Diagnostic Findings in the 1992 Epornitic of Neurotropic Velogenic Newcastle Disease in Double-crested Cormorants from the Upper Midwestern United States


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SUMMARY. Neurotropic velogenic Newcastle disease (NVND) occurred in juvenile double-crested cormorants, Phalacrocorax auritus, simultaneously in nesting colonies in Minnesota, North Dakota, South Dakota, and Nebraska and in Lakes Michigan, Superior, Huron, and Ontario during the summer of 1992. Mortality as high as 80%-90% was estimated in some of the nesting colonies. Clinical signs observed in 4- to 6wk-old cormorants included torticollis, tremors, ataxia, curled toes, and paresis or weakness of legs, wings or both, which was sometimes unilateral. No significant mortality or unusual clinical signs were seen in adult cormorants. Necropy of 88 cormorants yielded no consistent gross observations. Microscopic lesions in the brain and spinal cord were consistently present in all cormorants from which Newcastle disease virus (NDV) was isolated. Characteristic brain lesions provided rapid identification of new suspect sites of NVND. Lesions were also present in the heart, kidney, proventriculus, spleen, and pancreas but were less consistent or nonspecific. NDV was isolated at the National Wildlife Health Center from 27 of 93 cormorants tested. Virus was most frequently isolated from intestine or brain tissue of cormorants submitted within the first 4 wk of the epornitic. Sera collected from cormorants with neurologic signs were consistently positive for NDV antibody. The NDV isolate from cormorants was characterized as NVND virus at the National Veterinary Services Laboratories, Ames, Iowa. The NVND virus was also identified as the cause of neurologic disease in a North Dakota turkey flock during the summer of 1992. Although no virus was isolated from cormorants tested after the first month of submissions, brain and spinal cord lesions characteristic of NVND were observed in cormorants from affected sites for 2 mo, at which time nesting colonies dispersed and no more submissions were received. Risk to susceptible populations of both wild avian species and domestic poultry makes early recognition and confirmation of NVND in wild birds a priority.

RESUMEN. Hallazgos diagnósticos en el brote de la enfermedad de Newcastle velogénico neurotrópico en cormoranes de doble cresta en la zona norte del medio oeste de los Estados Unidos en 1992.

Durante el verano de 1992 se presentó un brote de Newcastle velogénico neurotrópico en cormoranes jóvenes de doble cresta (Phalacrocorax auritus), simultáneamente en Minnesota, Dakota del Norte, Dakota del Sur y Nebraska y en los Lagos Michigan, Superior, Hurón y Ontario. La mortalidad en algunos de los brotes alcanzó 80-90%. Los signos clínicos observados en cormoranes de 4 a 6 semanas de edad incluyeron torticollis, temblores, ataxia, dedos torcidos y paresia o debilidad de patas y alas, algunas veces unilateral. En cormoranes adultos no se observó mortalidad significativa o signos clínicos anormales. En la necropsia de 88 cormoranes, no se observaron lesiones macroscópicas consistentes. Las lesiones microscópicas en el cerebro y médula espinal estuvieron consistentemente presentes en todos los cormoranes de donde se aisló el virus de la enfermedad de Newcastle. Las lesiones caracte-
Newcastle disease was first confirmed as a cause of widespread mortality in free-flying birds in 1990, when mortality of double-crested cormorants, Phalacrocorax auritus, was reported in Alberta, Saskatchewan, and Manitoba (26). Mortality in double-crested cormorants was again caused by Newcastle disease in 1992 in the United States as well as in Canada. A previous report documented virus characterization and lesions in cormorants from Michigan during the 1992 epornitic (4). We describe the diagnostic findings in cormorants from multiple sites in the upper midwestern United States during 1992, the first wild bird epornitic of Newcastle disease virus (NDV) in the United States, with an emphasis on testing procedures that proved most reliable and efficient for identifying lesions and isolating virus.

