

Newcastle disease in wild water birds in western Canada, 1990

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Abstract

This report describes the investigation of mortality of double-crested cormorants (*Phalacrocorax auritus*), white pelicans (*Pelecanus erythrorhynchos*), and gulls (*Larus spp.*) in Alberta, Saskatchewan, and Manitoba during late summer 1990. Techniques used varied among areas, but virological and histopathological examination of birds was done in each area. The major clinical sign in cormorants was inability to fly, often with unilateral wing or leg paralysis. Focal nonsuppurative inflammation was present in the brain and spinal cord of cormorants and pelicans. Newcastle disease virus (NDV) was isolated from cormorants, a pelican, and a ring-billed gull (*Larus delawarensis*) from Saskatchewan. Cormorants from Alberta were positive for NDV in an immunofluorescent test. Most of the viruses were classed as velogenic and all had a similar monoclonal antibody profile to viruses from the 1970 to 1974 panzootic. Approximately half of cormorant, pelican, and gull eggs collected from affected colonies in the spring of 1991 had antibody to NDV. Antibody was also present in cormorant eggs from the Great Lakes. No unusual mortality was detected at any colony in 1991. Fledgling cormorants and gulls from colonies where mortality occurred in 1990 did not have antibody to NDV in June–July 1991. The overall extent of mortality among water birds and the source of the virus were not determined.

Résumé

La maladie de Newcastle chez les oiseaux aquatiques sauvages dans l'Ouest canadien en 1990

Cette étude rapporte les résultats d'une enquête sur les causes de mortalité chez les Cormorans à aigrettes

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(*Phalacrocorax auritus*), les Pélicans blancs (*Pelecanus erythrorhynchos*) et les Goélands (*Larus spp.*) de l'Alberta, de la Saskatchewan et du Manitoba, à l'été 1990. Des analyses virologiques et des examens histopathologiques ont été effectués selon différentes techniques dans chaque région. Le signe clinique le plus fréquent chez le Cormoran était son inaptitude à voler souvent accompagnée d'une paralysie unilatérale de l'aile ou de la patte. Les Cormorans et les Pélicans présentaient une inflammation focale non suppurée du cerveau et de la moelle épinière. En Saskatchewan, le virus de la maladie de Newcastle a été isolé chez les Cormorans, les Pélicans et les Goélands à bec cerclé (*Larus delawarensis*). En Alberta, le virus de Newcastle a été isolé chez les Cormorans par épreuve d'immunofluorescence. La majorité des virus isolés étaient de souche vélogénique et tous présentaient un profil d'anticorps monoclonaux semblable à ceux rencontrés lors de la panzootie de 1970–1974. Au printemps de 1991, presque la moitié des oeufs de Cormorans, de Pélicans et de Goélands provenant de colonies infectées avaient des anticorps contre le virus Newcastle. Des anticorps ont aussi été décelés dans les oeufs de Cormorans de la région des Grands Lacs. Aucune mortalité inhabituelle ne fut remarquée dans les colonies en 1991. Les oisillons Cormorans et Goélands, provenant des colonies avec mortalité en 1990, n'avaient pas de taux d'anticorps décelables contre le virus Newcastle en juin et juillet 1991. L'étendue globale de la mortalité chez les oiseaux aquatiques n'a pas été déterminée de même que la source du virus.

(Traduit par Dr Thérèse Lanthier)

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Introduction

Although virtually all birds are considered susceptible to infection with Newcastle disease virus (NDV) (1), and NDV has been isolated from many wild species (1,2), there are few reports of disease or mortality caused by NDV in free-living birds other than feral pigeons (*Columba livia*), recently captured teal (*Anas crecca*) (3), and double-crested cormorants (*Phalacrocorax auritus*) from the St. Lawrence River (4). This report describes an apparent epizootic among double-crested cormorants, white pelicans (*Pelecanus erythrorhynchos*), and gulls (*Larus spp.*) in western Canada during the summer of 1990, in which there was extensive mortality and in which velogenic NDV was isolated from some birds. Results of a limited serological survey of birds on some of the colonies during the following year are also reported.

