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ORTHO- AND PARAMYXOVIRUSES IN THE MIGRATORY WATERFOWL OF MICHIGAN

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Abstract: Four hemagglutinating agents were isolated from 100 cloacal samples collected from migratory water foul during the 1977 hunting season in Michigan. Three of the isolates are paramyxoviruses and they show no reactivity with antisera to Newcastle disease virus. The fourth isolate is an orthomyxovirus, A/Duck/Michigan/77 (Hsw1 Nav2).

Under experimental conditions two of the paramyxoviruses were recovered from the intestinal tract of chicks, and the third paramyxovirus was recovered from both the respiratory and intestinal tract of chicks. One paramyxovirus was pathogenic for chicks. The type A influenza virus was recovered from both the respiratory and intestinal tracts of chicks and caused subclinical infections.

INTRODUCTION

The surveillance of ortho- and paramyxoviruses of lower animals and birds is directed towards the elucidation of the natural history and ecology of these viruses.[4,5,9,10,13,14] Also, these studies may serve as a possible early warning system for myxoviruses in domestic animals.[2,10]

Previous virus surveillance studies have been conducted during the post-breeding,[3] wintering[12] and fall migration periods.[1,7,8,12] These studies have provided estimates of the prevalence of myxoviruses in waterfowl during specific times in their life cycle.

This study was conducted to determine the prevalence of ortho- and paramyxoviruses in the migratory waterfowl in Michigan during the fall migration. The samples were collected from three sites in lower Michigan during the 1977 hunting season (October-November).

MATERIALS AND METHODS

Sample Area

Three state game areas were sampled during the 1977 hunting season in Michigan: the Allegan State Game Area, Shiawassee River State Game Area, and Harsens Island — St. Clair Flats Wildlife Area. These areas are managed to provide food and sanctuary for waterfowl during fall migration. Regulations on these areas require that all hunters pass their game through check stations.

Sampling Procedure

Cloacal swabs were taken from each bird and placed in transport media. The transport media consisted of: nutrient broth, penicillin (200 μg/ml), streptomycin (4 μg/ml), and gentamicin (0.5 μg/ml). The samples were kept on ice in the field and then stored at -70 C in the laboratory. The sex, age and species of each bird was recorded. All samples were

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taken from birds that had been dead 8 h or less.

Virus Isolation

Each sample was inoculated by intra-amniotic route into five 10-day-old embryonated chicken eggs, 0.2 ml/egg. After 72 h incubation at 33 C, the amniotic and allantoic fluids were tested for hemagglutinin activity. Positive samples were reinoculated into eight eggs using intra-allantoic inoculation (a 10^{-1} dilution using transport media). The allantoic fluid from the second egg passages were checked for hemagglutinin activity after 48 h of incubation at 33 C.

The positive allantoic fluids were pooled and centrifuged at 500 x g for 10 min. The supernate was then stored in ampules at -70 C and lyophilized ampules at 4 C.

Virus Typing

Virus typing was done by Dr. Virginia Hinshaw at St. Judes Children's Hospital. See Hinshaw, *et al.*[3] for the procedures used in typing orthomyxoviruses. Paramyxoviruses were classified on the basis of their morphology under electron microscopy examination

In Vivo Studies

Chicken. For each isolate, six week-old chicks from Spafas-COFAL eggs were each inoculated intranasally with 0.2 ml of a 10^{-1} dilution of allantoic fluid (I1 = $10^{2.7}$ EID_{50}/ml, I2 = 10^{4} EID_{50}/ml, I3 = $10^{8.3}$ EID_{50}/ml, I4 = $10^{4.5}$ EID_{50}/ml). Cloacal swabs were taken on the third and seventh days. On the third day one chick was killed and a homogenate was prepared from the intestinal tract (a whole section of small intestine) and lungs. On the seventh day, all remaining chicks were killed and homogenates were prepared from their intestinal tracts and lungs.

Homogenates were prepared by grinding up the tissue in a minimum amount of transport media, usually about 2 ml. This mixture was centrifuged at 500 x g for 10 min. The supernate was collected and stored at -70 C.

All cloacal swabs and homogenates were inoculated by intra-amniotic route into 10-day-old embryonated chicken eggs (0.2 ml/egg). The amniotic and allantoic fluids were checked for hemagglutinin activity after 72 h incubation at 33 C.

Duck. When available, replication studies were also done on week-old ducks. Fertile eggs were collected from mallard *(Anas platyrhynchos)* nests and hatched in the laboratory. One duckling from each nest was killed and examined for maternal antibodies against the virus isolate used. Experimental procedures were identical to those used for the chicks.

RESULTS

A total of 100 cloacal samples was collected from migratory waterfowl during the 1977 hunting season in Michigan. Table 1 contains the species, sex and age distribution for the birds sampled at each area.

Four hemagglutinating agents were isolated from these samples. Three were identified as paramyxoviruses. They did not show any reactivity with antisera to NDV, and have not been classified any further. Also, two of the paramyxoviruses (I1 and I4) isolated required repeated amniotic passages before they could be grown allantoically. The fourth was an orthomyxovirus and was typed as influenza A/DUCK/MICH/77 (Hsw1 Nav2).