**MATERIALS AND METHODS**

**Field investigations.** The National Wildlife Health Center (NWHC) investigated mortality in double-crested cormorant nesting colonies at 29 sites in Minnesota and South Dakota and in Lakes Michigan and Huron in July 1992. Biosecurity precautions for field investigations and field necropsies followed methods described previously (12). Throughout the summer of 1992, field biologists and refuge managers were alerted to the mortality event in double-crested cormorants and were requested to send carcasses of sick or dead colonial nesting birds via overnight delivery to the NWHC for necropsy and virus isolation studies. Carcasses were received from Minnesota, Nebraska, North Dakota, and South Dakota and Lakes Michigan, Superior, and Huron.

**Necropsy.** Necropsies were performed at the NWHC on 88 double-crested cormorants in addition to the necropsies performed during field investigations. A protocol was developed to ensure complete and uniform testing of 54 cormorants for subsequent comparison and analysis of results. Three of these were cormorants from Florida in 1995 to ensure neurotropic velogenic Newcastle disease (NVND)-negative samples collected from cormorants outside the population of the 1992 migratory NVND population. The protocol included methods for bacteriology, virology, and histopathology. Personnel involved in necropsies at the NWHC were required to shower before leaving the biosafety level 3 necropsy facility. All liquid waste was heat treated prior to discharge, and ventilated air passed through high-efficiency particulate air filters before being released.

**Histopathology.** Samples of brain, spinal cord, esophagus, proventriculus, ventriculus, intestine, liver, pancreas, heart, trachea, lung, spleen, bursa of Fabricius, thymus, adrenal gland, thyroid gland, kidney, skeletal muscle, peripheral nerve, ovary, and eye were collected for histopathology. Tissues were placed in 10% neutral buffered formalin approximately 24 hr before being processed. Formalin-fixed tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. To characterize micro-
scoponic changes associated with NVND infections, a group of eight cormorants with NVND virus isolated and negative for salmonella (NVND cormorants) was compared with a group of nine cormorants collected from sites without isolation of NDV or salmonella. Microscopic changes characterized in this subset were then used to identify frequency of lesion occurrence in the larger sample of 54 cormorants necropsied.

**Virology/serology.** Virus isolation was attempted on 526 tissues that included trachea, lung, brain, liver, intestine, and spleen from 93 double-crested cormorants. Tissues were inoculated into Muscovy embryo fibroblast (MSDEF) cell cultures (9) and embryonating hen's eggs via the allantoic sac route (21). Trachea and lung tissues from individual cormorants were inoculated as a pool, as were liver and spleen samples. Brain and intestine were cultured separately. Amniotic-allantoic fluid was tested for presence of hemagglutinating virus by a previously described procedure (13). Virus isolates were identified as NDV by hemagglutination inhibition (5). Embryo mean death times (MDT) were determined by the method described by R. P. Hanson (13). Isolates of NDV were further characterized for pathogenicity in domestic chickens at the National Veterinary Services Laboratories (NVSL), Ames, Iowa (1,3,22).

Twenty-one double-crested cormorants with neurologic signs and eight without neurologic signs had serum collected for NDV antibody determination using hemagglutination inhibition (5). These cormorants were from three nesting sites in Lake Huron, one in Lake Superior, and one in Marsh Lake, Minn.

In 1995, an attempt was made to determine NDV status in nestlings from two of the 1992 NVND sites (Lake Michigan and Marsh Lake, Minn.). Sera were collected from 102 apparently normal cormorant chicks for NDV antibody determination. Samples of brain (86) and liver (96) were collected from pipping cormorants, and 37 samples each of liver, brain, and cloaca were obtained from 10-day-old cormorant chicks for virus isolation.

**Bacteriology.** Liver and intestine were cultured for aerobic bacteria using 5% sheep blood agar and eosin-methylene-blue agars (Difco Laboratories, Detroit, Mich.). Inoculated plates were incubated at 36 C for 48 hr. Air sacs were cultured on Sabouraud's dextrose agar (Difco) and incubated at 36 C for 5 days. Liver and intestine were also cultured for Salmonella sp. using selenite broth (Difco; with dulcitol substituted for lactose) incubated at 36 C for 24 hr and subcultured on xylose-lysine-deoxycholate and brilliant green agars (Difco). Identity of bacterial isolates was determined using API 20E system (Biomerieux Vitek, Inc., Hazelwood, Mo.). Salmonella sp. isolated were sent to the NVSL for identification of serotype using methods previously described (10). Botulism tests were conducted on heart blood from 24 cormorants using the standard mouse toxicity test (18).