Materials and methods

Morbidity and/or mortality was recognized water birds at different times during 1990 in the three prairie prov-

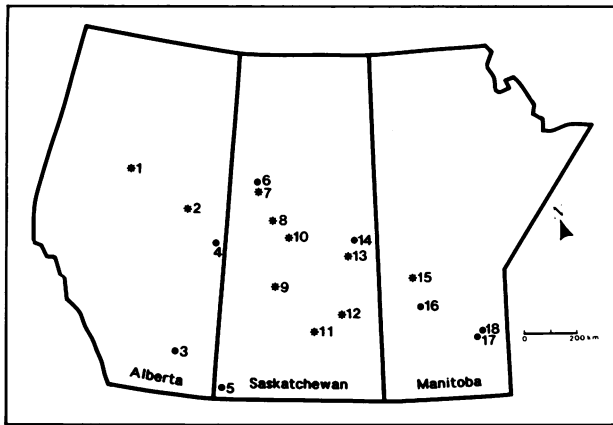


Figure 1. Locations in the prairie provinces of Canada referred to in text; sites marked with an asterisk are locations where mortality among waterbirds was recognized in 1990: 1 — Utikuma Lake; 2 — Lac La Biche; 3 — Lake Newell; 4 — Frog Lake; 5 — Cypress Lake; 6 — Churchill Lake; 7 — Kazan Lake; 8 — Dore Lake; 9 — Redberry Lake; 10 — Lavallee Lake; 11 — Last Mountain Lake; 12 — Margo; 13 — Tobin Lake; 14 — Suggi Lake; 15 — Lake Winnipegosis; 16 — Waterhen Lake; 17 — Lake Winnipeg, Riverton-Sandy Bar; 18 — Lake Winnipeg, Hecla Island.

inces. Because investigations were conducted independently, using different methods, the situation in each province will be considered separately. Locations on the prairies are shown in Figure 1.

Alberta

Mortality was first recognized among cormorants on nesting islands in Lac La Biche on August 2. Sick cormorants were seen throughout the month; by August 29, approximately 300 cormorants and three unidentified gulls had been found dead. Two dead and nine live cormorants, with varying degrees of paralysis, were submitted to the Animal Health Laboratory, Alberta Agriculture, Edmonton, for examination. Sera were collected and necropsies were performed. Liver from each bird was cultured routinely for bacteria. Tissues selected for microscopical examination were fixed in 10% neutral buffered formalin, processed routinely, sectioned at 5 μ , and stained with hematoxylin and eosin (H & E). Sera were tested for *Clostridium botulinum* toxin by intraperitoneal inoculation in mice (5) and for antibodies to NDV using a hemagglutination inhibition test (HI) (6) with egg-adapted NDV. Brain from four of the birds submitted alive was cryosectioned at 4–6 μ , incubated for 30 min at 22°C with chicken origin anti-NDV serum (Spafas, Storrs, Connecticut, USA), washed three times with phosphate buffered saline, incubated 30 min at 22°C with fluorescein-labelled antichick IgG (M + L) (Bio/Can Scientific, Mississauga, Ontario), washed, and counterstained with Evan's blue. Tracheal tissue from a chicken from which NDV had been isolated was used as a positive control, and brain from an uninfected chicken was used as a negative control. Sections were examined for cell-associated fluorescence. Portions of brain were frozen at –80°C and sent to the Animal Diseases Research Institute (ADRI), Agriculture Canada, Nepean, Ontario, for virus isolation.

During early August, mortality occurred among ducks and gulls on Utikuma Lake, and 754 ducks were collected around two nesting islands used by pelicans and cormorants. Four live ducks and a gull collected at this time were submitted to the Animal Health Laboratory, Fairview. Sera from these birds was tested for botulinum toxin. On September 4, a sick cormorant and a dead gull were submitted to the laboratory at Fairview. These birds were handled in the manner described for those submitted to the laboratory at Edmonton.

