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## AN OUTBREAK OF DUCK VIRUS ENTERITIS (DUCK PLAGUE) IN ALBERTA

J. A. HANSON<sup>1</sup> and N. G. WILLIS<sup>2</sup>

**Abstract:** duck plague (Duck virus enteritis) was diagnosed in a resident population of Muscovy ducks (*Cairina moschata*) on a small game farm in Alberta. This disease has not been reported previously in Canada. Clinical signs consisted of cyanosis, depression and acute death. Necropsy of two Muscovy ducks revealed lesions typical of the disease. There were ulcerations with pseudomembranes in the small intestine, ulcerations with caseous plaques in the esophagus and eosinophilic intranuclear inclusion bodies in the spleen.

Clinical disease with mortality was reproduced in young ducklings injected with tissue homogenates from field cases. All surviving inoculated ducklings seroconverted to highly significant titres of neutralizing antibodies to duck virus enteritis (DVE) virus. All attempts to isolate the agent in embryonating duck eggs or primary tissue cultures of duck and chicken kidney were negative. Identification of the DVE virus was accomplished by serum neutralization with ducklings as the host system.

### INTRODUCTION

The name "Duck Plague" was first proposed at the 14th International Veterinary Congress in London in 1949 for an acute, highly fatal disease of ducks, caused by a virus occurring in the blood and all organs.<sup>4</sup> The virus is presumed to belong in the Herpesvirus group.<sup>1</sup> This disease has been studied at the Institute for Infectious Diseases, Utrecht, Netherlands since that time. Cases may have occurred in the Netherlands as early as 1923, but were misdiagnosed as fowl plague.<sup>4</sup> The disease has been confirmed in Belgium, Britain, Netherlands and India, and suspected in France and China. The first diagnosed case in the United States occurred in a flock of White Pekin ducks (*Anas platyrhynchos domesticus*) in the concentrated duck producing areas of Long Island, New York in 1967.<sup>3,8</sup>

Epornitic studies indicate the virus of DVE may have entered the United States

in imported exotic anseriformes and was transmitted from farm to farm by free-flying ducks with fomites playing an important secondary role. The disease may have been present but undetected in the United States before January, 1967.<sup>5,9,10</sup>

An epornitic of DVE was reported in an outdoor aviary in San Francisco, California in 1972.<sup>11</sup> Also, recently an outbreak of DVE occurred in wintering wild waterfowl at the Lake Andes National Refuge, North Dakota in January of 1973.<sup>11</sup>

This report describes the first known occurrence of DVE in Canada.

### HISTORY

On 17 May, 1974, the owner of a small game farm near Edmonton, Alberta reported an acute, highly fatal disease occurring in his small flock of Muscovy ducks (*Cairina moschata*). Illness had

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been evident in the flock for 4 days and five birds of a flock of seven had died. It was suggested that the bird which died on 17 May be submitted to the diagnostic laboratory. The previous mortality had been incinerated. The two remaining Muscovy ducks did not show clinical signs but were later released by the owner and taken to the diagnostic laboratory for postmortem examination.

#### **PATHOLOGIC EXAMINATION:**

Postmortem examinations were performed on the birds submitted to the diagnostic laboratory. The two live birds obtained on May 24th were bled and then euthanized by intravenous injection of barbiturate. Portions of liver, heart, spleen, esophagus and small intestine were placed in buffered 10% formalin, embedded in paraffin, sectioned at 7  $\mu$ m thickness and stained with hematoxylin and eosin. Portions of liver, spleen, heart, cloaca and intestine were collected from these birds by aseptic technique for bacteriologic and virologic examination.

Bacteriologic examination was conducted by contacting sterile glass spreaders to the cut surfaces of the liver, spleen, heart and intestinal mucosa. These were then streaked onto blood and MacConkey agar plates and incubated aerobically at 37 C. Virologic examination was undertaken in two separate laboratories at Edmonton, Alberta and Hull, Quebec. Initially, at the Edmonton laboratory, suspensions of an homogenate of liver, spleen and intestinal tissues from the original submission were emulsified in nutrient broth. After centrifugation at 10,000 rpm for 20 min, 0.2 ml of the supernatant was injected by triple inoculation into each of five embryonating chick eggs. Also, 0.5 ml of this supernate was injected into the peritoneal cavity of six one-day-old White Pekin ducklings.

