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NEWCASTLE DISEASE VIRUS IN WATERFOWL IN WISCONSIN

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Abstract: Newcastle disease virus was isolated from the cloaca of 1-5% of live-trapped waterfowl in Wisconsin in the fall from 1978-1980. Antibody to NDV was detected in 8% of the birds tested, with no apparent difference between sex and age classes. Experimental infection resulted in persistence of virus shedding for months after exposure. Lack of detectable antibody in some of the experimentally infected birds suggests that reported antibody prevalence may not be indicative of the true prevalence of the infection. Isolation of NDV for the last 9 years as well as the detection of antibody in waterfowl over 25 years ago, suggests a well-adapted host-parasite relationship. Experimental evidence of virus persistence in individual mallards *(Anas platyrhynchos)* provides a mechanism for maintenance of the virus in the wild population.

INTRODUCTION

Thirty years ago, the first evidence of Newcastle disease virus (NDV) in waterfowl came from a serologic survey in California of wild ducks for poultry diseases (Quartrup et al., 1957). Antibody was found in the northern pintail *(Anas acuta)* and American wigeon *(Anas americana)*. In the 1960's antibody was also found in mallards *(Anas platyrhynchos)* and Canada geese *(Branta canadensis)* in the Midwest (Bradshaw and Trainer, 1966; Palmer and Trainer, 1970). In the early 1970's, an extensive survey of wild birds was conducted in conjunction with the outbreak of exotic NDV in California, and virus was isolated from several species of ducks (Pearson and McCann, 1974), although not the virulent pathotype found in chickens. Since then NDV has been isolated from hunter-killed and live-trapped waterfowl in the Atlantic and Mississippi flyways and in Canada (Slemons and Easterday, 1975; Webster et al., 1976; Bahl et al., 1977; Hinshaw et al., 1980). In North America, evidence of infection based either on serology or isolation of virus has been found in ten species of ducks including mallard (Bradshaw and Trainer, 1966; Slemons and Easterday, 1975; Bahl et al., 1977; Kocan et al., 1979; Hinshaw et al., 1980), American wigeon (Quartrup et al., 1957; Kocan et al., 1979; Hinshaw et al., 1980), northern pintail (Quartrup et al., 1957; Pearson and McCann, 1975; Hinshaw et al., 1980), wood duck *(Aix sponsa)* (Slemons and Easterday, 1975; Webster et al., 1976), green-winged teal *(Anas crecca)* (Rosenberger et al., 1974; Pearson and McCann, 1975), blue-winged teal *(Anas discors)*, gadwall *(Anas strepera)* (Hinshaw et al., 1980), cinnamon teal *(Anas cyanoptera)*, lesser scaup *(Aythya affinis)* (Pearson and McCann, 1975), American black duck *(Anas rubripes)* (B.C. Easterday, pers. comm.), in addition to the Canada goose (Bradshaw and Trainer, 1966; Palmer and Trainer, 1970; Rosenberger et al., 1974; Slemons and Easterday, 1975) and the American coot *(Fulica americana)* (Slemons and Easterday, 1975).

Serologic evidence of NDV infection in waterfowl has been found in Australia (Hore et al., 1973), and India (Chandra et al., 1973) while virus has been isolated in England (Alexander et al., 1979), France (Hannoun, 1977), Japan (Kida and Yanagawa, 1979) and Iran (Bozorgmehri-Fard and Keyvanfor, 1979). Virus isolation

has also been made from colony nesting migratory seabirds in U.S.S.R. (L'vov et al., 1975; Sazonov et al., 1975) and Alaska (B.C. Easterday, pers. comm.), suggesting that in migratory birds the virus may not be limited to Anatidae and Rallidae.