Saskatchewan

Morbidity among cormorants was noted near the north end of Last Mountain Lake during July, and four dead cormorants were submitted to the Department of Veterinary Pathology, Western College of Veterinary Medicine, on August 2. These birds were found on land and were described as being weak and with some as having difficulty holding the head erect. Between August 10 and 20, nine additional cormorants, together with four ring-billed gulls (*L. delawarensis*), a mallard (*Anas platyrhynchos*), and two Canada geese (*Branta canadensis*), were submitted from this lake. The duck, gulls, geese, and three of the cormorants were from a large island near the north end of the lake that had nesting cormorants, gulls, and pelicans. The other six cormorants were from an island close to the western shore of the lake near the town of Penzance. On September 10, two pelicans, one dead and the other unable to fly, found on a small wetland near Margo were submitted by a conservation officer. On September 11, three sick and two dead pelicans and ten dead cormorants were collected at a colony on Dore Lake. Between September 12 and 15, one dead and four partially paralysed cormorants were submitted from Tobin Lake. On September 13, four cormorants, three pelicans, and a horned lark (*Eremophila alpestris*) found sick or dead on an island in Lavallee Lake in Prince Albert National Park were submitted by wardens of the Canadian Park Service. On September 25, a sick pelican was submitted from a nesting colony in Redberry Lake, and on October 2, carcasses of a pelican, an unidentified gull, and an adult snow goose (*Anser caerulescens*) were submitted from the same site.

Blood was collected from live birds, and sera were tested for antibodies to NDV by HI. Sera from the Canada geese, a gull, and mallard from Last Mountain Lake were tested for botulinum toxin. Where specimens were suitable, necropsies were performed and tissues were collected for virus isolation, and bacteriological and histopathological examination. Tissue that usually included liver and lung, and less commonly, intestine, spleen, and kidney from individual birds were cultured routinely for bacteria. Tissues were fixed in neutral buffered 10% formalin and prepared routinely for microscopical examination.

Tissues from some birds were submitted immediately for virological examination; tissues from others were held frozen at –80°C until being submitted. Brain was submitted from all birds; liver, spleen, and lung were collected from most birds; and intestine was less commonly included among tissues

for virology. Tissues were pooled, ground in phosphate-buffered saline, frozen at -80°C , thawed, centrifuged at low speed to remove tissue fragments, and then filtered, with the final filter having a pore size of $0.22\ \mu$. Filtrate (0.15 mL) was inoculated into the allantoic sac of each of six hen eggs containing a nine-day-old embryo. The eggs used were from hens negative by HI for antibodies to NDV. Eggs were incubated and checked daily for mortality. At seven days postinoculation, allantoic fluid was harvested, pooled from the eggs used for each inoculum, and tested for hemagglutination using guinea pig erythrocytes. Where embryos had died, allantoic fluid was examined for virus particles by transmission electron microscopy. In cases in which the embryos died shortly after inoculation, inoculation was repeated using eggs containing 12-day-old embryos. Material was passaged through eggs three times with negative results before a conclusion was reached that no virus was present. Virus isolates were identified as NDV using HI with antibody to NDV. Viruses identified as NDV were sent to ADRI for confirmation of their identity and pathogenicity determination. Three tests for assessment of pathogenicity of NDV (6) were performed there on each isolate: mean death time in embryonated eggs (MDT), and pathogenicity indices based on clinical disease and mortality in day-old chicks inoculated intracerebrally (ICPI) and six-week-old chicks inoculated intravenously (IVPI). Classification of a virus as lentogenic, mesogenic, or velogenic was based on the combined results of the three tests as follows:

Test	Lentogenic	Mesogenic	Velogenic
MDT (h)	>90	60 - 90	<60
ICPI	<0.5	0.5 - 1.5	1.0 - 2.0
IVPI	0	0.08 - 1.0	>2.0

Virus isolates were subsequently sent to the International Reference Laboratory, Central Veterinary Laboratory, Ministry of Agriculture, Fisheries and Food, Weybridge, Surrey, England, for serological grouping, using a panel of monoclonal antibodies (7,8).

