# SEROLOGIC EVIDENCE OF NEWCASTLE DISEASE VIRUS IN CANADA GEESE

S. F. Palmer and D. O. Trainer
Department of Veterinary Science
University of Wisconsin
Madison, Wisconsin 53706

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## SUMMARY

Newcastle disease virus (NDV) hemagglutination-inhibition (HI) titers of 1:20 or higher were detected in 31% of 3,010 sera from migratory and nonmigratory Canada geese (*Branta canadensis*). HI titers ranged to 1:1280, and virus neutralization (VN) titers ranged to $10^{6.3+}$. Reactor-prevalence was 1% among flightless juveniles and higher in older geese. Prevalence of NDV reactors did not differ consistently between migratory and nonmigratory Canada geese or between sexes.

The correlation of NDV HI and VN titers and the predominant resistance of the Canada goose HI substance to heat-trypsin-periodate treatment suggested that a reasonable indication of previous exposure of Canada geese to NDV is an HI titer of 1:20.

A probable isolation of NDV was made from the spleen of a wild Canada goose.

## INTRODUCTION

The Canada goose *(Branta canadensis)* is an important natural resource valued by hunters, ornithologists, and naturalists. By manipulation of hunting pressure and enlargement and improvement of waterfowl refuges, management programs have increased the size and altered the behavior of many migratory Canada goose populations. In addition, a growing number of small migratory and nonmigratory local flocks have been established across the United States.

Because knowledge is limited on infectious diseases in Canada geese, Bradshaw and Trainer (4) began a serologic study to deter-

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Table 1. Waterfowl refuges where Canada goose blood samples were collected for NDV serologic study (1965–1967).

| Refuge | Flock status | Vicinity | Date of collection |
|---|---|---|---|
| Crex Meadows | C | Grantsburg, Wis. | 7-65, 7-66 |
| Bay Beach Park | B | Green Bay, Wis. | 7-65, 7-66 |
| Brown County | B | Green Bay, Wis. | 7-66 |
| Horicon Marsh | A | Horicon, Wis. | 4-65, 11-65, 4-66 |
| Horseshoe Lake | A | Miller City, Ill. | 1-65, 1-67 |
| Kellogg | B | Battle Creek, Mich. | 7-66 |
| Necedah | A | Necedah, Wis. | 10-65 |
| Oak Orchard | A-B | Batavia, N.Y. | 7-67 |
| Sand Lake | A | Columbia, S. Dak. | 10-66 |
| Seney | A | Seney, Mich. | 7-66 |
| Squaw Creek | A | Mound City, Mo. | 1-66, 11-66 |
| Swan Lake | A | Sumner, Mo. | 1-66, 11-66 |
| Trimble | B | Trimble, Mo. | 6-66 |
| Upper Canada | A | Morrison Is., Ont. | 8-65 |
| Wilson Hill | A-B | Massena, N.Y. | 7-67 |

[A] = migratory.
[B] = free flying, essentially nonmigratory.
[C] = captive.

mine some of the infectious diseases of the changing Canada goose populations. The detection of Newcastle disease virus (NDV) hemagglutination-inhibition (HI) and virus neutralization (VN) substances in Canada goose sera collected in Wisconsin and Illinois stimulated investigation of the NDV specificity of the serologic reactors and the geographical distribution of the NDV-inhibiting substance among Canada geese.

## MATERIALS AND METHODS

**Collection of sera.** Between January 1965 and July 1967, 3,010 Canada goose sera were obtained in 23 collections on 16 national, state, and municipal waterfowl refuges. Table 1 lists refuges where blood samples were collected, giving the status of each flock (i.e. migratory, nonmigratory, captive). Geese were captured in baited swim-in traps, cannon-net traps, or during the molt, in corrals. Geese were sexed and assigned to age groups: "juvenile" (less than 1 year old), "yearling" (1 to 2 years old) or "adult" (2 years and older). Occasionally all geese more than 1 year old were grouped as "adults."

Blood samples were collected by venipuncture. Sera were heat-treated in a water-bath for 30 min at 56 C and stored at –20 C until tested.

**Serologic procedures.** The GB strain of NDV was used as antigen in HI and VN tests. Sera were tested with the standard NDV Beta-HI procedure (13) in white plastic serologic plates. Canada goose erythrocytes were satisfactorily agglutinated by NDV and were substituted for chicken erythrocytes in the HI procedure to

avoid nonspecific agglutination of chicken erythrocytes by some goose sera. HI end-points of 1:20 or higher were considered positive.