Wild waterfowl appear to be relatively resistant to NDV strains that are highly virulent for chickens, based on experimental infections in which only NDV isolates obtained from chickens have been used; relatively large doses were administered intravenously in order to produce disease or mortality in the mallard (Friend and Trainer, 1972) and Canada goose (Spalatin and Hanson, 1975). The inoculated birds shed little or no virus. In a severe outbreak of Newcastle disease in a zoologic park, ducks and geese were relatively unaffected with 10% or less morbidity and mortality (Estudillo, 1972). However, outbreaks of Newcastle disease have occurred in domestic ducks in Hong Kong with mortality as high as 95% (Higgins, 1971).

Most of the sampling and isolation of virus from waterfowl has been done in the fall of the year when transmission of the virus may be facilitated by the concentration of birds that occurs before and during migration. Virus can be readily isolated from the cloaca suggesting an intestinal infection and possible fecal-oral route of transmission in the aquatic environment of the birds.

The mechanism by which the virus is maintained in the population is not known. Isolation of virus from the intestinal contents of ducks 6 months after exposure (Winmill and Haig, 1961) suggests that a persistent infection may be involved or that reinfection occurs.

This study was undertaken to examine the host-parasite relationship involving Newcastle disease virus and selected species of waterfowl. The study included a survey of wild waterfowl to determine the prevalence of NDV using virus isolation and serology and a study of experimental infection of mallards using isolates from wild mallards.

MATERIALS AND METHODS

Collection and Testing of Samples. Access to live waterfowl being banded was provided through the cooperation of the Wisconsin Department of Natural Resources and the U.S. Fish and Wildlife Service. Birds were banded and classified according to species, sex and age by the respective personnel on various city, state and federal refuges in Wisconsin. Bay Beach is a city park in Green Bay; Collins Marsh, Eldorado Marsh and Grand River Marsh are State Department of Natural Resources wildlife refugees; and Necedah and Horicon are national wildlife refugees. All are located in central or eastern Wisconsin. Age classes were juveniles or young of the year and adults. Canada geese, mallards, wood ducks, and coots were sampled. Except for the resident population of Canada geese at Bay Beach, tested in June, most of the samples were obtained in late August and September. Tracheal and cloacal swabs were taken from the Canada geese in Bay Beach in 1978. Thereafter, only cloaca swabs were taken. Blood samples were taken from the brachial vein of half to all the birds swabbed.

Swabs were placed in 2 ml of transport medium consisting of phosphate buffered saline (PBS) with 50% glycerin (Webster et al., 1976) or 0.5% bovine serum albumin (S. McGregor, pers. comm.), 10,000 units penicillin, 10,000 μg streptomycin and 300 μg tylocine per ml, and maintained on wet ice. Upon arrival in the laboratory within several hours after collection, swabs were frozen at -70 C until tested. From 0.2 to 0.3 ml of transport medium was inoculated intraallantoically into 9 to 10-day-old embryonated chicken eggs. Eggs were incubated at 37 C and candled daily to remove dead eggs. After 72 h of incubation, eggs were chilled, opened and the

allantoic fluids harvested were tested in standard hemagglutination (HA) test. Allantoic fluid from eggs showing hemagglutination was stored at -70 C until tested.

Virus identification. HA titrations and HA inhibition (HI) tests were performed as described by Cottral (1978). Each HA agent was diluted to contain approximately 8 HA units per 50 μl. Twenty-five μl of virus suspension and 25 μl of NDV antiserum were allowed to react at room temperature for 30 min, then tested for HI by the addition of 50 μl of 0.5% chicken red blood cells (rbc's). Similarly, HA agents not inhibited by NDV antiserum were tested against antiserum to known avian paramyxoviruses and influenza A viruses.

Serum Testing. Blood was allowed to clot, the serum decanted and frozen at -20 C. Serum was heat-treated at 56 C for 30 min and tested using micro HI test (Cottral, 1978). Serial two-fold dilutions of sera were reacted with 4 HA units of NDV-LaSota for 30 min at room temperature. Chicken rbc's were added and the test was read after an additional 30-45 min. Titers were expressed as the reciprocal of the dilution of sera at which there was complete inhibition of HA. Only titers 1:10 or greater were considered positive.

