auritus auritus,* many of which nest in Saskatchewan (Hatch and Weseloh, 1999). The birds were killed by shotgun and blood was collected from the heart or coelomic cavity using plastic disposable pipettes.

Additional serum samples were collected from 45 nestlings (1- to 7-wk-old) of the Florida subspecies. These birds were removed from tree-nests or branches near nests within seven different breeding colonies (Fig. 1) in Florida during the breeding seasons (March–July) of 1998 and 1999. The ages of the nestlings were estimated using culmen measurements (Dunn, 1975). Blood was collected from the jugular or brachial vein (using a 26 or 27 gauge needle) into sterile screw-cap tubes; sera were allowed to separate from clots at room temperature and then stored at −40 C until antibody analysis by the hemagglutination-inhibition method (Beard, 1989), using 0.5% chicken erythrocytes and 8 hemagglutinating units (HA) of the LaSota/B1 vaccine strain of ND virus. One hundred and twenty-six egg samples were collected during the same time period from 11 breeding colonies in Florida, including the seven breeding colonies from which the serum samples were collected (Fig. 1).

One egg per nest was collected and later opened in the laboratory to determine stage of development. Egg yolks were removed and the approximate age of the embryo, if present, was determined (Hanbidge and Fox, 1996). The yolks were stored at −40 C. Prior to analysis, the yolks were thawed and extracted with chloroform (Piela et al., 1984).

The detection limit for antibodies to ND virus was a titer of 5 for serum and 4 for yolk. Titer values were expressed as the reciprocal of the highest dilution of serum or yolk in which antibody activity was observed.

Sites from which serum samples were collected in Alabama were the Walter F. George Reservoir (n = 5), Limestone Creek (n = 5) and Lubbub Creek (n = 12). Sites in Mississippi were Lake Whittington (n = 5), Lake Chotard (n = 4), Lake Washington (n = 44), Little Mossy Lake (n = 47), and Malmaison Wildlife Management Area (n = 61).

Sites from which serum samples were collected in Florida were the freshwater colonies at Lake Griffin (n = 3), Rabbit Island (n = 1), and an unnamed reclaimed phosphate mine pond (n = 3). Serum sam-

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ples were also collected from saltwater colonies at Sandy Hook Key (n = 21), Tarpon Bay Keys (n = 5), Banana River Aquatic Preserve (n = 10), and I25 Marker Spoil Island (n = 2). Sites from which egg samples were collected in Florida included the freshwater colonies at Lake Griffin (n = 16), Rabbit Island, Lake Kissimmee (n = 6), unnamed reclaimed phosphate mine pond (n = 1), Orange Lake (n = 3), Gourd Neck area (n = 3), and Medard Park Island (n = 1). Egg samples were also collected from saltwater colonies at Sandy Hook Key (n = 28), Tarpon Bay Keys (n = 17), Banana River Aquatic Preserve (n = 24), I25 Marker Spoil Island (n = 3), and Anclote Keys (n = 24).

Samples of tissues (brain, kidney, intestine, lung, and liver) from 18 migratory cormorants from Alabama and Mississippi were removed with sterile scissors and placed into sterile plastic bags, sealed, and held at −80 C until analysis. Tracheal and cloacal swabs were collected from 202 migratory cormorants and 51 nestlings in Florida. Tracheal and cloacal samples were made using sterile dacron polyester-tipped swabs moistened with sterile phosphate-buffered saline (pH 7.4). After sampling, the swabs were stored in vials with Dulbecco's minimal essential medium supplemented with 4% fetal bovine serum, penicillin (100 ug/mL), streptomycin (100 ug/mL), amphotericin (10 ug/mL) and gentamycin (10 ug/mL) and held at −80 C until analysis.