To substantiate the presence of NDV antibody in the goose sera and to determine the most feasible HI titer to be considered a valid indication of prior NDV exposure, 90 sera were titrated with both the HI test and the standard Alpha virus-neutralization

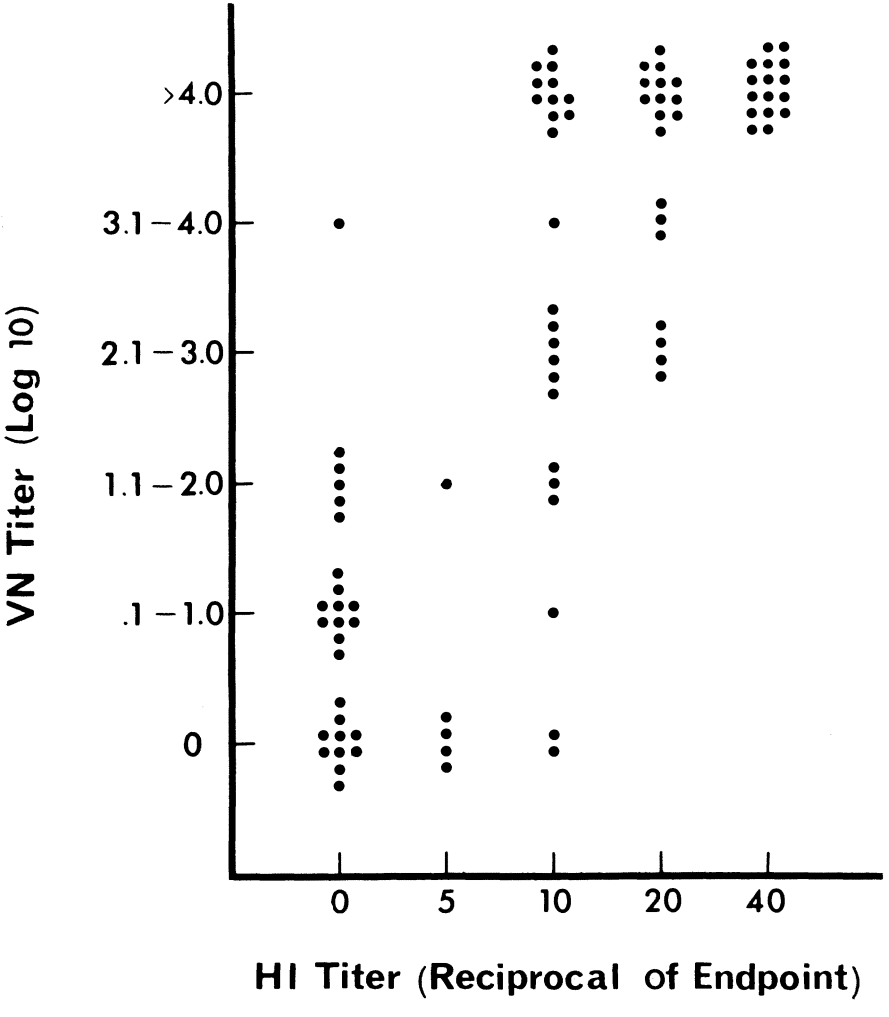


Fig. 1. A comparison of virus-neutralization and hemagglutination-inhibition antibody titers of 90 sera collected from wild and captive Canada geese.

test (13). Sera were diluted 1:2 for use in the VN test, which was conducted in embryonated chicken eggs. VN titers, corrected for serum dilution, were recorded as $\log_{10}$ of NDV neutralized.

To determine the specificity of the HI substance, 63 sera with HI titers of 1:40 or higher and 19 sera from geese experimentally exposed to NDV were further treated with trypsin, periodate, and heat to remove nonspecific inhibitors (NSI) (7). In the HI titration, treated and untreated portions of each serum were tested in twofold dilutions beginning with 1:15.

**NDV isolation.** To confirm the presence of NDV in Canada geese, we attempted to isolate NDV from geese shot at Horicon Marsh during the 1966 hunting season and geese which apparently succumbed to lead poisoning at Fox Lake, Wisconsin (December 1966). Fifty-nine spleens, 39 brains, bone marrow from 26 femurs, and 10 livers were cultured for NDV.

Tissues were ground in TenBroek grinders, and approximately 10% suspensions of each tissue were prepared in tryptone broth containing 1000 units penicillin and 1000 $\mu$g streptomycin per ml. One-tenth ml of each preparation was inoculated intra-allantoically into each of ten 9-to-10-day-old embryonated chicken eggs. Eggs were incubated 5 days at 37.5 C and candled twice daily. Allantoic fluids from every egg which died after 24 hours postinoculation and every surviving egg were harvested after cooling 24 hours at 4 C. Allantoic fluids of eggs inoculated with each individual preparation were pooled, two eggs per pool. Each pool was tested for hemagglutination (HA) with a micro-adaptation of the HA test (13,14). HA-positive allantoic fluids were tested by neutralization in embryonated eggs and by HI with known NDV-immune sera to ascertain that an isolate was NDV. Any suspect isolate was titrated in embryonated eggs to determine the approximate killing time and titer of virus produced.