Experimental Infections

Animals. Day old mallards were obtained from a commercial supplier and raised at the Charmany Experimental Farm. Some of the birds were hatched in the Department of Veterinary Science from eggs laid by ducks maintained on Charmany Experimental Farm.

Viruses. NDV isolates used to experimentally infect mallards had been recovered from wild mallards. Stocks were maintained at -70 C prior to use. The infectivity was determined by the method of Reed and Muench (1938). Virus isolations from swabs and serologic techniques were as described above. Geometric mean titers were determined by adding the value of one to all reciprocal titers and transforming these adjusted values to logarithms (base 10) according to the method described by Friend and Trainer (1972).

Organ cultures. Sections of organs used for explants were removed, washed at least three times in PBS, minced and placed in 60 mm petri dishes or flasks. Medium consisted of 5 ml of Medium 199 with 20% fetal calf serum, 2× MEM vitamins and amino acids, 1000 units penicillin, and 1000 μg streptomycin per ml (Lennette and Schmidt, 1979). Cultures were incubated at 37 C in a CO_2 incubator. One half the medium was removed from each culture and replaced after 10 days, and changed every 3-4 days thereafter. The medium removed was frozen at -20 C until inoculated into embryonated eggs for virus isolation. Explant cultures were maintained for at least 6 weeks unless contamination intervened.

Experiment 1. Two male and three female adult mallards were exposed to NDV $2 \times 10^{3.5}$ EID_{50} either orally or cloacally. Tracheal, esophageal and cloacal swabs were taken every 2 days for 2 weeks and twice a week thereafter. Birds were bled once a week for 5 wk and then every 3 wk for 13 wk. Birds were maintained uncaged in an isolation room on a natural light cycle.

Experiment 2. In this experiment mallards were divided equally between caged and uncaged groups, half male and half female. Eight birds in each group were exposed orally or cloacally to 2×10^8 EID_{50} NDV. Tracheal, esophageal and cloacal swabs and blood samples were taken periodically for 19 wk post exposure (PE). Some of the birds with detectable antibody titers were killed and explant cultures made from tissues of trachea, cecum, testis, ovary and oviduct.

Experiment 3. Seven to 10 day-old mallard ducklings were exposed orally to 10^6 - 10^7 EID_{50} of NDV. Five birds were killed on days 1 through 7, and three birds on day 8. Tracheal, esophageal and

cloacal swabs were obtained as well as samples of blood, lung, liver, spleen, kidney and intestine. Tissues were ground in glass tissue grinders (Ten-Broeck), centrifuged and the supernatant was inoculated into embryonated chicken eggs for virus isolation. Serum samples were tested for the presence of maternal antibody.

RESULTS

Survey of waterfowl. The identification of hemagglutinating viruses isolated from birds swabbed from 1978 to 1980 is recorded in Table 1. Hemagglutinating viruses were isolated from less than 5% of the Canada geese, but up to 55% of the ducks. The virus isolation ratio from adult and juvenile mallards was 1:4. The difference was statistically significant using the chi square ($P<.001$). Twenty-eight isolates of Newcastle disease virus were made. Seventy isolates were identified as avian paramyxoviruses other than Newcastle disease virus, 174 were influenza A virus isolates, and three remain to be identified.

NDV antibodies at a level of 1:5 was found in only two of the Canada goose sera collected in 1980. However, an-

TABLE 1. Hemagglutinating viruses isolated from migratory waterfowl in Wisconsin in **1978-1980.**