Virus isolation attempts from swabs and tissues were carried out using 10-day-old embryonated specific pathogen-free chicken eggs (Charles River-SPAFAS, Inc., North Franklin, Connecticut, USA), as described in Hitchner (1989). Approximately half of the swab samples were submitted to the USDA National Veterinary Services Laboratories in Ames, Iowa, for analysis, utilizing similar techniques.

Fisher's exact test was performed using SAS (SAS Institute, 1988) to determine differences in antibody prevalence of serum samples from wintering cormorants by gender and season and differences in antibody prevalence in eggs from resident Florida cormorants by colony type and stage of incubation of the egg. Significant differences were declared at $P \leq 0.05$.

Eighty-six of the 183 sera (47%) collected from migratory cormorants in Alabama and Mississippi were positive for antibodies to ND virus. The range of titers was from 5 to 40. The difference in observed antibody prevalence in sera collected from females (n = 84) in early winter (November and December, n = 50, 26% prevalence) and late winter (February and March, n = 34, 56% prevalence) was significant (P = 0.0007).

Ninety-eight of 126 (78%) egg yolks collected from breeding colonies were positive with titers ranging between 4 and 256. The difference in antibody prevalence between eggs collected from fresh-water colonies (63%, n = 30) and salt-water colonies (82%, n = 96) was significant (P = 0.041). This difference was not attributed to variations in the developmental stages of the eggs sampled in the two types of colonies. The percentages of eggs in each developmental stage from fresh-water and salt-water colonies were comparable even though total sample sizes were different (Johnson, 1999). The prevalence of positive eggs in the early developmental stages (1 to 7 days) was high (100% for eggs from fresh-water colonies and 99% for salt-water colonies). The higher prevalence of positive eggs in salt-water colonies may have been a result of differences in densities of birds in the two colony types. Salt-water colonies in Florida, for example, have greater numbers of cormorants than do fresh-water colonies (Runde et al., 1991), which may enhance the rate of transmission of the ND virus from bird to bird. Differences in species composition of other colonial nesting birds (wading birds, pelicans, anhingas, etc.) in the two colony types may play a role; this needs to be investigated.

None of the 45 sera collected from cormorant nestlings in Florida breeding col-

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onies was positive. This was possibly because most of the nestlings sampled were older than 15 days; only five nestlings were 14-days-old or younger. Meteyer et al. (1997) found that passive antibodies to ND virus were not detectable in cormorants over 14 days of age. Conversely this may be an indication that the nestlings in these colonies had not been exposed to ND virus.

There were no clinical signs of ND in any of the cormorants from which samples were collected. No virus was isolated from tissues or from cloacal and tracheal swab samples from migratory cormorants in the winter roosts in Alabama and Mississippi or from cloacal and tracheal swabs collected from nestlings in the Florida breeding colonies. This was not surprising since ND virus has been isolated infrequently utilizing swab samples from wild birds in the absence of an epizootic (Stallknecht et al., 1991). The isolation of ND virus from apparently healthy double-crested cormorants has been documented only in experimentally infected birds (Kuiken et al., 1998b).

Detection of maternally derived antibodies in 78% of the eggs of resident Florida double-crested cormorants is evidence that these birds had been exposed to ND virus and may be involved in the epizootiology of ND in North America. As no virus was isolated from any of the Florida subspecies of cormorants in this study, the question remains as to the identity of the strain or pathotype of the virus or viruses to which the birds had been exposed. In addition, other species of birds may be involved in the transmission of the virus. It is possible that members of these species transmit the virus to double-crested cormorants and other species. Double-crested cormorants in Florida breed in mixed-species colonies that include brown pelicans (*Pelecanus occidentalis*), anhingas (*Anhinga anhinga*), cattle egrets (*Bubulcus ibis*), and numerous wading birds, including such threatened species as roseate spoonbills (*Ajaia ajaja*) and wood storks (*Mycteria americana*) (Runde et al., 1991). The involvement of other species in the transmission of ND and attempts to isolate and characterize the ND virus in cormorants in Florida and other Gulf states should be the focus of further study.

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