

AN EPIDEMIC OF NEWCASTLE DISEASE IN DOUBLE-CRESTED CORMORANTS FROM SASKATCHEWAN

Authors: Kuiken, Thijs, Leighton, Frederick A., Wobeser, Gary, Danesik, Karen L., Riva, José, et al.

Source: Journal of Wildlife Diseases, 34(3) : 457-471

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-34.3.457>

The BioOne Digital Library (<https://bioone.org/>) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (<https://bioone.org/subscribe>), the BioOne Complete Archive (<https://bioone.org/archive>), and the BioOne eBooks program offerings ESA eBook Collection (<https://bioone.org/esa-ebooks>) and CSIRO Publishing BioSelect Collection (<https://bioone.org/csiro-ebooks>).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Digital Library 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 is an innovative nonprofit that 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.

AN EPIDEMIC OF NEWCASTLE DISEASE IN DOUBLE-CRESTED CORMORANTS FROM SASKATCHEWAN

Thijs Kuiken,^{1,3} Frederick A. Leighton,¹ Gary Wobeser,¹ Karen L. Danesik,¹ José Riva,² and Robert A. Heckert²

¹ Canadian Cooperative Wildlife Health Centre, Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon SK S7N 5B4, Canada

² Canadian Food Inspection Agency, Animal Diseases Research Institute, Virology Section, 3851 Fallowfield Road, P.O. Box 11300, Station H, Nepean, Ontario K2H 8P9, Canada

³ Corresponding author (e-mail: kuiken@admin3.usask.ca)

ABSTRACT: A Newcastle disease epidemic in double-crested cormorants (*Phalacrocorax auritus*) occurred in July and August 1995, during a 1994–96 study of a breeding colony of this species on Doré Lake (Saskatchewan, Canada). Clinical signs and mortality were observed from a tunnel-and-blind system, and moribund and freshly dead birds were examined virologically. Yolks from cormorant eggs and sera from cormorants and other birds were tested for hemagglutination inhibiting antibodies to Newcastle disease virus (NDV). Evidence of Newcastle disease was limited to juvenile double-crested cormorants, despite close contact with other birds, including American white pelicans (*Pelecanus erythrorhynchos*) and gulls (*Larus* spp.). Clinical signs included limb, head or neck paralysis, head or body tremors, ataxia, and blindness; pathogenic NDV was isolated from affected birds. The mortality rate of juvenile cormorants was 32 to 64%, which was high relative to overall first-year mortality in years without epidemics. Thirty-seven of 63 (59%) cormorant sera collected during the epidemic tested positive for antibodies to NDV. Antibody status of cormorant egg yolks depended on stage of incubation, likely due to changes in the amount of water in the yolks. The departure of juvenile cormorants from their nests at 4 wk of age, resulting in an increased contact rate among individuals, may have been important in triggering the epidemic.

Key words: Newcastle disease, avian paramyxovirus type 1, double-crested cormorant, *Phalacrocorax auritus*, epidemiology.

INTRODUCTION

Pathogenic Newcastle disease virus (NDV) caused epidemics in double-crested cormorants (*Phalacrocorax auritus*)—hereafter referred to as cormorants—in Quebec in 1975 (Cleary, 1977), in western Canada in 1990 (Wobeser et al., 1993), and in western Canada, the Great Lakes area, and north-central USA in 1992 (Roffe, 1992; Heckert, 1993). The main clinical signs were neurologic, and only juveniles were affected. The virus spread to other wild bird species (Wobeser et al., 1993) and to domestic poultry (Mixson and Pearson, 1992). The presence of pathogenic NDV in wild birds in North America is of concern because of (1) mortality in cormorants, (2) potential mortality in other wild bird species in contact with cormorants, and (3) potential economic impact on the poultry industry.

As part of a disease study of a cormorant breeding colony in Saskatchewan from

1994 to 1996, we had the rare opportunity to observe the start of a Newcastle disease (ND) epidemic in 1995 and to follow closely its course for 1 mo. Herein, we (1) describe the epidemic, including clinical signs, contact with other bird species, mortality rate, virus isolation and serology, and (2) compare results among the three consecutive breeding seasons.

MATERIALS AND METHODS

Study area

The study area was Doré Lake (Saskatchewan, Canada; 54°46'N, 107°17'W); the lake has three islands, designated as Island A, Island B, and Rock Island, each with ground-nesting colonies of cormorants (Fig. 1). Besides cormorants, American white pelicans (*Pelecanus erythrorhynchos*)—hereafter referred to as pelicans—breed on Island A, herring gulls (*Larus argentatus*) and California gulls (*L. californicus*) breed on Island B, and Caspian terns (*Sterna caspia*), ring-billed gulls (*L. delawarensis*), herring gulls, and California gulls breed on Rock Island. The main study site was Island

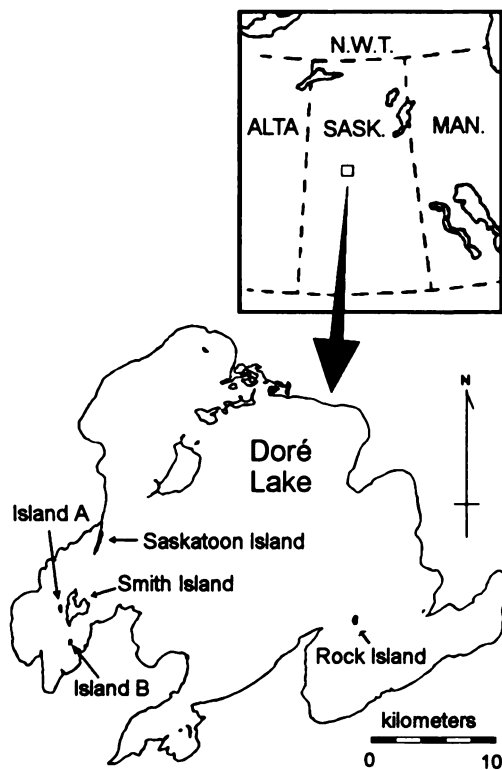


FIGURE 1. Map of Doré Lake (Saskatchewan, Canada). Island A, Island B, and Rock Island have breeding colonies of double-crested cormorants.

A, a 300 × 100 m island in the south-west part of Doré Lake (Fig. 2).