Manitoba

Mortality in cormorants was first reported to the Veterinary Diagnostic Laboratory, Winnipeg, at the end of August when three young-of-the-year birds (i.e., hatched in 1990) found by officers of the Department of Natural Resources, together with four found by citizens within Winnipeg or on commercial poultry farms, were submitted to the laboratory. The birds found by citizens were unable to fly and some had unilateral wing dysfunction.

A meeting of commercial fishermen and government representatives was held in mid-September in the village of Winnipegosis to define the extent of mortality among cormorants on Lake Winnipegosis. In late September, seven cormorants were collected by officers of the Department of Natural Resources near Lake Winnipegosis. The birds were euthanized and necropsies were performed. Tissue samples were sent

to ADRI for virus isolation. Portions of brain from these birds were fixed in 10% neutral buffered formalin and prepared for microscopical examination.

Serological survey, 1991

At a meeting of concerned agencies in November 1990, it was agreed to conduct a serological survey during the summer of 1991 to assess the extent of exposure to NDV among water birds. For ease of collection, egg yolk rather than serum was used in an HI test for antibody, as has been done for domestic poultry (9,10). Eggs were collected, under appropriate permits, during May 1991 from sites indicated in Table 1. Cormorant eggs were obtained from several colonies in the Great Lakes through cooperation with Canadian Wildlife Service personnel. Upon receipt in the laboratory, eggs were opened, and the yolk was removed, mixed, and frozen at -80°C until used. For testing, yolk was diluted 1:10 with 0.85% saline and then adsorbed twice with thrice-washed guinea pig erythrocytes to remove nonspecific hemagglutinins. After adsorption, yolk samples were tested for HI activity. The NDV used was a B₁ strain used routinely in the Virology Laboratory, Department of Veterinary Microbiology, Western College of Veterinary Medicine. In addition to controls normally used in HI tests, egg yolk from an immunized adult domestic hen that had an HI titer of 1:320 in both serum and yolk and yolk from a second domestic hen with no detectable titer in either yolk or serum were used as positive and negative controls, respectively. We have found that HI titers in yolk, used in the manner described, were the same as or one dilution lower than in the serum of hens vaccinated against NDV (Pensaert and Wobeser, unpublished data). Dilutions of yolk of from 1:10 to 1:40 were tested. Between June 17 and 24, 1991, blood was collected by personnel of Manitoba Agriculture and Manitoba Natural Resources from nesting cormorants on Lake Winnipegosis and Hecla Island, Lake Winnipeg; ring-billed gulls at Riverton-Sandy Bar, Lake Winnipeg; and herring gulls (*L. argentatus*) from Lake Winnipegosis. On July 30, 1991, blood was collected from 20 fledgling cormorants at Lavallee Lake. Serum from these birds was tested by HI for antibodies to NDV.

Results

Clinical signs in affected birds

The most obvious feature at most sites, other than presence of dead birds, was cormorants acting abnormally or in unusual locations. Cormorants were found walking along roads, in fields and farm yards (sometimes at a considerable distance from water), and in one instance in Saskatchewan, swimming among bathers at a public beach. Some birds appeared unafraid or unaware of humans, while others tried to escape but were unable to fly. The latter birds flopped along the surface and dove when approached closely. Many had paralysis of one wing, with the affected wing held tightly to the body or extended slightly; others had unilateral leg paralysis, with the affected leg flexed and the foot clenched tightly (Figure 2). No description of sick gulls was available; affected pelicans were weak and unable to fly.