Isolates 1 and 3 were isolated from mallard ducks *(Anas platyrhynchos)*, isolate 2 was from a pintail duck *(Anas acuta)*, and isolate 4 was from a black duck *(Anas rubripes)*. Table 2 gives the species, sex, and age of the bird from which each isolate was obtained. It also gives the area from which the sample was collected.

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TABLE 1. Sex and age distribution of species sampled.

Species	#Sampled	Male			Female		
		Total	Adult	Juv.	Total	Adult	Juv.
Anas acuta (Pintail)	9	2	—	2	5	—	7
Anas creca (Green-winged Teal)	10	4	2	2	6	4	2
Anas platyrhynchos (Mallard)	60	39	23	16	21	11	10
Anas rubripes (Black duck)	5	4	2	2	1	—	1
Anas ribripes x platyrhynchos (Black-Mallard Cross)	1	1	—	1	—	—	—
Aythya collaris (Ring-neck duck)	1	1	1	—	—	—	—
Branta canadensis (Canada geese)	11	4	2	2	7	4	3
Mergus cucullatus (Hooded merganser)	3	1	1	—	2	—	2

In Vivo Studies

Isolate 1: Paramyxovirus. All chicks appeared healthy throughout the experiment. Hemagglutinating agents were recovered from both the cloacal swabs and the intestinal homogenates, but not from the lung homogenates.

Isolate 2: Paramyxovirus. On the second day one chick appeared ill. The clinical signs included: dyspnea, muscular dysfunction, anorexia, diarrhea, and feather loss. Feather loss was common to all chicks in this experiment. On the third day the ailing chick was dead. A second chick showing similar signs was killed on the third day, and homogenates were made from the lungs and intestinal tract. The remaining chicks did not develop clinical signs.

Hemagglutinating agents were recovered from the cloacal swabs and intestinal homogenates but not from the lung homogenates.

Isolate 3: A/DUCK/MICH/77 (Hsw1 Nav2)

Chicken. The chicks appeared healthy throughout the experiment. Hemagglutinating agents were recovered from the cloacal swabs, intestinal homogenates, and lung homogenates.

Duck. The ducklings appeared healthy throughout the experiment. Hemagglutinating agents were recovered from the cloacal swabs and intestinal homogenates, but not from the lung homogenates. The ducklings used in the experiment had no detectable antibodies against the isolate prior to infection.

Isolate 4: Paramyxovirus

Chicken. The chicks appeared healthy throughout the experiment. Hemagglutinating agents were recovered from the cloacal swabs, intestinal homogenates, and lung homogenates.

Duck. The ducklings appeared healthy throughout the experiment, and hemagglutinating agents were not recovered from any of the samples. The ducklings used in the experiment had no detectable antibodies against the isolate prior to infection.

DISCUSSION

A/DUCK/MICH/77 is the first type A influenza virus isolate with the Hsw1

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TABLE 2. Viral Isolates.

Isolate #	Sample Area	Species	Sex	Age	Viral Type
I-1	2	*Anas platyrhynchos* (Mallard)	F	AD	Paramyxovirus
I-2	2	*Anas acuta* (Pintail)	F	JUV	Paramyxovirus
I-3	3	*Anas platyrhynchos* (Mallard)	M	AD	A/DUCK/MICH/77 (Hsw1 Nav2)
I-4	3	*Anas rubripes* (Black duck)	M	JUV	Paramyxovirus

2 — Shiawassee River State Game Area
3 — Harsens Island — St. Clair Flats Wildlife Area

and Nav2 envelope antigen combination. This isolate adds to the wide diversity in neuraminidase subtypes already associated with the Hsw 1 antigen in wild ducks.[4] The diversity of neuraminidase associated with the Hsw 1 antigen in avian species provides additional evidence for recombination of avian influenza viruses under natural conditions

The A/DUCK/MICH/77 virus was recovered from both the respiratory and intestinal tract in chickens, and the intestinal tract in ducks in the *in vivo* studies. Previous studies have demonstrated that avian influenza viruses replicate in both the respiratory and digestive tracts.[11,16] The failure to recover virus from the respiratory tract of the ducks does not exclude the possibility of this virus replicating in the respiratory tract due to the small sample size.

Paramyxoviruses are routinely isolated from waterfowl,[6,7,8,15] however, with the exception of Newcastle disease virus, very little is known about their prevalence or pathogenicity in domestic or wild fowl. In the chick experiments all three paramyxoviruses were recovered from the intestinal tract, and I-4 was also recovered from the respiratory tract. The I-2 isolate produced clinical signs of infection, and in one case resulted in death. Research on these viruses and their potential pathogenicity to domestic and wild fowl is needed.

Four hemagglutinating agents were isolated from 100 field samples. Three of the isolates were paramyxoviruses and one was a type A influenza virus. The number of paramyxoviruses isolated is considerably higher than reported in previous studies. Hinshaw *et al.*,[3] reported the isolation of 107 hemagglutinating agents with only one being identified as a paramyxovirus. The majority of researchers studying avian myxoviruses are currently using allantoic inoculations as their method of viral isolation. Two of the paramyxoviruses isolated in this study would not have been isolated using allantoic inoculations. The I-4 isolate also required several amniotic passages before it would grow allantoically. This indicates that researchers interested in both ortho- and paramyxoviruses should use amniotic inoculations as the preferred method of viral isolation.

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