Monitoring of reproduction, morbidity, and mortality

Observations were made on Island A from within an 88-m-long tunnel-and-blind (TAB) system (Fig. 2; Kuiken et al., 1997) every third day from 1 June to 1 September 1994 to 1996. About 10% of the total nesting area of cormorants was visible from within this system. All cormorant and pelican nests within about 6 m of the TAB system were marked with numbered stakes at the beginning of each breeding season. At each visit, we recorded the number of eggs and chicks in each marked nest, pres-

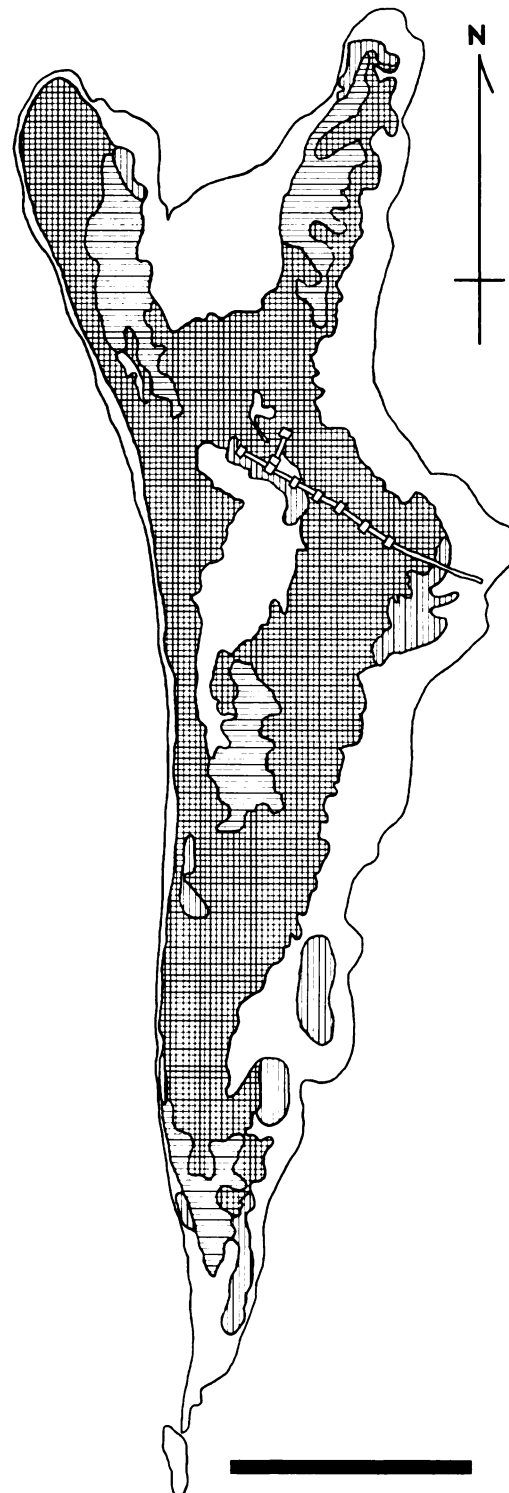


FIGURE 2. Location of the tunnel-and-blind system and nesting areas of double-crested cormorants (horizontal lines), American white pelicans (vertical lines), and both species (cross-hatching) on Island A at Doré Lake. This map was modified from an aerial photograph taken in June 1996. Bar = 50 m.

ence of sick or dead birds, and presence of birds other than cormorants or pelicans. The location of carcasses was recorded in relation to the numbered stakes or TAB system to prevent counting them more than once per visit. Whenever possible, carcasses were classified as fresh or old. Criteria for designating carcasses as fresh were convex shiny eyes, clean plumage, red musculature, and glistening viscera. Criteria for designating carcasses as old were desiccation, flattening, and soiled plumage. In some cases, only skin, bones, esophagus and stomach were present when carcasses were first observed. We assumed that fresh carcasses were of birds that had died <3 days previously, and old carcasses were of birds dead for ≥ 3 days. In some cases it was not possible to determine the state of decomposition of carcasses because they were either too far away from the TAB system or partly hidden from view; these carcasses were classified as unknown. At the end of each breeding season, we counted carcasses and nests on Island A. We corrected for the disappearance of nests after fledging by determining the proportion of marked nests that were still visible, and dividing the total number of nests counted on Island A by this proportion.

During a ND epidemic in 1995 (23 July to 24 August), we also visited the cormorant colonies on Island B (28 July and 18 August) and on Rock Island (14 August), and walked along the shores of the adjacent Smith Island and Saskatoon Island (20 to 24 August) to look for sick and dead birds (Fig. 1).

We estimated the mortality rate of juvenile cormorants from Island A in the period of the ND epidemic as the number of deaths during the period divided by the number alive at the beginning of the period. The number of deaths during a period, or cumulative mortality, is not the same as the number of carcasses observed at the end of that period, because of carcass disappearance due to scavenging and decomposition (Wobeser, 1994). Because we only counted the total number of cormorant carcasses on Island A at the end of the epidemic, we used a correction factor α for carcass disappearance. To determine α , we divided the number of juvenile cormorant carcasses found in the area visible from the TAB system at the end of the epidemic by the cumulative mortality in that area during the epidemic. The sum of the number of fresh carcasses seen at each visit during the epidemic provided a minimum estimate of the cumulative mortality; the sum of the number of fresh carcasses and carcasses of unknown stage of decomposition provided a maximum estimate. We used the average of these two values. Including this correction factor, the formula was: mortality rate = number

of carcasses observed at end of period / ($\alpha \times$ number alive at beginning of period).

Virological examination

In 1994, samples for virus isolation were collected from 13 juvenile and one adult cormorant found moribund or dead on Island A (7 July to 1 September). In 1995, samples were collected from five cormorants with clinical signs of ND collected on Island A (23 and 24 July), and from the following individuals of other species found dead or moribund on Island A, Island B, Rock Island, or Smith Island (16 July to 24 August): 10 juvenile pelicans, two adult ring-billed gulls, two juvenile herring gulls, one juvenile dabbling duck, probably a mallard (*Anas platyrhynchos*), one juvenile Caspian tern, one red-necked grebe (*Podiceps grisegena*) of unknown age, one adult common merganser (*Mergus merganser*), and one juvenile great blue heron (*Ardea herodias*). In 1996, samples were collected from one adult pelican found moribund on Island A on 16 June, two juvenile cormorants found moribund on Island A (29 July and 19 August), and one juvenile ring-billed gull found dead on Rock Island (23 August).

Samples for virus isolation were stored in liquid nitrogen until processing. Virus isolation was attempted on pooled samples of brain, trachea, lung, liver, kidney, spleen, jejunum, and femoral bone marrow from each bird according to the method described by Wobeser et al. (1993) for samples from Saskatchewan. Confirmation of virus identity and assessment of pathogenicity (Alexander, 1988) was done on a pool of NDV-positive first-passage allantoic fluid samples from embryonating chicken eggs inoculated with tissue suspensions from the five cormorants from 1995.

Examination for antibodies to NDV

Examination for hemagglutination inhibiting (HI) antibodies to NDV was carried out on yolk and serum samples. We arbitrarily collected 28 to 147 cormorant eggs per year from marked nests around the TAB system between 25 and 29 May from 1994 to 1996. Only one egg was collected per nest. Eggs were opened at the blunt end by use of scissors and length of incubation was estimated as: (1) marginal vein not yet visible grossly at 0–1 day (Romanoff, 1960), (2) marginal vein clearly visible as a red ring in the yolk sac and crown-rump length of embryo ≤ 10 mm at 2–8 days (Van Scheik, 1985), (3) crown-rump length of embryo > 10 mm and ≤ 20 mm at 9–13 days (Van Scheik, 1985), and (4) crown-rump length of embryo > 20 mm and ≤ 50 mm at 14–25 days (Van

Scheik, 1985). The yolk was separated from the white by use of a kitchen egg separator. One ml of yolk was collected by use of a 1-ml insulin syringe without a needle and was diluted 1:10 in 0.01 M phosphate-buffered saline (pH 7.4).