**Statistical analysis.** Fisher's exact test (27) was used to test the null hypothesis that the frequency of histologic lesions in cormorants was "independent" of salmonella isolation status. Fisher's exact test was also used to test for "independence" between the presence of encephalitis and occurrence of other frequently found lesions in the cormorants. To account for the many tests conducted using Fisher's exact test, and to provide an experiment-wise P-value of 0.05, a Bonferroni adjustment was made for the number of statistical tests conducted (n = 9) providing a critical P-value of 0.05/9 (0.006) to establish significance (16). Confidence intervals for the sensitivity and specificity for each of the lesions that was significantly related to encephalitis were based on the exact binomial confidence interval (27). McNamar's test for paired or related samples (8) was used to test the null hypothesis that the proportion of birds diagnosed with NVND was the same for virus isolation compared with histopathology.

**RESULTS**

**Field investigations.** Clinical signs observed in sick juvenile double-crested cormorants included torticollis, tremors, ataxia, curled toes, and paralysis or weakness of legs, wings, or both, which was sometimes unilateral. No unusual mortality or clinical signs were seen in adult cormorants. Mortality in juvenile cormorants was estimated to be 80%–90% in midwestern colonies. Most nesting colonies in the Great Lakes were difficult to monitor on a regular basis because of geographic distribution, but young-of-the-year mortality in the Great Lakes colonies was estimated to be 10%–30%.

**Gross necropsy findings.** Of the 54 cormorants used to characterize necropsy findings, 30 were female and 22 were male (sex was not recorded for two birds). Thirty-six cormorants were classified as immature, 14 as subadult, and three as adult. Age was not determined for one cormorant. Forty-seven cormorants were in fair to good body condition, two were in poor condition, and five were emaciated. No consistent gross lesions were reported in the cormorants at necropsy. Serosanguineous fluid flowed from the cut surface of lungs from 17% of the cormorants. Mildly enlarged livers were seen in 20% of the cormorants and four of these had white foci (8%). Large spleens (approximately 3 cm long) were seen in 26% of the cormorants, and 33% had spleens that were mottled.
deep red to red in the subcapsular region. Pale foci were seen in the hearts of 6% of the cor-
morants. Tracheas were red in six cormorants, but all six of these cormorants were euthanati-
zied by cervical dislocation. Occasional dull red foci could be seen on the intestinal mucosa of 20% of the cormorants; these foci could be re-
moved with gentle rubbing. One cormorant had a suppurative conjunctivitis. Two cormo-
rants had focal air sac lesions consistent with aspergillus air sacculitis.

**Histopathology.** Lesions characteristic for NVND in the subset of virus isolation-positive and salmonella-negative cormorants were found in the brain, spinal cord, heart, and kidney. Multifocal Purkinje cell necrosis, with neuron-
ophasia and cell loss, was observed in brains from all cormorants with NVND virus isolated. The associated gliosis in the molecular layer was often so extensive that the margin of the molecu-
lar and granular cell layer was obscured (Fig. 1A). Lymphoplasmacytic vasculitis, peri-
vascular cuffs, and gliosis were also found in the brain from all NVND cormorants. The vessel-
associated inflammation was seen more fre-
quently in the brain stem or white matter of the cerebellum (88%) than in the cerebrum (56%). Vasculitis ranged from occasional lymphocyte and plasma cell accumulation within the vessel wall with endothelial cell swelling and vacuolation (Fig. 1B) to vessels that were diffi-
cult to identify because of dense lymphoplasm-
acytic inflammation and thick perivascular cuffs. Meningitis with perivascular orientation was seen in 28% of the brain sections and 37% of spinal cord sections. Gliosis and vasculitis of the grey matter were found in all spinal cord sections from NVND cormorants that had grey matter included. Inflammation described in the brains and spinal cords of NVND cormorants was not seen in nine normal cormorants. Mild perivascular hemorrhage was seen in the brains of 50% of NVND cormorants and 33% of normal cormorants.