Table 1. Results of hemagglutination-inhibition test for antibodies to Newcastle disease virus in the yolk of eggs collected during May 1991 and in serum from nestling water birds collected during June and July 1991

Location	Species ^a	Specimen (n)	Number with titer			
			<1:10	1:10	1:20	1:40
Alberta						
Lac La Biche	C	egg (30)	12	9	6	3
	G	egg (5)	4			1
Lake Newell	C	egg (30)	26	4		
	G	egg (6)	4		1	1
Frog Lake	C	egg (30)	29	1		
	G	egg (9)	8		1	
Saskatchewan						
Last Mountain Lake	C	egg (30)	10	9	5	6
Lavallee Lake	C	egg (27)	13	12	2	
		serum (20)	20			
Churchill Lake	P	egg (10)	2		1	7
	C	egg (30)	4	10	8	8
Manitoba						
Lake Winnipeg Hecla I.	C	egg (32)	7	3	2	18
		serum (15)	15			
		egg (3)	1		2	
Riverton- Sandy Bar	RBG	egg (9)	9			
		serum (6)	6			
		egg (8)	4			4
Waterhen Lake	C	egg (30)	11	9	9	1
		serum (16)	16			
	HG	egg (7)			1	6
		serum (8)	8			
Ontario						
Lake Superior Granite I.	C	egg (9)	1		1	7
Lake Huron S. Watcher I.	C	egg (11)	3		4	4
Michigan						
Lake Michigan Little Gull I.	C	egg (9)	3		3	3
		egg (20)	2	8	5	5
St. Marten's Shoal	C	egg (22)	4	1	6	11
Lake Huron Scare Crow I.	C	egg (22)			2	13
		egg (4)			3	1

^aC — double-crested cormorant, P — white pelican, G — unidentified gull, RBG — ring-billed gull, HG — herring gull

Estimation of mortality

The total number of birds affected in 1990 is unknown. The number of dead birds at some sites was estimated; these estimates were not based on sampling, and are only included to give an indication of the extent of the mortality. The 300 cormorants found dead at Lac La Biche, Alberta, represented about 10% of the breeding population. There were 30–75 pelicans present during the period, but no sick or dead pelicans were seen. Only one dead cormorant and a dead gull were examined from Utikuma Lake, Alberta. Twelve cormorant colonies at other locations in Alberta were visited during August and September with no unusual mortality being observed. The estimated mortality in Saskatchewan included 2000 cormorants and approximately 50 ring-billed gulls at Last Mountain Lake; approximately 1000 cormorants and 30 pelicans at

Dore Lake; about 2000 cormorants, 75 pelicans, and a few gulls at Lavallee Lake; and 160 cormorants, 36 pelicans, 1730 ring-billed gulls, and 380 California gulls (*L. californicus*) at Redberry Lake. The number of dead and affected birds at Tobin Lake was not estimated. Anecdotal information from a pilot suggested there were many dead cormorants about a colony in Kazan Lake. No unusual mortality was observed on cormorant colonies on Suggi and Cypress Lakes, when these sites were checked during early September. No estimate was made of mortality in Manitoba, but commercial fishermen reported that many sick cormorants unable to fly were seen during late summer on Lake Winnipegosis and that many dead young cormorants were present on breeding colonies on some islands in Lake Winnipeg.

Table 2. Results of attempts to isolate Newcastle disease virus from water birds in Saskatchewan during 1990

Site	Species ^a	No. NDV isolated from/ No. cultured for virus	Pathogenicity ^b
Last Mountain Lake	C	2/4	mesogenic, velogenic
	G	1/3	velogenic
Tobin Lake	C	1/5	velogenic
Lavallee Lake	C	0/1	
	P	1/3	velogenic
	HL	0/1	
Margo	P	0/1	
Redberry lake	P	0/1	

^aC — double-crested cormorant, G — ring-billed gull, P — white pelican, HL — horned lark