We bled and banded 25 to 81 cormorants from Island A per year between 1994 and 1996. Juvenile cormorants that had not yet left their nests were caught by hand. Juvenile cormorants that had left their nests and adult cormorants were trapped from within the TAB system by hand or by use of a telescopic rod with a blunt hook at the end (Kuiken et al., 1997). Birds were bled from the brachial vein, banded with a size 8 U.S. Fish and Wildlife Service aluminum band (Laurel, Maryland, USA), and released. In case of moribund birds, free-flowing blood was collected from the jugular vein after cervical dislocation. Serous fluid from the heart lumen or celomic cavity was collected from 10 cormorant carcasses from Island A. We also bled four cormorants from Rock Island (14 August 1995) and one cormorant from Island B (18 August 1995); all were juveniles with clinical signs of ND.

Blood samples were collected from other bird species as well. In 1994, we bled nine pelicans and one ring-billed gull, all juveniles. Before the ND epidemic in 1995, we bled one duckling, probably a mallard, and two juvenile pelicans. During the ND epidemic in 1995, we bled one great blue heron, two herring gulls, one ring-billed gull, five pelicans, and one Caspian tern, all juveniles. In 1996, we bled three juvenile and one adult pelican. All birds were from Island A, except for one herring gull from Island B, the great blue heron, duckling, and one ring-billed gull from Smith Island, and the Caspian tern from Rock Island.

Yolk and serum samples were frozen in liquid nitrogen until further processing. They were held at 56 C for 30 min, adsorbed once (serum) or twice (yolk) with guinea pig erythrocytes to remove nonspecific hemagglutinins, and tested for HI antibodies to NDV (Beard, 1989) using guinea pig erythrocytes. Eight hemagglutination units of NDV B1 strain were used as antigen. An HI titer of $\geq 1:20$ was considered positive (Brown et al., 1990).

We captured 28 newly-hatched cormorants from Island A (19 and 27 June 1995) and raised them in captivity so that we could measure the HI antibody titer to NDV in their serum over time without repeated disturbance of the breeding colony. These cormorants were taken from nests from which eggs had been collected previously that season for measurement of HI antibody titer to NDV in yolk, and choice of nests was biased towards those containing eggs with high antibody titers. Twenty-two of 28

eggs (79%) in nests from which newly-hatched cormorants were taken were seropositive for NDV, with titers ranging from $<1:20$ to 1:160 (geometric mean titer: 51). Birds were bled when captured and weekly thereafter for at least 5 wk. Newly-hatched birds were bled from the jugular vein; older birds were bled from the medial metatarsal vein or brachial vein.

Statistical analysis

Statistical analysis was done with Statistix software (Analytical Software, 1996). We tested the hypothesis that presence of antibody in egg yolk was independent of length of incubation and collection year by use of a log-linear model for multidimensional contingency tables (Zar, 1996). Following this, we tested the conditional independence of each of the three variables from the other two. We also tested the hypothesis that presence of antibody in yolk from eggs incubated <2 days was independent of collection year by use of chi-square analysis (Zar, 1996).

RESULTS

Clinical signs in cormorants

Juvenile cormorants with clinical signs of ND were first seen on Island A on 23 July 1995 and were seen at each visit thereafter until the end of the observation period on 24 August 1995. Clinical signs of ND were not seen in adult cormorants nor in any other bird species in 1995, nor in any birds in 1994 or 1996.

The most obvious clinical signs of ND were partial paralysis (paresis) or complete paralysis of one or more limbs. Signs varied with severity of paralysis and number and combination of limbs affected. All possible variations were observed.

Cormorants with mild unilateral leg paralysis limped, occasionally stumbled, and occasionally stood on the affected leg with toes curled (knuckling). Cormorants with severe unilateral leg paralysis held the affected leg tucked up against the body or partly or completely stretched out forwards, backwards or sideways, with toes held curled or straight (spastic paralysis). They hopped on the unaffected leg and maintained balance by flapping their wings in the air or leaning on the ground with

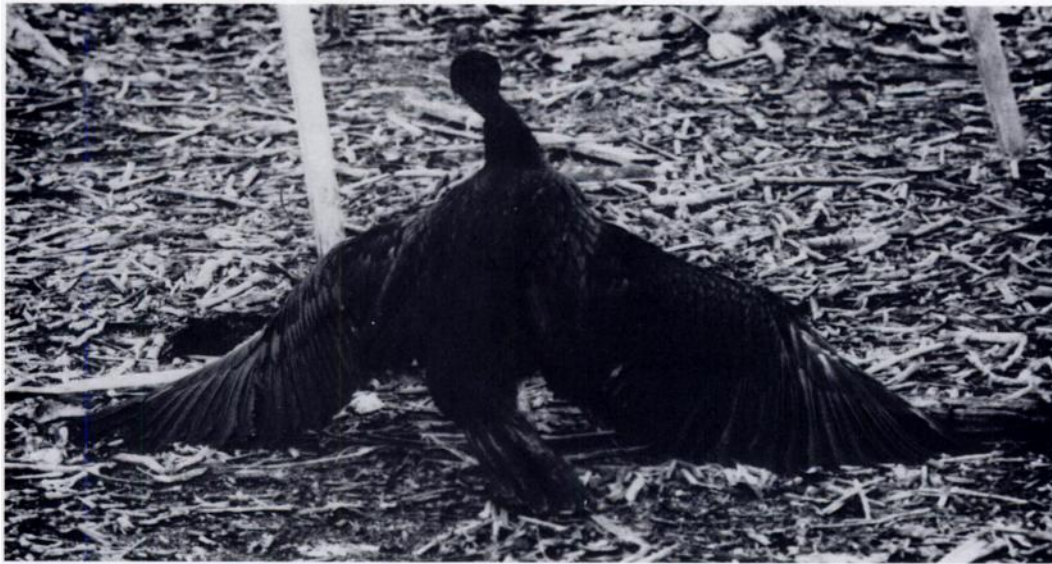


FIGURE 3. Double-crested cormorant with bilateral leg paralysis due to Newcastle disease. It is trying to move forward by use of its wings pivoted against the ground, and is leaning on the ground with its tail.

wings, tail, or both. They were capable of flying and swimming, but were unable to dive. They often rested in sternal recumbency, whereas normal cormorants usually rest standing. Cormorants with bilateral leg paralysis sometimes showed knuckling

or sat on their intertarsal joints, but were usually seen in sternal recumbency. They tried to move forwards by use of beak and wings pivoted against the ground (Fig. 3), but made very slow progress.

Birds with unilateral wing paralysis let the affected wing hang loose by the side of the body or held it tucked against the body in the same way as an unaffected wing in resting position. In such cases, the abnormality only became visible when the birds carried out behavior in which they normally would use both wings, such as drying their wings, flying, or hopping onto a log or other object. Then, they spread the normal wing fully and the other partially or not at all (Fig. 4). They could not fly and, in cases in which the lame wing hung loose, they had difficulty swimming and diving. A less apparent consequence of unilateral wing paralysis was that birds falling on their back had difficulty in righting themselves (Fig. 5). Birds lying on their back paddled vigorously with their legs, swung their tail and head back and forth, pushed off the ground with the functioning wing, and in this way were eventually able to right themselves. Cormorants with bilateral wing paralysis were



FIGURE 4. Double-crested cormorant with unilateral wing paralysis due to Newcastle disease. The normal wing is spread and the affected wing is held close to the body.