Myocardial lesions in the NVND cormo-
rants included myofiber necrosis, lymphoplasm-
acytic inflammation, and nonsuppurative vas-
culitis. Mild lymphoplasmacytic interstitial ne-
phritis and subcapsular splenic hemorrhage were also observed only in NVND cormorants.

Inflammation, primarily mild multifocal nonsuppurative vasculitis or perivasculitis, was seen inconsistently in lung, skeletal muscle, esophagus, peripheral nerve, conjunctiva, and connective tissue of the eye from NVND cormorants. This vessel-associated inflammation was not seen in normal cormorants.

Lesions in pancreas, liver, proventriculus, and adrenal gland were seen in both NVND and normal cormorants. These lesions included ran-
dom lymphoplasmacytic inflammation in the pancreas and mild multifocal nonsuppurative periportal inflammation, lymphoplasmacytic vasculitis, and perivasculitis in the submucosa, muscle layer, and serosa of the proventriculus. Occasional cormorants had areas of mild adre-
nal gland hemorrhage.

One NVND and one normal cormorant had liver flukes with associated granulomatous in-
flammation. The fluke was identified as Am-
phimerus sp. using previously described criteria (17).

Although spleen, thymus, and bursa ap-
ppeared hypocellular, no obvious active necrosis could be confirmed, and changes could not be differenti-
ated from autolysis or stress-related changes. No inflammation was seen in ovary, small intestine, thymus, thyroid gland, bursa of Fabricius, or trachea of either NVND or nor-
mal cormorants.

Frequencies of lesions described above in brain, spinal cord, heart, kidney, pancreas, pro-
ventriculus, spleen, liver, and miscellaneous tis-
sues (lung, skeletal muscle, peripheral nerve, conjunctiva, and regional connective tissue of the esophagus and eye) were quantified for all 54 cormorants necropsied regardless of isolation status of NVND virus or salmonella (Table 1). The P-values were not determined for pro-
ventricular lesions because of the small sample size. We used Fisher's exact test with a Bonferroni adjustment for multiple comparisons and found no significant association (P > 0.006) between the lesions listed in Table 1 and isolation of Salmonella spp. with a range of P-values from 0.028 for interstitial nephritis to 1.0 for pan-
creatic inflammation. Fischer's exact test, with encephalitis as the criteria for classifying a cor-
morant as histologically positive for NVND, was used to determine if there was significant association with other frequent lesions found in the cormorants. Significant association (<0.006) was found with Purkinje cell necrosis, myelitis, and myocarditis (Table 1). The range of P-values for tissue lesions was from <0.001 for Purkinje cell necrosis and myelitis and
Fig. 1. Brain lesions from double-crested cormorants collected during 1992 NVND epornitic in the United States. (A) Extensive gliosis within the molecular layer (m) of the cerebellum associated with Purkinje cell necrosis (arrows) and disruption of the margin with the granular layer (g). Bar = 40 μm. (B) Early vasculitis with lymphocytes and plasma cells (thick arrow) within the vascular wall and endothelial cell swelling and vacuolation (thin arrow). Bar = 20 μm.
Table 1. Frequent histopathologic lesions seen in 51 double-crested cormorants collected during 1992 NVND epizootic in the United States and their relation to salmonella isolation and in relation to typical NVND lesions found in the brain.