## RESULTS

To determine whether the NDV HI substance detected in Canada goose sera was antibody, we titrated the NDV-neutralizing activity of selected sera and treated other Canada goose sera with a procedure designed to remove NSI of myxoviruses.

Ninety goose sera with HI titers of 1:40 or less were titrated for VN activity in embryonated chicken eggs, and the NDV VN and HI titers of each serum were compared (Fig. 1). Sera with HI titers of 1:10 to 1:40 generally contained at least 100-to-1000-fold more NDV neutralizing substance per ml than did sera with

Table 2. Newcastle disease virus hemagglutination-inhibition study of Canada goose sera, summarized by age and area of collection.

| Area of collection | Collection date | HI test results[A] | | | |
|---|---|---|---|---|---|
| | | Juvenile | Yearling[B] | Adult | Total[C] |
| Summer | | | | | |
| Trimble | 6-66 | 0/84 | 3/46(07) | 22/47(47) | 25/177(14) |
| Green Bay | 7-65 | 0/32 | . | 8/27(30) | 9/59(15) |
| Green Bay | 7-66 | 0/115 | . | 7/15(47) | 7/130(05) |
| Crex Meadows | 7-65 | 0/07 | . | 9/25(36) | 11/44(25) |
| Crex Meadows | 7-66 | 1/42(02) | 1/3(33) | 4/19(21) | 6/64(09) |
| Seney | 7-66 | . | 13/32(41) | 72/116(62) | 92/158(58) |
| Kellogg | 7-66 | 1/47(02) | 9/50(18) | 43/74(58) | 53/171(31) |
| Wilson Hill | 7-67 | 6/179(03) | 6/21(63) | 17/27(63) | 35/227(16) |
| Oak Orchard | 7-67 | 0/10(00) | 3/6(50) | 26/37(70) | 29/53(55) |
| Upper Canada | 8-65 | . | . | . | 5/63(08) |
| Fall migration | | | | | |
| Necedah | 10-65 | 19/52(37) | . | 30/51(59) | 50/106(47) |
| Sand Lake | 10-66 | 1/23(04) | . | 1/9(11) | 2/32(06) |
| Horicon Marsh | 11-65 | 9/71(13) | . | 109/262(42) | 119/336(35) |
| Swan Lake | 11-66 | 2/11(18) | 17/66(26) | 81/205(40) | 100/282(35) |
| Squaw Creek | 11-66 | 2/10(20) | 6/13(46) | 21/37(57) | 30/61(48) |
| Winter | | | | | |
| Poynette | 12-65 | . | . | 7/72(10) | 7/72(10) |
| Horseshoe Lake | 1-65 | . | . | . | 36/80(45) |
| Horseshoe Lake | 1-67 | 101/196(51) | . | 85/137(62) | 186/333(56) |
| Swan Lake | 1-66 | 46/234(20) | . | 58/190(31) | 104/424(25) |
| Squaw Creek | 1-66 | 1/10(10) | . | 1/8(12) | 2/18(11) |
| Spring migration | | | | | |
| Horicon Marsh | 4-65 | 4/19(21) | . | 7/14(50) | 11/33(33) |
| Horicon Marsh | 4-65 | 7/28(25) | . | 19/59(32) | 26/87(30) |
| Total | | 200/1170(17) | 64/237(27) | 627/1431(44) | 944/3010(31) |

[A]HI end-point dilution of 1:20 considered to be positive; number of positive sera/number of sera tested (reactor prevalence in percent).

[B]Yearling geese sometimes included with adults.

[C]Includes geese which were not aged.

Table 3. Distribution of NDV HI titers in sera of juvenile, yearling, and adult Canada geese.

| HI Titer[A] | Age Classes | | |
|---|---|---|---|
| | Juvenile (1170 total) | Yearling (237 total) | Adult[B] (1431 total) |
| 0 | 60[C] | 42 | 20 |
| 5 | 14 | 15 | 18 |
| 10 | 09 | 17 | 18 |
| 20 | 07 | 14 | 19 |
| 40 | 04 | 06 | 12 |
| 80 | 03 | 05 | 08 |
| 160 | 02 | 01 | 03 |
| 320 | 01 | 01 | 01 |
| >320 | 01 | 00 | 02 |

[A]HI titer expressed as the reciprocal of the end-point dilution.
[B]Adult age class contains some yearling geese.
[C]Percentage of each age class with each HI titer.

HI titers of 0 or 1:5. There was an abruptly increasing gradient of neutralizing substance between sera with HI titers of 1:5, which predominantly had low VN titers, and sera with HI titers of 1:40, which all had VN titers of greater than $10^4$. VN titers ranged as high as $10^{6.3+}$.