^bSee text for definition of pathogenicity

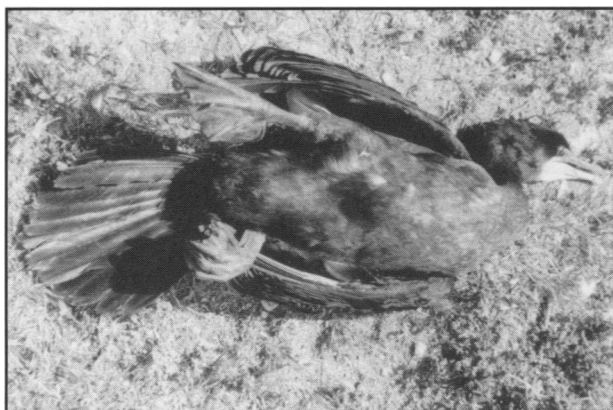


Figure 2. Live cormorant from Last Mountain Lake, Saskatchewan, with unilateral spastic paralysis of the right leg. Newcastle disease virus was isolated from this bird.

Virological findings

Virus isolation was attempted from five cormorants and one gull in Alberta. No NDV was isolated; however, brain from the four cormorants tested was positive for NDV antigen by indirect fluorescent antibody test. Virus isolation was attempted from two cormorants in Manitoba, but no NDV was isolated. Virus isolation was attempted from 21 birds of different species in Saskatchewan, and NDV was isolated from six of these (Table 2). The pathogenicity of five of these viruses was characterized using MDT, ICPI, and IVPI. The initial virus isolate from a cormorant was classed as mesogenic, based on a low IVPI (0.52), although the values for MDT (41.1 h) and ICPI (1.51) were within the range considered to be velogenic. The other four isolates tested were velogenic. The five viruses tested for pathogenicity, together with an additional isolate obtained from a cormorant from Last Mountain Lake, were examined in the Central Veterinary Laboratory, Weybridge, England. All were confirmed as NDV by HI test and were antigenically similar when tested with a panel of 27 monoclonal antibodies (7,8). The viruses were in monoclonal antibody group A, previous members of which have been velogenic viscerotropic viruses, typical of the 1970 to 1974 panzootic in poultry.

Pathological, bacteriological, and serological findings
Cormorants with both adult and subadult plumage were among those examined in Alberta and Saskat-

chewan. All pelicans examined were young-of-the-year. No specific gross lesions were found in pelicans or cormorants. Most of the birds were in poor bodily condition with little or no fat; however, five adults examined in Alberta were in good bodily condition. The alimentary tract was empty of ingesta in all birds that were necropsied, and dark tarry material, suggestive of digested blood, was present in the stomach and intestine of many. The cormorants and pelicans were heavily parasitized by lice and had many nematodes (*Contraecum* spp.) in the gizzard. Five of the cormorants examined in Saskatchewan had excess clear fluid within the pericardial sac. Seven cormorants, two pelicans, and two gulls examined in Saskatchewan were not suitable for study because of putrefaction. Four other cormorants were not suitable for histological examination. The most commonly found microscopical changes were in the central nervous system. Twelve of 18 cormorants from Saskatchewan, seven of 12 cormorants from Alberta, and one gull from Alberta from which tissues were examined microscopically had nonsuppurative encephalitis and/or myelitis. The lesions were mild and focal, consisting of small accumulations of lymphocytes surrounding one or a few tiny vessels. Cormorants from Alberta also had focal gliosis in the grey matter of the cerebellar folia with loss of Purkinje cells. Similar mild focal encephalitis or myelitis was found in two of 12 pelicans from Saskatchewan. Many birds, including those with encephalomyelitis, had vacuolation and separation of white matter, particularly in the cerebellar folia. The pelican from which NDV was isolated had focal encephalitis, as well as focal lymphocytic infiltration in the pancreas and liver. One of three cormorants from which NDV was isolated had focal encephalomyelitis, another had inflammatory changes restricted to the cerebellum. No lesions were found in the nervous system of the third bird from which virus was isolated, but it had foci of necrosis in the liver. Several birds had small foci of lymphocytic inflammation in one of more organs, but no consistent pattern was evident. No microscopic lesions were found in the brain of cormorants examined in Manitoba. Type C botulism was diagnosed in the ducks and gull from Utikuma Lake, and in a Canada goose, ring-billed gull, and mallard from Last Mountain Lake, Saskatchewan. None of the