<table>
<thead>
<tr>
<th>Lesion Lesion</th>
<th>+</th>
<th>-</th>
<th>+</th>
<th>-</th>
<th>P-value</th>
<th>Positive brain lesion in same bird</th>
<th>No brain lesion in same bird</th>
<th>P-value</th>
</tr>
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<tr>
<td>Encephalitis</td>
<td>9</td>
<td>1</td>
<td>29</td>
<td>11</td>
<td>0.416</td>
<td>26/26</td>
<td>0/6</td>
<td>&lt;0.001</td>
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<td>Purkinje cell necrosis</td>
<td>5</td>
<td>0</td>
<td>21</td>
<td>6</td>
<td>0.555</td>
<td>10/14</td>
<td>1/11</td>
<td>&lt;0.001</td>
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<tr>
<td>Myelitis</td>
<td>7</td>
<td>1</td>
<td>23</td>
<td>11</td>
<td>0.402</td>
<td>13/24</td>
<td>0/6</td>
<td>0.002</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>9</td>
<td>0.667</td>
<td>10/14</td>
<td>0/8</td>
<td>0.002</td>
</tr>
<tr>
<td>Interstitial nephritis</td>
<td>6</td>
<td>1</td>
<td>8</td>
<td>16</td>
<td>0.028</td>
<td>13/24</td>
<td>0/6</td>
<td>0.024</td>
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<tr>
<td>Pancreatic lymphoid foci</td>
<td>8</td>
<td>1</td>
<td>24</td>
<td>4</td>
<td>1.0</td>
<td>27/27</td>
<td>5/7</td>
<td>0.037</td>
</tr>
<tr>
<td>Proventricular pericarditis</td>
<td>4</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td></td>
<td>10/10</td>
<td>2/2</td>
<td></td>
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<tr>
<td>Subcapsular splenic hemorrhage</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>27</td>
<td>0.332</td>
<td>7/33</td>
<td>0/7</td>
<td>0.317</td>
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<tr>
<td>Periportal inflammation</td>
<td>9</td>
<td>1</td>
<td>26</td>
<td>11</td>
<td>0.414</td>
<td>24/35</td>
<td>4/11</td>
<td>0.008</td>
</tr>
<tr>
<td>Perivascular inflammation</td>
<td>misc. tissues</td>
<td>5</td>
<td>9</td>
<td>4</td>
<td>27</td>
<td>0.111</td>
<td>12/32</td>
<td>0/11</td>
</tr>
</tbody>
</table>

*No grey matter included in one spinal cord section examined.

1No brain stem or cerebellum in one brain section examined without inflammation.

2Misc. tissues = lung, skeletal muscle, peripheral nerve, conjunctiva, and regional connective tissue of esophagus and eye.

0.002 for myocarditis to 0.317 for subcapsular splenic hemorrhage. Sensitivity and specificity for the three significant tissue lesions were 100% for both sensitivity (CI = 86.6%-100%) and specificity (54.1%-100%) for Purkinje cell necrosis, 86.7% (69.3%-96.2%) sensitivity and 90.9% (58.7%-99.8%) specificity for myelitis, and 71.4% (41.9%-91.6%) sensitivity and 100% (63.1%-100%) specificity for myocarditis.

**Virology/serology.** NDV was isolated and identified by the NWHC from 27 of 93 cormorants representing 10 nesting sites in Minnesota, South Dakota, and Nebraska and in Lakes Michigan, Superior, and Huron. NDV was isolated primarily in embryonating hen's eggs and occasionally in MSDEF. Virus isolates were identified as NDV, and most mean death times in embryonating hen's eggs ranged from 45 hr to 65 hr with two extremes at 30.2 hr and 72 hr.

The NVSL classified the 1992 cormorant NDV isolate as neurotropic and velogenic for domestic chickens. These findings were confirmed in subsequent pathogenicity evaluation of the 1992 cormorant and turkey isolates using different chicken strains with different routes of inoculation (15).

Isolation rates during the 1992 epornitic were highest in intestinal samples (26%) followed by brain (20%). Virus was isolated from only 12% of trachea/lung samples and liver/spleen samples. When NVND virus was isolated from trachea/lung or liver/spleen samples, it was always isolated from intestine, brain or both as well.