Following the initial gross examination, portions of liver, spleen and intestine were frozen and sent to the Animal Diseases Research Institute "E", Hull, Quebec, for further virologic examination. The fro-

zen tissues were divided into two pools, the original submission (specimen A) and material from the euthanized ducks (specimen B). A 10% suspension of each was prepared in a nutrient broth containing antibiotics, and centrifuged at 1000 x g. The resulting supernatant fluid was inoculated into 10 nine-day-old embryonating chicken eggs by the stab technique,<sup>2</sup> 10 seven-day-old embryonating chicken eggs by the yolk sac route and 10 nine-day-old embryonating White Pekin duck eggs by the chorioallantoic membrane route. Three serial blind passages were conducted in each of these systems at seven day intervals. The same supernatant fluid was also inoculated onto primary chicken kidney tissue cultures with two subsequent blind passages of fluid and cells in this system. Finally, 3 ten-day-old White Pekin ducklings for each specimen pool were pre-bled and inoculated intraperitoneally with 0.5 ml of the supernate, and orally with 0.2 ml. These birds were observed clinically for 21 days. Subsequent to this they were again sampled for serum and challenged, along with controls, by an intraperitoneal injection of 0.5 ml of virulent DVE virus as a 10% duck liver suspension.

Alpha serum neutralization tests were conducted by using the chicken embryo attenuated DVE virus in embryonating chicken eggs and in a micro chicken kidney tissue culture system. Serum collected from the ducklings prior to the inoculation with the specimen, and at 21 days after inoculation, was heat-inactivated and diluted 1:4. The difference between the logarithm<sub>10</sub> titre of the virus in the presence of the pre-inoculation serum of a bird and in the presence of its post-inoculation serum, represents the neutralization index. Both the attenuated and the virulent DVE viruses were obtained from Dr. W. K. Butterfield of the Plum Island Animal Disease Laboratory, Greenport, New York, and have been maintained at the Animal Diseases Research Institute, Hull, Quebec by passage in embryonating chicken eggs and adult ducks, respectively, with storage of egg material and liver tissue at -70 C.

**GROSS PATHOLOGIC FINDINGS:**

All three Muscovy ducks submitted for necropsy appeared in good physical condition and had abundant deposits of subcutaneous fat. The most significant lesions in the dead bird were in the digestive tract and consisted of numerous punctate ulcers in the mucosa of the small intestine. These were rather evenly distributed throughout its length and consisted of necrotic plaques which, when removed, revealed a red, ulcerated lesion. Distinctive red annular bands, which appeared as areas of congestion or hemorrhage in the mucosa, were also noted in the small intestine. There was a pronounced diphtheritic cloacitis with similar changes evident in the salpinx and the rectum.

Blood vessels of the ovary of this bird were distended and many follicles appeared congested and hemorrhagic. The spleen appeared to be dark in color. There were no ocular, esophageal or proventricular lesions evident and the liver was grossly normal.

One of the live birds submitted had yellowish-white crusted plaques adherent to the mucosal surface of the upper one-third of the esophagus. When these were removed, distinctive hemorrhagic erosions were evident. The liver of this bird appeared to be somewhat swollen, whereas the spleen appeared shrunken and dark in color.

The lesions in the second live bird consisted of hyperemic annular bands within the small intestine. The rectum, near its border with the cloaca, was extremely hyperemic. Other lesions were not noted in this or the previous bird.

**HISTOLOGIC:**

There were no lesions evident in the esophagus of the bird which died from the disease. Esophageal lesions in one of the live birds submitted for examination consisted of punctate mucosal ulcerations. Hemorrhage and inflammatory cell infiltration were prominent.

Congestion and hemorrhage were prominent in the spleens of the three birds.

Necrosis of reticular cells with intranuclear inclusion bodies was evident in the spleen of the dead bird. Perivascular granulocytic infiltrations were noted in the spleens and livers of the three birds. An amorphous, hyaline-like material was present in the lymphoid follicles of the spleen of one bird.