Location	Number sampled	Number isolations	Newcastle	Influenza	Para-myxovirus
		1978			
Bay Beach, Green Bay (Canada geese)	219	3 (1%)	1	1	1
Grand River Marsh (mallards)	71	24 (34%)	0	7	17
Eldorado Marsh (mallards)	84	15 (18%)	2	3	11
Collins Marsh (mallards)	147	28 (19%)	7	15	6
Necedah NWR (mallards)	38	11 (29%)	1	5	4
(wood ducks)	50	0	0	0	0
Horicon Marsh NWR (coots)	158	4 (3%)	4	0	0
		1979			
Grand River Marsh (mallards)	100	55 (55%)	2	47	6
Collins Marsh (mallards)	295	76 (26%)	6	55	14
Horicon Marsh (Canada geese)	218	10 (5%)	1	1	8
		1980			
Bay Beach (Canada geese)	138	0	0	0	0
Collins Marsh (mallards)	94	50 (53%)	4	40	4

tibodies were found in 5-16% of the mallards sampled (Table 2). No difference in antibody prevalence was detected between immature (9%) and mature (9%) or between male (8%) and female (10%) mallards. Titers were relatively low, extending up to 1:40.

Experiment 1. Virus was isolated from experimental mallards on days 2 and 4 PE. No further isolations were made until day 14. Thereafter, virus continued to be isolated up to 10 wk (Fig. 1) from tracheal, esophageal and cloacal swabs. Three wk PE, one week after virus shedding resumed, hen mallards started egg-laying and continued through the period of virus isolation. Three orally exposed birds continued to shed virus in the presence of circulating antibody and two cloacally exposed birds continued to shed virus without developing antibody. Since this experiment involved few birds, all free in a single room, the experiment was repeated.

Experiment 2. Virus was isolated on days 2, 4 and 6 PE (Fig. 2). No further isolations were made from any of the birds with the exception of one caged female mallard 14 wk PE, one caged male 15 wk PE and one non-caged female 16 wk after exposure. Isolations were made after the onset of egg-laying at 11 wk PE. Six out of eight birds in each group produced detectable antibodies, which reached a peak at day 15 PE and decreased thereafter. After laying started,

TABLE 2. NDV hemagglutinating inhibiting antibody in free-flying waterfowl.

	Number tested	Number with antibody	% Prevalence
1978			
Bay Beach (Canada geese)	20	0	0
Grand River Marsh (mallards)	38	0	0
Eldorado Marsh (mallards)	85	4	5
Collins Marsh (mallards)	147	11	8
Necedah NWR (mallards)	86	4	5
1979			
Grand River Marsh (mallards)	98	5	5
Collins Marsh (mallards)	162	20	12
1980			
Bay Beach (Canada geese)	137	0	0
Collins Marsh (mallards)	89	14	16

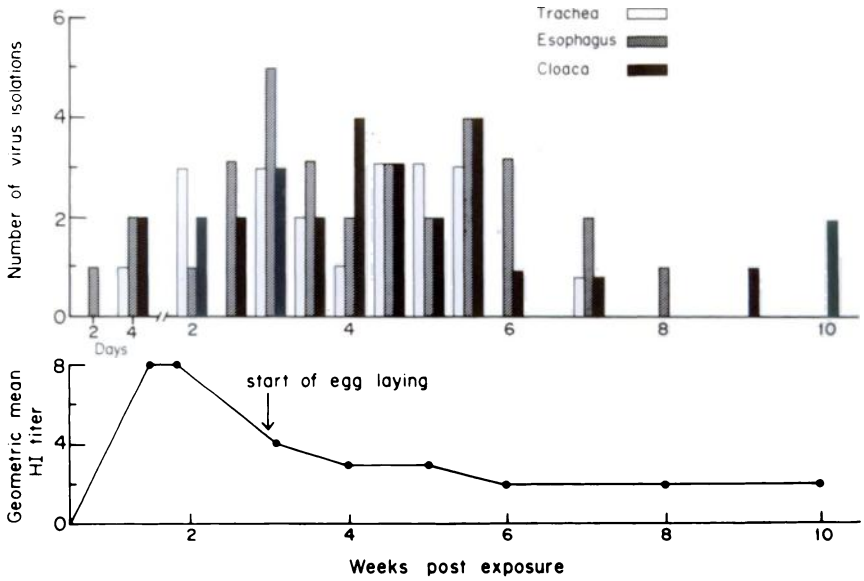

FIGURE 1. Experimental NDV infection in five mallards.

antibody titers in two females in the uncaged group increased 3-4 fold. No virus was isolated from the organ cultures taken at termination 20 wk PE.