Twenty-seven cormorants from which NVND virus was isolated were submitted between June 29 and July 29, 1992. During the first week of submissions, NVND virus was isolated from 82% of tissues submitted (18/22), representing all cormorants tested. No submissions from this die-off were received during the second week of this period. By the third week, virus isolation rates dropped to 20% (16 of 83 tissues tested), representing 55% of the 22 cormorants sampled. The number of submissions continued to decrease during the fourth week, with 26% (8 of 31) of the tissues yielding virus. No virus was isolated from 26 cormorants (104 tissues) submitted to the NWHC after the fourth week of the epornitic.
In late August 1992, the NWHC received three cormorants from Devil's Lake, N. Dak. Brain lesions consistent with NVND were present in the cormorants, but virus was not isolated from tissue samples. However, a turkey flock <4 miles from Devil's Lake became infected with NVND virus. At that time, the United States Animal and Plant Health Inspection Service collected additional samples from sick cormorants, and NVND virus was isolated at the NVSL.

Comparison of histopathology and virus isolation showed that all cormorants (16) with virus isolated had brain and spinal cord lesions consistent with NVND. There were no cases where a bird was virus positive and histopathology negative, and 13 cormorants were both histopathology negative and virus negative. However, 25 cormorants had histologic lesions of NVND but were negative for virus. During the epornitic, the proportion of suspect NVND-positive birds (41 of 54 = 76%) based on characteristic histopathologic lesions was higher (McNamar's test, Z = 5.0, P < 0.001) than that based on virus isolation (16 of 54 = 30%)

All serum samples collected from 21 cormorant chicks showing neurologic signs had antibody to NDV, whereas only three of eight chicks without neurologic signs had NDV antibody. When sera and tissues were submitted from cormorant chicks with neurologic signs, virus was isolated from half (three of six). Serum titers among the cormorants with neurologic signs ranged from 1:10 to >1:10,240, whereas titers from cormorants without neurologic signs ranged from <1:10 to 1:320.

In 1995, NDV serum antibody titers of 1:10–1:20 were detected in normal chicks up to 13 days of age. In normal cormorants 14–33 days of age, NDV serum antibody titers were <1:10. NDV was not isolated from the tissues and swabs collected from pipping cormorants or 10-day-old chicks.

**Bacteriology.** *Salmonella typhimurium* or *Salmonella typhimurium-copenhagen* was isolated from 20 of 72 cormorant livers cultured. Ten salmonellapositive cormorants had brain lesions consistent with NVND infection. Numerous bacteria were isolated from liver, intestine, or brain, including six additional Enterobacteriaceae (including *Escherichia coli*), five *Staphylococcus* spp. (not *aureus*), three *Streptococcus* spp., and two *Vibrio* spp. (not *cholera*). None of the bacteria isolated was thought to have contributed significantly to the cause of death based on necropsy findings and histopathology. *Aspergillus fumigatus* was isolated from air sacs of two cormorants. Botulism tests run on heart blood from 24 cormorants were negative.

**Parasitology.** *Contracaecum* sp. (nematode) was seen occasionally in the ventriculus. *Hysterocephora* sp. and *Drepanocephalus* sp. (trematodes) and cestodes were occasionally identified in the intestine.

**DISCUSSION**

Applying the standard NDV pathogenicity assessments to infer pathogenicity of that isolate in species other than poultry may not be accurate in all cases. This pathogenicity index was developed in chicken systems to classify NDV isolates in relation to domestic poultry. This classification system does not necessarily reflect the disease syndrome a particular isolate might produce in other avian species because it has been shown that any individual NDV isolate can show a variety of clinical signs and pathogenicity in different species (2). In this particular case, however, clinical signs, histopathology, and mortality as high as 90% in some nesting colonies provide evidence that this NDV isolate was neurotropic and velogenic in cormorants as well.

Inflammation in the molecular layer of the cerebellum associated with neuronophagia of Purkinje cells was a consistent lesion seen in the NVND -virus-positive cormorants submitted to the NWHC. This cerebellar lesion could explain clinical signs in cormorants with only mild generalized encephalitis. Purkinje cell necrosis was not noted in previous reports of lesions in double-crested cormorants with NVND (4,26). This cerebellar lesion has been described in chickens experimentally infected with a neurotropic strain of NDV (23). The nonuniform distribution of lesions throughout the central nervous system emphasizes the importance of including brain stem, cerebellum, and spinal cord for histopathology because lesions were absent in sections of cerebrum from some cormorants.