HI titers of 14 of the 63 NDV-positive Canada goose sera treated to remove NSI were unchanged, titers of 39 were reduced but still diagnostic, and titers of 10 were reduced below diagnostic levels. Thus, 84% of the treated sera retained diagnostic HI titers and 16% did not. All 19 sera from known-NDV-exposed Canada geese retained diagnostic HI titers.

Sera collected from 3,010 Canada geese in the central and eastern United States were tested for NDV HI antibody: 31% had HI titers of 1:20 or higher and were considered to indicate prior exposure to NDV (Table 2). Reactor prevalence was, in general, higher among adults (44%) than among yearlings (27%) and higher among yearlings than among juveniles (17%) (Table 2). While only 1% of juveniles were positive during the summer, reactor prevalence among juveniles was substantially higher during the fall, winter, and spring. There were no consistent differences in reactor prevalence between migratory and nonmigratory geese which were not attributable to the age composition of the flocks, and no marked differences between sexes.

Except for a scarcity of high HI titers in flightless juveniles, the range of HI titers was similar in the three age groups (Table 3). HI titers were detected as high as 1:1280.

NDV was isolated from one of 24 spleens collected from Canada geese involved in a lead-poisoning epizootic at Fox Lake, Wisconsin, in December 1966. During the first passage the virus

produced HA at 1:1280 to 1:2560 dilutions in 2 of 10 eggs, but did not kill the embryos. A second isolation attempt from the original spleen suspension produced the same results. The second passage of the isolate in embryonated eggs caused mortality by 120 hours, but not by 96 hours. A death time of this length is characteristic of lentogenic strains of NDV (13). The second-passage allantoic fluid contained 8.8 $CELD_{50}$ per ml. The virus titer was reduced from 8.8 to 3.8 $CELD_{50}$ per ml if incubated with equal volumes of NDV antiserum. Immune serum reduced the HA titer of the isolate 40-fold.

## DISCUSSION

There are several indications that the HI substance detected in Canada geese is predominantly NDV-specific antibody:

A) Nonspecific NDV hemagglutination, in amounts which would interfere with routine evaluation, have not been reported in avian sera (2). NDV HI titers of 1:160 and higher and VN titers of over $10^{6.3}$ detected in Canada geese greatly exceed levels of NDV inhibition previously reported to be nonspecific in poultry (8) or suggested to be nonspecific in Canada geese (11).

B) Domestic geese are susceptible to natural and experimental NDV infection (2,10), and Canada geese produce VN and HI antibody in response to experimental inoculation (3,11,12).

C) The heat-stable NSI of myxoviruses which are active in whole sera are sensitive to trypsin-periodate treatment (1,9). The HI substance detected in Canada geese appears to be predominantly antibody since it was resistant to treatment at 56 C for 30 min, and in 84% of 63 sera was resistant to additional heat-trypsin-periodate treatment, which would normally destroy mucoprotein NSI.

D) Heat-stable NSI (except for the $\alpha$-inhibitor of certain influenza A2 strains) has not been reported to inhibit both the infectivity and hemagglutination of myxoviruses (1,9) as does the substance detected in Canada geese.

E) The prevalence of HI in Canada goose sera increases with the age of the geese tested, as would be observed if the populations were periodically exposed to NDV. The presence of nonspecific virus inhibitors may depend on the animal species, the individual animal, age, or the physiological state of the individual (1). The presence of the NDV HI substance in Canada geese appears to be correlated with increasing age but not with sex, stage of sexual activity, or migration. Since most Canada geese do not become

sexually mature until their third year, the apparent increase in NDV reactor prevalence in juveniles between July and October is most logically attributable to NDV exposure. The summer and fall serum collections, however, represent different populations of geese and may not be comparable.

The isolation of a lentogenic strain of NDV from a Canada goose requires further substantiation. Although inoculum was prepared on disinfected benches in microbiological transfer rooms equipped with ultraviolet lamps, and although no spurious isolations of NDV were evident in our previous or concurrent studies, we must reserve total acceptance of this isolate because extensive NDV research in the same facility makes contamination a possibility. The isolate should be examined with the series of procedures designed by Hanson *et al.* (6) to determine whether it is identical with any other lentogenic strains in use in the laboratory. Further isolation attempts should rigorously follow criteria for valid virus isolation (5).

Although NDV antibody appears to be geographically widespread in Canada geese, the manner in which the geese are exposed to the virus and the significance of NDV to goose populations are unknown. Although geese feed in grain fields around refuges and could occasionally encounter contaminated poultry offal, there is no evidence to suggest a continuing relationship between the epizootiology of Newcastle disease in domestic fowl and in Canada goose populations. Geese for this study were obtained from the northern-breeding migratory populations only after the geese had migrated southward, and no group of geese was observed throughout a year (Table 1). Observation of a single goose population during at least one entire year may elucidate the epizootiology of NDV in Canada geese.

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