FIGURE 5. Double-crested cormorant with unilateral wing paralysis due to Newcastle disease. It is lying on its back and has difficulty in righting itself. Note the scuff marks on the ground caused by the tail movements.

unable to right themselves if they fell on their back.

Cormorants with unilateral leg paralysis and unilateral (either ipsi- or contralateral) wing paralysis were able to hop on one leg and maintain balance by flapping the functioning wing in the air or leaning on the ground with it, and by leaning on the ground with beak and tail. They were able to swim slowly but were unable to dive, and, if they fell on their back, could right themselves only with great difficulty. Cormorants with more than two paralyzed limbs were only able to move short distances and were usually found lying in sternal recumbency.

Paralysis of the head and neck was rarely seen. One bird walked with its head and anterior half of the neck hanging vertically, as if the anterior neck muscles were paralyzed. When it stood still, it rested the tip of the beak on the ground beside its right

foot. Another bird was apparently unable to tuck its head between its shoulders, as cormorants normally do when resting, and instead held its head to one side of its breast.

The head of many affected birds trembled constantly. Such birds were often unable to peck accurately, for example at an approaching hand. One bird with head tremors repeatedly bobbed its head up and down. Two birds with unilateral leg paralysis had trembling of the whole body: one constantly, the other for about 30 sec after it had been fed.

Other signs of neurologic disease included circling, excessive elevation of the legs during walking (goose stepping), and an unsteady gait. Some affected birds apparently lost their fear of humans and avian predators such as ravens (*Corvus corax*). Others were found in unusual places, such as around human dwellings. Several

birds walked from our camp site on Smith Island along a path through the forest towards the middle of the island.

Ocular involvement was common in affected birds. Clinical signs included closure of the eyelids, watery exudate, a plaque of yellow-white friable material in the conjunctival sac, and reddened and opaque cornea, sclera, and third eyelid. Two birds had bilateral pupillary dilatation and were apparently blind: they walked without coordination, constantly bumping into objects, and did not react to an approaching hand.

Other common, non-specific, clinical signs included depression, soiled and damaged plumage, cloacal area matted with grey-green droppings, outside of beak caked with dirt, laborious breathing—in one case with beak opened at each inspiration, emaciation—more apparent in the last 2 wk of observations, many lice on the plumage and in the oral cavity, and leeches in the oral cavity, nasal cavity, or conjunctival sac.

There was abundant contact between affected juvenile cormorants and other cormorants and pelicans on Island A. Normal juvenile cormorants pecked at and clutched various body parts of affected cormorants, which often lacked defensive behavior. Adult cormorants continued to feed affected juveniles as long as they were able to beg. Immediately before and after being fed, juvenile pelicans pecked at any birds in their proximity, including cormorants with signs of ND. Juvenile pelicans also tried to steal food from juvenile cormorants being fed by adult cormorants. Both juvenile cormorants and juvenile pelicans regularly pecked at and extensively mouthed carcasses lying on the ground, and in one case a juvenile pelican ate the intestine of a juvenile cormorant carcass. Large shallow pools of water in the center of Island A formed as a result of heavy rains between 29 July and 1 August. These pools and the shallow water along the shore of Island A were used extensively by both juvenile cormorants and juvenile pel-

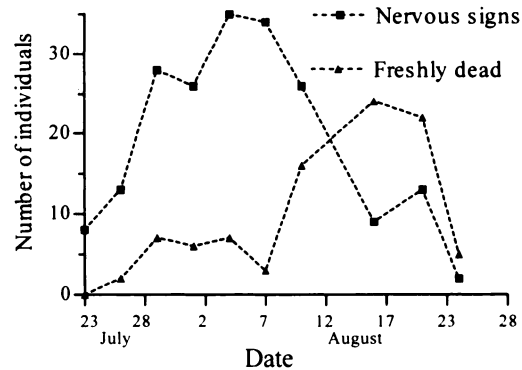


FIGURE 6. Morbidity and mortality of juvenile double-crested cormorants during the Newcastle disease epidemic in 1995 in the area visible from the tunnel-and-blind system on Island A at Doré Lake.

icans. Many partly paralyzed cormorants remained on the shoreline and died there because they were unable to get out of the water after swimming.

Course of morbidity and mortality in cormorants

The number of juvenile cormorants with clinical signs of ND observed from the TAB system reached a peak about 2 wk after the start of the epidemic, and the number of freshly dead carcasses reached a peak about 10 days later (Fig. 6). Between 20 and 24 August 1995, we found 1,005 juvenile cormorant carcasses: 972 on Island A, 32 on the shore of Smith Island, and one on the shore of Saskatoon Island (Fig. 1). We assumed that they were from the Island A colony, and had died of ND. The number of cormorant nests counted on Island A on 24 August 1995 was 3,219, and the proportion of nests marked in May that were visible on that date was 0.72 ($n = 232$). The estimated number of cormorant nests on Island A in 1995 was $3,219 \div 0.72 = 4,499$. The mean number of juveniles surviving to 3 wk of age per nest, excluding those nests from which eggs and juveniles were collected, was 1.38 ($n = 101$, $SD = 1.00$, range 0 to 3.5), so that the estimated number of juveniles surviving to 3 wk on Island A was $4,499 \times 1.38 = 6,208$. The median hatching date in 1995 on Island A was 16 June ($n = 191$,

TABLE 1. Mortality of juvenile double-crested cormorants during the Newcastle disease epidemic of 1995 in the area visible from the tunnel-and-blind system on Island A at Doré Lake.

Date		Number of carcasses observed				Cumulative mortality	Total number of carcasses observed/mean cumulative mortality
		State of decomposition			Total		
		Fresh	Old	Unknown			
July	23	0	0	0	0	0 (0-0) ^a	—
	26	2	0	0	2	2 (2-2)	1
	29	7	0	5	12	12 (9-14)	1
August	1	6	1	12	19	24 (15-32)	0.8
	4	7	13	3	23	32 (22-42)	0.7
	7	3	10	18	31	44 (25-63)	0.7
	10	16	7	4	27	62 (41-83)	0.4
	16	24	9	9	42	91 (65-116)	0.5

^a Mean (minimum-maximum) where the minimum was calculated as the sum of fresh carcasses seen at each visit and the maximum was calculated as the sum of fresh carcasses and carcasses of unknown state of decomposition seen at each visit.

first quartile 13 June, third quartile 23 June, range: 10 June to 8 July), so that the median date on which the surviving juveniles were 3-wk-old was 7 July. No mortality was observed from the TAB system between this date and 23 July, when the ND epidemic was first noted, so that the number of juveniles surviving to 3 wk of age is a reasonable estimate of the number of juveniles alive at the beginning of the epidemic. During the course of the epidemic, the total number of juvenile cormorant carcasses observed from the TAB system at each visit progressively underestimated the calculated cumulative mortality (Table 1). At the end of the epidemic, the number of cormorant carcasses present around the TAB system was 50% of the cumulative mortality, so $\alpha = 0.5$ (Table 1). Therefore, the mortality rate of juvenile cormorants of Island A from ND from 23 July to 24 August 1995 was approximately: number of deaths observed at end of period/ $(\alpha \times \text{number alive at beginning of period}) = 1,005/(0.5 \times 6,208) = 0.32$.