Submucosal hemorrhage, with necrosis of overlying epithelium and formation of epithelial plaques, was noted in the intestine of the dead bird. The epithelial plaques consisted of eosinophilic necrotic cellular debris containing bacterial colonies. Many villi were degenerate and necrotic with sloughing of epithelium into the lumen of the intestine.

Perivascular granulocytic infiltrations, with focal areas of coagulation necrosis, were noted in the livers. Hepatocytes in these foci frequently were swollen with large cytoplasmic vacuoles and nuclear degeneration.

**MICROBIOLOGIC:**

Bacteriologic findings were not considered to be significant. Moderate growths of nonhemolytic coliforms were isolated from the liver and heart of the dead bird. Coliforms and alpha hemolytic streptococci were isolated from the intestines of the three birds.

At the Edmonton laboratory no mortality was encountered after five serial passages in embryonating chicken eggs or in ducklings. At post-mortem examination the only significant changes were focal areas of necrosis in the livers of three of the six ducklings. No significant bacterial pathogens were identified from these ducklings. However, blood samples drawn from the birds prior to euthanasia had neutralization indices to DVE virus of at least 3.0-3.5.

The initial isolation attempts at the Animal Diseases Research Institute with two tissue pools submitted by the Edmonton laboratory resulted in no abnormality in either embryonating eggs or tissue cultures during three serial passages of each specimen pool. Also, no signs of clinical

disease were shown by any one of the inoculated ducklings during a 22 day observation period. Upon challenge with virulent DVE virus, all the ducklings originally inoculated with tissue material from the dead bird completely resisted the virulent challenge while the control birds and the birds inoculated with tissue material from the live birds died in four days of acute duck virus enteritis. In addition, pre-challenge serum samples from the birds that resisted the challenge showed neutralization indices to DVE virus of at least 3.5. All pre-inoculation sera and the pre-challenge sera from birds that succumbed to the challenge were negative for neutralizing antibodies to DVE virus.

A second isolation attempt was made with the original material from the dead bird. In this attempt, 1.0 ml of supernatant fluid was inoculated intraperitoneally and intramuscularly into eight one-day-old White Pekin ducklings. The birds began dying five days after inoculation and by the ninth day all were dead. Gross pathological appearance was typical for DVE.<sup>7</sup> Material harvested from these birds was inoculated into nine-day-old embryonating duck eggs by the stab technique and by the dropped chorioallantoic membrane route, as well as onto primary chicken kidney and White Pekin duck kidney tissue cultures. Five serial tissue culture passages and three egg passages failed to demonstrate either a cytopathic effect or embryo mortality. However, material harvested from both the first and third duck egg passages was capable of inducing a fatal disease in inoculated White Pekin ducklings.

Liver tissue harvested from the inoculated ducklings was used in an alpha neutralization test conducted in ducklings. Birds were inoculated intraperitoneally with serial 10-fold dilutions of supernatant fluid mixed with equal quantities

of either negative serum or DVE antiserum. The DVE antiserum neutralized 100 duck lethal dose<sub>50</sub>'s relative to the negative serum of the lethal agent, thus identifying it as DVE virus.

#### DISCUSSION

Considerable difficulty was experienced in attempting to isolate the virus from specimen material even though a variety of systems and serial passages were made. The only system which revealed the presence of the virus was inoculated ducklings; however, mortality was not inevitably produced. In all inoculated ducklings that survived, the presence of the virus was indicated by a definite seroconversion. The virus was identified by neutralization of infectivity for ducklings. Embryonating duck eggs did not reveal the presence of this isolate by embryo mortality even though it was demonstrated in ducklings that the virus was being serially passaged. These observations may be a reflection of a reduced pathogenicity of this particular strain of the agent.

The source of introduction of the virus was not clearly established. No additions of waterfowl had been made to this game farm for at least six months prior to the outbreak of disease although wild birds had free access to the pond. Also, the mortality rate was not abnormally high during the preceding winter months. The possibility that DVE virus had been introduced to the captive flock by wild migrating waterfowl must be given serious consideration. This is especially true since mallard ducks banded and released from the Lake Andes region of South Dakota were recorded in Alberta in the spring of 1974. Also worthy of note is the observation that the retrieval of banded birds has located Lake Andes birds in the four Western Canadian Provinces and the North West Territories and Yukon (J. S. Tener, Personal Communications).

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