Experiment 3. Virus was isolated from tracheal, esophageal and cloacal swabs and the intestine of mallard ducklings and occasionally from lung, liver, spleen, or kidney (Table 3). There were no overt signs of infection or mortality. Antibody that did not protect against infection was detected in one bird prior to exposure.

DISCUSSION

In the fall of the year in Wisconsin paramyxoviruses and myxoviruses were isolated from more than 50 percent of the waterfowl, particularly ducks, that were swabbed. Similar findings have been obtained in the fall by Hinshaw and Webster in the prairie province of Alberta, Canada (Hinshaw et al., 1980).

More virus isolations in this study were made from juvenile birds. This higher frequency is consistent with what would be expected among susceptible young recruits in an infected population.

Thirty-four of the isolates made during the period of 1978-1980, were Newcastle disease virus. From 1-5% of the mallards were infected. This isolation rate for NDV is similar to that found by others (Slemons and Easterday, 1975; Hinshaw et al., 1980).

Only Newcastle disease virus was isolated from the American coot in this study. The virus has been isolated also from American coots by National Wildlife Health Laboratory personnel (D.E. Docherty, pers. comm.) and Slemons and Easterday (1975) in the U.S. and evidence of the virus in common coots *(Fulica atra)* was shown serologically by Chandra et al. (1973) in India.

Although Canada geese from Bay Beach were a resident population with only a few migrants, a low prevalence of virus and antibody was detected. This is in contrast to the NDV antibody prevalence of 5-15% found during the

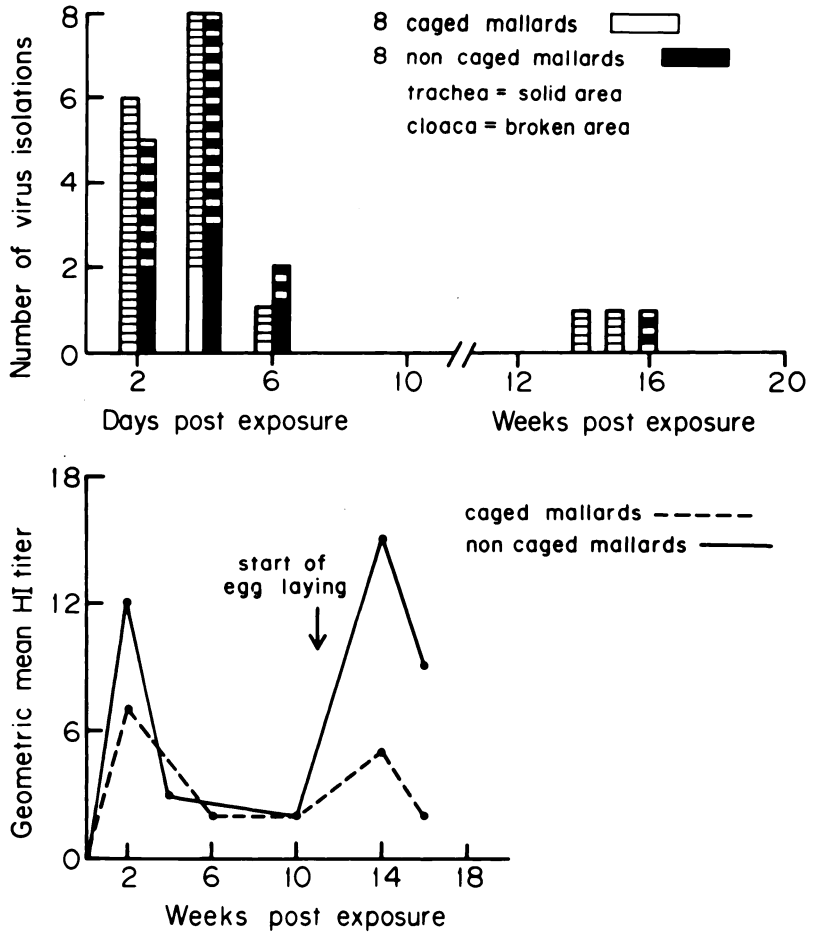

FIGURE 2. Experimental NDV infection in mallards.