The behavior and development of unaffected juvenile cormorants on Island A is summarized here to put the epidemic in the context of the cormorant's reproductive cycle. Juvenile cormorants started wandering among the nests and mingling with birds from other nests on 14 July

1995 (median age = 4 wk), and by 17 July, most of them were no longer on their nests. They were very curious, pecking at sticks, feathers, and other objects on the ground. On 20 July, they were first seen at the water's edge, and on 23 July, some were swimming and diving in the shallows next to Island A. On 26 July, juvenile cormorants were first seen flying a few meters in the air, and by 3 August, many were flying around Island A and swimming and diving in the water around the island. They also started catching fish from about this date onwards, although adult cormorants were still seen feeding juveniles until the end of the observation period. On 16 August there appeared to be fewer juvenile cormorants on Island A than on the previous visit, and their number decreased progressively until 24 August, when <100 juveniles were seen on Island A out of several thousand juveniles fledged.

Morbidity and mortality also were noted on the other two cormorant colony sites on Doré Lake. On Island B, which had 49 cormorant nests, we found one juvenile cormorant carcass on 28 July 1995, and two juvenile cormorants with unilateral wing paralysis and 17 juvenile cormorant carcasses on 18 August 1995. On Rock Island, which had 1,120 cormorant nests, we found 27 juvenile cormorants with signs of

lameness and 169 juvenile cormorant carcasses on 14 August 1995.

Observations of other bird species

Several bird species, besides cormorants and pelicans, were present on or around Island A during the epidemic, and had contact with affected cormorants. Up to 200 juvenile and adult herring, California, and ring-billed gulls were present on Island A at each visit. Most roosted at the water's edge or in the open area in the center of Island A (Fig. 2). They often swam in the shallows and in the stagnant pools in the central open area, walked in the nesting areas, and fed on cormorant and pelican carcasses. One to three ravens were present at each visit, usually feeding on a cormorant or pelican carcass in the central open area, or sitting in a tree. Once, several American crows (*Corvus brachyrhynchos*) were seen feeding on a cormorant carcass. An immature bald eagle (*Haliaeetus leucocephalus*) was seen at Island A twice during the epidemic, once soaring over the island, the other time sitting in a tree. Occasionally, but not during the epidemic, an immature bald eagle was seen scavenging on cormorant and pelican carcasses on Island A and drinking in the shallows. A pair of American coots (*Fulica americana*) with their chicks, four pairs of red-necked grebes with their chicks, and up to 20 yellow-headed blackbirds (*Xanthocephalus xanthocephalus*) were seen regularly among the bulrushes (*Scirpus lacustris*) around Island A, and had presumably nested there. Up to 22 mallards were regularly seen swimming in the shallows and feeding in the mud at the water's edge. Occasionally, a common goldeneye (*Bucephala clangula*), a green-winged teal (*Anas crecca*), and several American wigeon (*Anas americana*) were seen swimming in the shallows. Three semi-palmated plovers (*Charadrius semipalmatus*) and five sandpipers (*Calidris* sp.) were seen once at the water's edge.

Morbidity and mortality were noted in

pelicans, gulls, and terns during the epidemic. On Island A, we found one adult and 638 juvenile pelican carcasses, 18 moribund juvenile pelicans, one adult herring gull carcass, two adult California gull carcasses, three juvenile herring or California gull carcasses, and one adult and one juvenile ring-billed gull carcass on 24 August 1995. On Island B, we found one adult herring gull carcass on 28 July 1995, and one juvenile and one adult herring gull carcass on 18 August 1995. On Rock Island, we found one juvenile Caspian tern with a broken humerus, 11 ring-billed gull carcasses, 11 herring or California gull carcasses, and one adult Caspian tern carcass on 14 August 1995.

Virological examination

Newcastle disease virus was isolated from the five cormorants collected from Island A on 23 and 24 July 1995. The virus (designated PMV-1/cormorant/Saskatchewan-Canada/2035/95) had an intracerebral pathogenicity index of 1.61 and an intravenous pathogenicity index of 1.23, and was classified as pathogenic on the basis of an intracerebral pathogenicity index >0.7 (Commission of the European Communities, 1993). Newcastle disease virus was not isolated from any birds sampled in 1994 or 1996, nor from birds other than the cormorants sampled in 1995.

Examination for antibodies to NDV

Yolk samples were more difficult to collect from incubated eggs (≥ 2 days of incubation) than from freshly laid eggs (< 2 days of incubation) because embryonic development and higher fluidity and volume of the yolk increased the risk of rupturing the yolk sac. The antibody status of cormorant eggs, their length of incubation and year of collection (Table 2) were not all mutually independent (Pearson's $\chi^2 = 151.80$, 17 df, $n = 225$, $P < 0.00001$). Antibody status was dependent on length of incubation, holding collection year constant (Pearson's $\chi^2 = 43.41$, 9 df, $n = 225$, $P < 0.0001$), but independent of collection

TABLE 2. Hemagglutination inhibiting antibody titer to Newcastle disease virus in yolks of double-crested cormorant eggs from Island A at Doré Lake.

Year	Estimated length of incubation (days) ^a	Prevalence	Geometric mean titer	Titer				
				<1:20	1:20	1:40	1:80	1:160
1994	0-1	70 (10) ^b	44	3	2	3	1	1
	2-8	40 (10)	34	6	1	3	0	0
	9-13	40 (5)	20	3	2	0	0	0
	14-25	67 (3)	20	1	2	0	0	0
	Total	54 (28)	33	13	7	6	1	1
1995	0-1	93 (27)	41	2	6	13	5	1
	2-8	60 (60)	39	24	13	13	8	2
	9-13	23 (35)	22	27	7	1	0	0
	14-25	24 (25)	32	19	3	2	1	0
	Total	51 (147)	37	72	29	29	14	3
1996	0-1	68 (49)	26	16	22	10	1	0
	2-8	0 (1)	<20	1	0	0	0	0
	Total	66 (50)	26	17	22	10	1	0

^a Length of incubation was estimated as (1) marginal vein not yet visible at 0-1 day, (2) marginal vein visible as red ring on yolk sac and crown-rump length of embryo ≤ 10 mm at 2-8 days, (3) crown-rump length of embryo > 10 mm and ≤ 20 mm at 9-13 days, and (4) crown-rump length of embryo > 20 mm and ≤ 50 mm at 14-25 days.

^b Percent positive (number of yolks tested).

year, holding length of incubation constant (Pearson's $\chi^2 = 11.98$, 8 df, $n = 225$, $P = 0.15$). Length of incubation was dependent on collection year, holding antibody status constant (Pearson's $\chi^2 = 116.21$, 12 df, $n = 225$, $P < 0.00001$). If only eggs of < 2 days of incubation were considered, there was a significant difference in antibody status between years ($\chi^2 = 6.20$, 2 df, $n = 86$, $P = 0.045$), due to the high prevalence (93%) in 1995 (Table 2).