Table 3. Results of hemagglutination-inhibition test for antibody to Newcastle disease in serum from sick birds from Saskatchewan, 1990

Location	Species	HI titer
Last Mountain Lake	Cormorant	1:30
	Cormorant	1:480 ^a
	Ring-billed gull	1:60
	Ring-billed gull	<1:20
	Canada goose	<1:20
	Canada goose	<1:20
Margo	Mallard	<1:20
	Pelican	<1:20
Dore Lake	Pelican (3)	<1:20
Tobin Lake	Cormorant (2)	<1:20
	(2)	1:20
	Pelican	<1:20

^aNDV isolated from this bird

sera from cormorants at Lac La Biche, Alberta, tested positive for botulinum toxin. No bacterial growth was obtained from the liver of birds that arrived alive at the laboratories in either Alberta or Saskatchewan. Salmonellae were isolated from the intestine, but not from liver, of two cormorants and two pelicans that appeared to have died shortly prior to collection, and from the liver of five cormorants that had been dead for some time prior to collection in Saskatchewan. *Plesiomonas shigelloides* was isolated from the intestine of two cormorants and three pelicans and in small numbers from the liver of two pelicans. *Pasteurella multocida* was isolated in very small numbers from the liver of one pelican found dead.

Sera from sick birds from four locations in Saskatchewan and from Lac La Biche, Alberta, were tested for antibodies to NDV (Table 3). Only eight birds had a titer \geq 1:20; NDV was isolated from the bird with the highest titer. More than 50% of cormorant, pelican, and gull eggs collected in 1991 from all locations, except Lake Newell and Frog Lake in Alberta, had a HI titer of 1:10 or greater (Table 1). None of the nestling gulls or cormorants captured during late June 1991 in Manitoba or the 20 cormorants captured during July 1991 on Lavallee Lake had antibodies to NDV.

Discussion

The number of birds involved in the epizootic among water birds in the prairie provinces in 1990 is unknown, and it is impossible to estimate the role of NDV in this occurrence. Botulism was diagnosed in ducks, geese, or gulls at two sites, but not in cormorants. Isolation of velogenic NDV from three species at three different sites and immunofluorescent evidence of NDV at two other sites suggest that this agent was widespread.

This is not the first time that NDV has been associated with birds of the order Pelecaniformes. The source of infection was never established conclusively in an outbreak of Newcastle disease among poultry in northern Scotland between 1949 and 1951 (11,12), but fishermen reported dead cormorants in the sea and, on many of the farms, cormorants (*P. carbo*, *P. aristotelis*) had been shot for food by farmers

“7–14 days previously” and “fowl had either been fed the offal or had access to it” (12). Approximately 40% of sera from cormorants shot in the vicinity of confirmed outbreaks among poultry during 1949 had “positive or doubtful H.I. titers” to NDV (12). Newcastle disease virus was isolated from bone marrow of some of these birds. The six viruses isolated all killed four to five-day-old chicks; three produced symptoms and lesions in adult chickens, the others caused seroconversion without illness (11). In 1975, NDV characterized as velogenic (MDT = 48 h, ICPI = 1.9, IVPI = 2.3) was isolated from double-crested cormorants found dead in the St. Lawrence River, near Rivière du Loup and Trois Pistoles, Quebec (4). Newcastle virus of unknown pathogenicity was isolated from cormorants (*P. carbo*) in the Volga River delta of the former Soviet Union (13).