The inflammation characteristic of NVND in the brain and spinal cord of double-crested
cormorants appears to be unique and specific. No correlation was found between NVND lesions and presence of salmonella. Salmonella typhimurium and Salmonella typhimurium-copenhagen have been isolated from the livers of 35 cormorants submitted to the NWHC over 20 years that had died of various causes (NWHC, unpubl. data). Isolation of salmonella from cormorants in this report is regarded as an incidental finding.

The combination of lymphoid foci in the heart, kidney, pancreas, and proventriculus and subcapsular hemorrhage in the spleen was found in NVND cormorants with encephalomyelitis. These lesions are not, however, pathognomonic for NVND. Although not reported in cormorants, increased lymphoid foci in the pancreas and lymphoid foci in the myocardium and muscle layer of the proventriculus in young chickens with encephalitis are diagnostically significant for avian encephalomyelitis, a picornavirus (6,19). Meningitis, when present, may help distinguish NVND from avian encephalomyelitis. Lymphoid foci were found in pancreas of normal as well as NVND virus-infected cormorants. Although the cellular infiltrate in the pancreas tended to be more diffuse and less circumscribed in the NVND cormorants, the inflammation could not be clearly distinguished from that seen in cormorants without NVND. Lymphoid foci have also been reported previously in pancreas from chickens infected with NDV (2) as well as in normal birds (6).

Virus isolation is necessary for confirmation of new sites of NVND virus infection. However, the rate of virus isolations dropped precipitously as the epornitic progressed. The drop in isolation rate occurred regardless of the tissue type cultured; however, when virus was isolated, either the intestine or brain or both were consistently positive. Although no viruses were isolated by the NWHC from cormorants collected after July 1992, microscopic evidence of viral infection was seen in cormorants submitted to the NWHC through August, at which time the nesting colonies dispersed and submissions ended. The low virus isolation rate late in the epornitic indicates a need for increased sampling efforts if confirmation by virus isolation is to be successful.

Although maternal antibody was detected at low titers in clinically normal, virus-negative hatchling cormorants in 1995, antibody titer was no longer detectable in this population at 14 days of age. This would imply that cormorants greater that 2 wk of age with antibody titer to NDV have or have had active NDV infection. All serology from 1992 double-crested cormorants with neurologic signs had detectible antibody to NDV, indicating that serology can also be used as a nonlethal way of determining NDV exposure in a cormorant population older than 13 days. However, because NDV was isolated from only 50% of the antibody-positive birds submitted with serum and tissue, the need still exists for an extensive virus isolation effort to confirm NVND infection.

Parasites identified in intestine and observed microscopically in liver were considered incidental because significant gross and microscopic lesions were not observed.

Velogenic Newcastle disease epornitics in the United States, historically associated with importation of exotic birds, have cost the poultry industry and state and federal programs millions of dollars (11,24,25). It is interesting that nucleotide sequence analysis of the 1992 NVND virus isolate is most closely related to NDV isolates of psittacine origin and is similar to the 1970 outbreak in California (20) described by Walker and Utterback in the references cited above.

The simultaneous occurrence of NVND-associated mortality in juvenile cormorants from geographically distant nesting colonies and the small foraging range reported for double-crested cormorants once they reach their nesting grounds (7) suggest exposure of the affected adult population at some point in the flyway prior to arrival in the nesting grounds. Double-crested cormorants winter in the southern United States and Mexico and nest in the northern United States and Canada. This international migration may have contributed to the difficulty in determining the source of the 1992 NVND epornitic. Monoclonal antibody binding patterns and nucleotide sequence analysis of cormorant NDV isolates from 1990 and 1992 were similar, which might also suggest the virus is maintained in the migrating cormorant population (14).

In conclusion, in the face of a confirmed NVND epornitic, identification of newly infected areas through histopathology and serol-
ology can provide rapid information needed to increase biosecurity to prevent potential spread of NVND to susceptible wildlife and poultry and to increase sampling intensity to improve opportunities for virus isolation and confirmation. Samples of choice include intestine and brain for virus isolation in embryonating hen's eggs and cerebellum, brain stem, spinal cord, and heart for histopathology.

REFERENCES


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