During the ND epidemic, 37 of 63 (59%) of cormorant sera tested positive for antibodies to NDV, with titers of 1:20 or 1:40 (Table 3); all positive sera were from

5- to 9-wk-old birds (Table 4). The highest prevalence and the highest geometric mean titers were found during the first 2 wk of the epidemic (Table 3). However, bird capture was strongly biased towards birds with clinical signs of ND, which were easier to catch, so that these samples do not accurately represent the juvenile cormorants of the Island A colony. There was no clear relationship between the presence of clinical signs of disease and titer (Table 5). Sera from three of four cormorants from Rock Island collected during the ND epidemic tested positive for antibodies to NDV, one with a titer of 1:40, two with a

TABLE 3. Relationship between sampling date and hemagglutination inhibiting antibody titer to Newcastle disease virus in sera of double-crested cormorants from Island A at Doré Lake during the Newcastle disease epidemic in 1995.

Sampling date	Prevalence	Geometric mean titer	Titer		
			<1:20	1:20	1:40
23 to 29 July	63 (19) ^a	28	7	6	6
30 July to 5 August	84 (19)	27	3	9	7
6 to 12 August	57 (7)	24	3	3	1
13 to 19 August	29 (18)	20	13	5	0
Total	59 (63) ^b	26	26	23	14

^a Percent positive (number of sera tested).

^b This includes four sera which were collected from two cormorants which had been bled at least once before where one was bled twice and the other four times.

TABLE 4. Relationship between age and prevalence of hemagglutination inhibiting antibody titer to Newcastle disease virus in double-crested cormorants from Island A at Doré Lake.

Age class	Age ^a (wk)	Prevalence		
		1994	1995	1996
Juvenile	0	0 (6) ^b	10 (20) ^c	nd ^d
	1	0 (6)	0 (11)	0 (17)
	2	0 (17)	0 (9)	0 (3)
	3	0 (8)	0 (6)	nd
	4	0 (13)	0 (4)	0 (4)
	5	0 (4)	57 (14)	nd
	6	0 (11)	84 (19)	0 (1)
	7	0 (8)	67 (12)	nd
	8	0 (12)	38 (8)	nd
	9	0 (2)	33 (15)	nd
Adult	10	0 (1)	nd	nd
		0 (2)	0 (3)	nd
Total		0 (90) ^e	34 (121) ^f	0 (25)

^a The age of juvenile cormorants captured in their nest was based on the estimated hatching date for that nest. The age of juvenile cormorants captured outside their nest was based on the median hatching date of the marked cormorant nests (20 June 1994, 16 June 1995, 29 June 1996).

^b Percent positive (number of sera tested).

^c All 20 were the first sera taken from 20 juvenile cormorants captured on Island A and taken into captivity.

^d No data.

^e Eight of the 11 were the first sera taken from eight juvenile cormorants captured on Island A and taken into captivity.

^f This includes 23 sera taken from free-living cormorants which had been bled at least once before where 10 were bled twice, two were bled five times, and one was bled six times.

^g This includes four sera taken from free-living cormorants which had been bled at least once before where one bird was bled twice and the other was bled four times, each at intervals of 1 wk. One of the paired sera had a titer of 1:20 and the titers of the others was <1:20.

titer of 1:20. The serum from the cormorant from Island B tested negative.

The only cormorant sera that tested positive for antibodies to NDV before or after the ND epidemic were from two 2-day-old birds taken into captivity in 1995 (Table 4). Both had a titer of 1:20. The titer of the sibling egg of one of these chicks was 1:40, that of the other was <1:20. One of the seropositive chicks tested negative 1 wk later, and the other died a few days after sampling. It was dehydrated and emaciated; histological lesions of Newcastle disease, such as non-suppurative encephalitis and neuronal necrosis, were absent (T. Kuiken et al., unpublished data). Sera from birds other than cormorants all tested negative for antibodies to NDV.

DISCUSSION

The clinical signs of ND described here correspond to those reported in previous ND epidemics in cormorants (Cleary, 1977; Wobeser et al., 1993; Banerjee et al., 1994; Meteyer et al., 1997). Most of the nervous signs may be attributed to cerebellar lesions (Clippinger et al., 1996). However, some suggest lesions in other parts of the nervous system. Knuckling suggests damage to sensory or motor pathways of the peripheral nerve, spinal cord, brain stem, or cerebral cortex. Loss of fear and walking into the forest may be interpreted as changes in learned behavior, which suggest cerebral dysfunction. Blindness, in the absence of structural changes

TABLE 5. Relationship between clinical signs and hemagglutination inhibiting antibody titer to Newcastle disease virus in sera of double-crested cormorants from Island A at Doré Lake, during the Newcastle disease epidemic in 1995.

Clinical signs	Prevalence	Geometric mean titer	Titer		
			<1:20	1:20	1:40
Nervous disease	66 (41) ^a	33	14	15	12
Emaciated/moribund/dead	36 (14)	20	9	5	0
None observed	63 (8)	26	3	3	2
Total	59 (63) ^b	26	26	23	14

^a Percent positive (number of sera tested).

^b This includes four sera which were collected from two cormorants which had been bled at least once before where one was bled twice and the other four times.

within the eye itself, suggests damage to the optic nerve or the cerebral pathways. Fixed dilated pupils also may be a result of damage to these structures or to the oculomotor nerve (Clippinger et al., 1996).

Placing the ND epidemic in the context of the cormorant's breeding cycle may help to identify risk factors. The traditional "mass action" formulation for the transmission of directly transmitted diseases states that "the rate of appearance of new infections is proportional to the product of susceptibles, X , times infectious cases, Y , thus $dY/dt = \beta XY$, the β being the rate of effective contact between individuals" (Fine et al., 1982). Assuming that NDV was carried to Island A by returning cormorants (Meteyer et al., 1997), infectious cases (Y) were present from the beginning of the breeding season. Juveniles were present from around 16 June, the median hatching date. Passive HI antibody to NDV, which correlates with protection of the host against NDV (Alexander, 1991), was not detectable in juveniles >1-wk-old (Table 4). This indicates that large numbers of susceptibles (X) were present from about 23 June. Until 17 July, most juveniles remained in their nests and only had direct contact with parents and siblings. After that date, the number of intra- and inter-species contacts increased greatly as the juveniles left their nests and wandered about the island. This greatly increased the rate of effective contact among individuals (β). The epidemic started on 23 July, 6 days later, which is the approximate incubation period for ND in chickens (Alexander, 1991). This sequence of events suggests that departure of juvenile cormorants from their nests, with the associated increase in contact rate among birds, was a critical initiating factor for the epidemic. If this also is true for other ND epidemics in cormorant breeding colonies, monitoring activities can be focused on times when the mean age of juveniles exceeds 5 to 6 wk.

Because juveniles leave nests on the ground at 4 wk of age, while they leave

nests in trees or on cliffs at 6 wk of age (Lewis, 1929), colonies with ground nests might have earlier and more severe ND epidemics than colonies with tree nests or nests on cliffs. This is consistent with the higher mortality in juveniles from ground nests than from tree nests during the ND epidemic in Quebec (Canada) in 1975 (Cleary, 1977). In more southerly colonies, where egg laying is less synchronized and age variation in juvenile cormorants is greater (Palmer, 1962), ND might occur in cormorants much younger than 4 wk of age due to contact with juveniles that hatched earlier and already have left their nests.