We are not aware of the use of egg yolk for serological surveys in wild birds, but the technique has been used to test chicken flocks for antibody to NDV (10). As is often the case with serology in wild animals, we do not have good reference standards for cormorants, pelicans, or gulls with which to establish the significance of the titers detected. In chickens, yolk and serum have similar HI titers (10,14, Pensaert and Wobeser, unpublished data). The majority of eggs from sites where mortality was recognized in 1990 had antibody, suggesting that exposure to NDV was common among birds that nested at these sites. Occurrence of antibody in cormorant eggs from sites in the Great Lakes, where no unusual mortality was recognized in 1990, was unexpected. No mortality was detected among birds at Lake Newell and Frog Lake in Alberta in 1990, and few eggs from these sites had a titer $<$ 1:10. None of the cormorant and gull nestlings examined in late June and July 1991 had detectable antibody, although eggs collected earlier from the same sites did. This suggests that passively acquired antibody wanes within four to six weeks after hatching and that the fledglings had not been exposed to NDV.

Several factors may have contributed to the failure to isolate NDV from many of the birds examined. Some of the birds may have been ill for other reasons, e.g., some fledgling cormorants may have been suffering from malnutrition related to death of the parents. In other instances, inappropriate tissues may have been used for virus isolation, as the virus may be localized. Perhaps most importantly, the birds from which virus isolation was attempted were collected weeks or months after abnormalities were first noted on the colonies. In pigeons with Newcastle disease, virus does not persist for more than three and five weeks in intestine and brain, respectively, while neurological disorders may persist for two to six months (15). Thus, some of the birds we examined may have had residual signs and lesions but have been free of virus.

There was no evidence that velogenic NDV occurred among domestic poultry in western Canada in 1990, although exposure could have occurred as a result of sick birds, particularly cormorants, entering farmyards. We had anecdotal information that one farmer in Saskatchewan kept a “sick” pelican in his chicken

house without experiencing any mortality among the chickens. Mortality of unknown cause was reported retrospectively in small farm flocks of chickens near Lake Winnipegosis in Manitoba, but no specimens were available from these occurrences. Major cormorant and pelican colonies in Alberta, Saskatchewan, and Manitoba were visited at least once during 1991 with no evidence being found of unusual mortality. About 40% of cormorants collected in the area of an outbreak of Newcastle disease in Scotland in 1949 had antibodies to NDV, but none of 32 cormorants collected in the same area in 1952 had detectable antibodies (12). These two findings suggest that NDV may not be continuously present among cormorants.

Impact of the mortality that occurred in 1990 on populations of cormorants, pelicans, and gulls was not assessed. Double-crested cormorants have increased dramatically in numbers in North America during the past two decades (16–18), and the number of white pelicans breeding in Canada has also increased markedly (19). Despite their conspicuous nature, little is known about causes or extent of mortality in these species and the only diseases that have been investigated in western Canada are the occurrence of pouch lice in pelican chicks (20,21) and avian cholera among cormorants at Lac La Biche, Alberta (22). Epizootics may go undetected in these species, because inaccessible areas are used for nesting and because human visitation to colonies is discouraged. In 1990, it was the occurrence of cormorants acting abnormally away from the colonies that first attracted attention in Saskatchewan. The occurrence at Lac La Biche, Alberta, may have been detected because of increased surveillance as a result of an outbreak of avian cholera in the colony in 1989.

Source of the viruses is unknown. The viruses isolated from cormorants and a gull from Saskatchewan reacted identically when tested with a panel of monoclonal antibodies. Other viruses with the same antigenic pattern (group A) have been viscerotropic velogenic and are typical of viruses from the 1970–74 panzootic among poultry (7,8). Cormorants winter in the Gulf of Mexico and as far south as Cuba and the West Indies, and white pelicans winter as far south as the coast of Central America (23). Either of these species, or gulls, might have been exposed to NDV during the winter and brought it north during migration. Alternatively, NDV may be enzootic among water birds.

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