TABLE 3. NDV isolations from swabs and tissues of mallard ducklings.[a]

	Days post exposure							
	1	2	3	4	5	6	7	8
Tracheal swab	2	5	5	5	3	-	3	2
Esophageal swab	2	4	4	5	2	1	3	-
Lung	-	-	1	-	-	-	1	-
Liver	-	-	-	1	1	1	1	-
Spleen	-	-	-	-	1	-	-	-
Kidney	-	-	1	-	-	-	-	-
Intestine	1	1	4	4	3	3	1	3
Cloacal swab	-	-	4	4	3	4	2	-

[a]Sample size was five for days 1 to 7, three for day 8 post exposure.

1960s when the Bay Beach population was tested previously (Palmer and Trainer, 1970).

Newcastle antibody prevalence in mallards ranged from 5-15%. The prevalence of antibodies did not appear to vary much over the years and is similar to that found previously (Bradshaw and Trainer, 1976; Kocan et al., 1979). However, interpretation is made difficult by the experimental observation that it is possible for a bird to become infected and shed virus without producing a detectable antibody response. If this occurs in the wild population, the reported antibody prevalence is indicative of the occurrence of the infection, but not the true prevalence of the infection.

Experimental infection of the mallard using NDV isolated from the wild birds was undertaken to determine the duration of the infection and investigate the possibility of a persistent infection, a mechanism of virus perpetuation that could maintain the infection in the wild population. Persistent infection or reinfection with viral shedding detectable for more than two mo was found in the first experiment. Since virus shedding resumed about a week before egg-laying commenced and continued through the egg-laying period, the involvement of the breeding cycle in triggering virus shedding was considered. Although there were only a few isolations made in the second experiment, these were again made after the onset of egg-laying in the hens. The exact mechanism involving the breeding cycle was not determined.

Virus isolations from swabs and tissues of mallard ducklings suggest an infection at least in the young that is not limited to the respiratory or digestive tracts. While there was no overt morbidity or mortality, more subtle measurements of disabilities were not made and the possibility that this infection may affect the survival of the young in wild populations merits study.

The report of antibody in waterfowl more than 25 years ago and isolation of the virus frequently during the last nine years, suggests that a stable coexistence has evolved. The virus appears widespread, having been isolated worldwide (Hannoun, 1977; Alexander et al., 1979; Kida and Yanagawa, 1979) from many different species of waterfowl and from apparently healthy birds (Pearson and McCann, 1975; Slemons and Easterday, 1975; Webster et al., 1976; Hinshaw et al., 1980).

Matumoto (1969) has described a number of parameters important in determining virus perpetuation in a population, related both to the population and to the individual host. The population parameters include: the size of the population, the population turnover, density of the population and the immunity level. The parasitic parameters include: transmissibility, duration of infection, generation time and prevalence of infection. The mallard is the most numerous wild duck in North America and probably worldwide (Bellrose, 1976). The average size of the North American population in May as determined from U.S. Fish and Wildlife Service breeding bird surveys is approximately 8,700,000. The population turnover is high, the fall population consisting of approximately half immature birds each year (Bellrose, 1976). The population density, although low during the breeding season in spring is higher during the remainder of the year, particularly in the fall and winter. The virus has been shown to be transmissible experimentally to contacts. However, the critical factor for perpetuation may be the duration of infection or the persistence of the virus in an individual host. A persistent virus infection in even a few individuals could maintain the virus in populations through the period of low density during breeding until the high population densities of the fall when transmission could occur more easily.

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