Spread of NDV between juvenile cormorants by direct and indirect contact may have been increased by their investigative behavior, including pecking at affected birds and at inanimate objects contaminated with droppings. Their intensive use of shallow water on and around Island A, and the tendency of affected birds to die on the shoreline, may have increased transfer of NDV by water.

Besides ND in juvenile cormorants on Island A, which was confirmed by virus isolation, the occurrence of this disease in juvenile cormorants on Island B and Rock Island may be inferred from the presence of birds with clinical signs consistent with ND on these islands and from positive sera of birds from Rock Island. This was later confirmed by histological and virological examination of those individuals (T. Kuiken et al., unpublished data). Because we did not study the interaction between birds from breeding colonies on Island A, Island B, and Rock Island, we can only speculate about the possible mechanisms of NDV transfer among these colonies. Island A is about 2 km from Island B, and these two islands are about 25 km from Rock Island (Fig. 1). First, adult cormorants from different colonies may have infected each other at common foraging areas, which may be up to 20 km from nesting sites (Hobson et al., 1989). Second, fledged juvenile cormorants with ND may

have swum or flown between Island A and Island B, and may have flown between these two islands and Rock Island, and so transferred NDV between colonies. Third, other bird species moving between colonies, such as gulls, may have transferred NDV. Finally, spread of NDV between Islands A and B may have occurred by air or water (Alexander, 1991).

The importance of ND as a cause of mortality for cormorants depends on several factors, including (1) age of bird affected, (2) mortality rate, and (3) whether mortality is additive or compensatory. Life history studies of six species of cormorants suggest that "a key to successful long-term survival in these birds is the maintenance of a considerable proportion of older and reproductively experienced birds in the breeding flock . . ." (Johnsgard, 1993). Because mortality in this and previous ND epidemics in cormorants was limited to juveniles (Cleary, 1997; Meteyer et al., 1997), ND may not be as important per bird lost as causes of mortality that also affect older birds, such as oil spills and pesticide poisoning (Johnsgard, 1993).

We calculated that the ND epidemic on Island A in 1995 had a mortality rate of 32% of juveniles. This is a minimum estimate that assumes that all carcasses present on the colony site on 24 August 1995 were found when searched for. Such success in finding carcasses is most unlikely. Given the brown-black color of the cormorant carcasses and the speed with which they were hidden by bird droppings and debris, it might be realistic to assume that we found only about 50% of cormorant carcasses present. In that case, the calculated mortality rate would be twice as high as the minimum rate, or 64%. This is higher than the overall first-year mortality rate for cormorants from Mandarte Island (British Columbia, Canada) which was 59%, calculated by subsequent-year sightings of birds banded as nestlings (van de Veen, 1973). It is impossible to speculate whether mortality from ND in juvenile cormorants is additive or compensatory,

because little is known about other causes of first-year mortality in cormorants, particularly after fledging (Johnsgard, 1993; Erwin, 1995).

Newcastle disease did not appear to be an important cause of morbidity or mortality in other wild bird species cohabiting with cormorants on Doré Lake. We found no clinical signs, or serological or virological evidence of ND in species other than the cormorant, despite potential transfer of NDV by direct contact with affected live and dead cormorants and indirect contact with their droppings on land and in water. During the ND epidemic in cormorants, unusual mortality in other species was seen only in juvenile pelicans. However, NDV was not isolated and necropsy findings suggested that they died of starvation of undetermined cause (T. Kuiken et al., unpublished data). Mortality in juvenile pelicans and gulls was recorded in association with the 1990 and 1992 epidemics in cormorants (Roffe, 1992; Wobeser et al., 1993). Pathogenic NDV was isolated from one pelican, which also had focal encephalitis, and one ring-billed gull from 1990 (Wobeser et al., 1993), but this was the only evidence that the virus caused mortality in those species. Virus was not isolated from any gulls or pelicans in 1992 (Roffe, 1992). There were no reports of ND outbreaks in poultry in Canada, the USA, or Mexico in 1995 (Welte, 1997), suggesting that NDV from the epidemic on Doré Lake in 1995 did not spread to poultry during or after the breeding season.

Statistical analysis of antibody titers to NDV in cormorant eggs indicates that the antibody status of the eggs was dependent on the incubation stage, and, once corrected for this variable, did not differ between years. The most likely explanation for the variation in antibody titers to NDV at different stages of incubation (Table 2) is the change in yolk concentration due to absorption of water from the albumen in the first half of incubation and loss of water from the yolk in the second half (Ro-

manoff, 1967). Transfer of antibodies from the yolk to the embryo and enzymatic breakdown of antibodies may also cause fluctuation in yolk antibody titer (Kramer and Cho, 1970). For comparison of antibody titers in eggs among years, examination should be limited to unincubated eggs. These have the additional advantage that yolk samples are easier to collect than from partly incubated eggs.

In chickens, egg yolk antibody titers to NDV correspond with serum titers of the dam (Heller et al., 1977). Thus, the significantly higher prevalence of antibody titer to NDV in eggs incubated <2 days in 1995 compared to 1994 and 1996 (Table 2) suggests that a higher percentage of adult cormorants had been in contact with NDV in that year. In chickens, HI antibodies to NDV usually become undetectable within a year after infection (Hanson, 1980). By extrapolation, the cormorants that had antibodies to NDV in May 1995 probably had contact with NDV some time after the 1994 breeding season. This is consistent with the assumption that in 1995 NDV was carried to Island A by returning cormorants infected during winter or migration.

Antibodies to NDV were present in sera of juvenile cormorants in July and August 1995, when there was a ND epidemic on Island A, but not in 1994 and 1996, when there was no epidemic (Tables 3 and 4); this corresponds with the findings of Wobeser et al. (1993) and Meteyer et al. (1997). It confirms the suggestion of Meteyer et al. (1997), that serology can be used as a non-lethal way of determining exposure to NDV in a cormorant population >1 to 2 wk of age, when passive antibodies become undetectable.

ACKNOWLEDGMENTS

We are grateful to T. Bollinger, F. Edquist, B. Hanbidge, G. Honing, S. Honour, R. Kerr, S. Kutz, R. Laliberté, P. Leighton, W. Lusimbo, R. Martinez-Molina, V. Misra, R. Nast, H. Philibert, A. Pocknell, C. Riddell, A. Scholten, I. Shirley, D. Vasconcelos, D. Welch, S. Wilson, A. Wobeser, and B. Wobeser for their help in

the field. We thank L. Hassard and B. Norman for initial isolation of the virus, A. Scholten for typing in data, C. Wheler for help with bleeding and treating newly-hatched cormorants, and the staff of the Animal Care Unit of the Western College of Veterinary Medicine for their excellent husbandry of captive cormorants. This project was supported by Saskatchewan Environment and Resource Management, the Canadian Wildlife Service (Environment Canada), the Wildlife Health Fund of the Western College of Veterinary Medicine, the Animal Health Trust of Canada, the Alberta Sports, Recreation, Parks and Wildlife Foundation, and the University of Saskatchewan.

LITERATURE CITED

- ALEXANDER, D. J. 1988. Newcastle disease diagnosis. In *Newcastle disease*, D. J. Alexander (ed.). Kluwer Academic Publishers, Boston, Massachusetts, pp. 147–160.
- . 1991. Newcastle disease and other paramyxovirus infections. In *Diseases of poultry*, 9th ed., B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid and H. W. Yoder, Jr. (eds.). Iowa State University Press, Ames, Iowa, pp. 496–519.
- ANALYTICAL SOFTWARE. 1996. *Statistix for Windows: User's manual*. Analytical Software, Tallahassee, Florida, 333 pp.
- BANERJEE, M., W. M. REED, S. D. FITZGERALD, AND B. PANIGRAHY. 1994. Neurotropic velogenic Newcastle disease in cormorants in Michigan: Pathology and virus characterization. *Avian Diseases* 38: 873–878.
- BEARD, C. W. 1989. Serologic procedures. In *A laboratory manual for the isolation and identification of avian pathogens*, 3rd ed., H. G. Purchase, L. H. Arp, C. H. Domermuth, and J. E. Pearson (eds.). Kendall/Hunt Publishing Company, Dubuque, Iowa, pp. 192–200.
- BROWN, J., R. S. RESURRECCION, AND T. G. DICKSON. 1990. The relationship between the hemagglutination-inhibition test and the enzyme-linked immunosorbent assay for the detection of antibody to Newcastle disease. *Avian Diseases* 34: 585–587.
- CLEARY, L. 1977. Succès de reproduction du cormoran à aigrettes, *Phalacrocorax auritus auritus*, sur trois îles du St-Laurent, en 1975 et 1976. M.Sc. Thesis, L'Université Laval, Ste-Foy, Quebec, 68 pp.
- CLIPPINGER, T. L., R. A. BENNETT, AND S. R. PLATT. 1996. The avian neurologic examination and ancillary neurodiagnostic techniques. *Journal of Avian Medicine and Surgery* 10: 221–247.
- COMMISSION OF THE EUROPEAN COMMUNITIES. 1993. Commission decision of 12 May 1993 laying down the criteria for classifying third countries with regard to avian influenza and Newcas-

- the disease. *Official Journal of the European Communities L* 137: 24–30.
- ERWIN, R. M. 1995. The ecology of cormorants: Some research needs and recommendations. *Colonial Waterbirds* 18 (Special publication): 240–246.
- FINE, P. E. M., J. L. ARON, J. BERGER, D. J. BRADLEY, H. J. BÜRGER, E. G. KNOX, H. P. R. SEELIGER, C. E. G. SMITH, K. W. ULM, AND P. YEKUTIEL. 1982. The control of infectious disease group report. In *Population biology of infectious diseases*, R. M. Anderson and R. M. May (eds.). Springer Verlag, Berlin, Germany, pp. 121–147.
- HANSON, R. P. 1980. Newcastle disease. In *Isolation and identification of avian pathogens*, S. B. Hitchner, C. H. Domermuth, H. G. Purchase and J. E. Williams (eds.). American Association of Avian Pathologists, College Station, Texas, pp. 63–66a.
- HECKERT, R. A. 1993. Newcastle disease in cormorants. *Canadian Veterinary Journal* 34: 184.
- HELLER, E. D., D. B. NATHAN, AND M. PEREK. 1977. The transfer of Newcastle serum antibody from the laying hen to the egg and the chick. *Research in Veterinary Science* 22: 376–379.
- HOBSON, K. A., R. W. KNAPTON, AND W. LYSACK. 1989. Population, diet and reproductive success of double-crested cormorants breeding on Lake Winnipegosis, Manitoba, in 1987. *Colonial Waterbirds* 12: 191–197.
- JOHNSGARD, P. A. 1993. *Cormorants, darters, and pelicans of the world*. Smithsonian Institution Press, Washington, D.C., 445 pp.
- KRAMER, T. T., AND H. C. CHO. 1970. Transfer of immunoglobulins and antibodies in the hen's egg. *Immunology* 19: 157–167.
- KUIKEN, T., G. WOBESER, F. A. LEIGHTON, I. SHIRLEY, AND L. BROWN. 1997. A modular tunnel-and-blind system to reduce investigator disturbance of breeding colonial waterbirds. *Colonial Waterbirds* 20: 532–536.
- LEWIS, H. F. 1929. The natural history of the double-crested cormorant (*Phalacrocorax auritus auritus* (Lesson)). Ph.D. Thesis, Cornell University, Ithaca, New York, 94 pp.
- METEYER, C. U., D. E. DOCHERTY, L. C. GLASER, J. C. FRANSON, D. A. SENNE, AND R. DUNCAN. 1997. Diagnostic findings in the 1992 epornitic of neurotropic velogenic Newcastle disease in double-crested cormorants from the upper midwestern United States. *Avian Diseases* 41: 171–180.
- MIXSON, M. A., AND J. E. PEARSON. 1992. Velogenic neurotropic Newcastle disease (VNND) in cormorants and commercial turkeys, FY 1992. Proceedings of the ninety-sixth annual meeting of the United States Animal Health Association 1992: 357–360.
- PALMER, R. S. 1962. Double-crested cormorant *Phalacrocorax auritus*. In *Handbook of North American birds: Loons through flamingos*, Vol. 1. R. S. Palmer (ed.). Yale University Press, New Haven, Connecticut, pp. 325–340.
- ROFFE, T. J. 1992. Neurotropic, velogenic Newcastle disease in double-crested cormorants. In Proceedings of the ninety-sixth annual meeting of the United States Animal Health Association 1992: 148–150.
- ROMANOFF, A. L. 1960. The avian embryo: Structural and functional development. The Macmillan Company, New York, New York, 1305 pp.
- . 1967. Biochemistry of the avian embryo: A quantitative analysis of prenatal development. Interscience Publishers, New York, New York, 398 pp.
- VAN DE VEEN, H. E. 1973. Breeding biology and demography of the double-crested cormorant *Phalacrocorax auritus*. Final-year Thesis, State University of Groningen, Groningen, The Netherlands, 25 pp.
- VAN SCHEIK, W. J. 1985. Thermal aspects of the reproductive ecology of the double-crested cormorant (*Phalacrocorax auritus*) in southern Alberta. Ph.D. Thesis, University of Alberta, Edmonton, Alberta, 120 pp.
- WELTE, V. R. (Editor). 1997. *Animal health yearbook 1995*. Food and Agricultural Organization, Rome, Italy, 279 pp.
- WOBESER, G. A. 1994. Investigation and management of disease in wild animals. Plenum Press, New York, New York, 265 pp.
- , F. A. LEIGHTON, R. NORMAN, D. J. MYERS, D. ONDERKA, M. J. PYBUS, J. L. NEUFELD, G. A. FOX, AND D. J. ALEXANDER. 1993. Newcastle disease in wild water birds in western Canada, 1990. *Canadian Veterinary Journal* 34: 353–359.
- ZAR, J. H. 1996. *Biostatistical analysis*, 3rd ed. Prentice Hall, Upper Saddle River, New Jersey, 662 pp.

Received for publication 8 September 1997.