ISOLATION OF MYXOVIRUSES FROM MIGRATORY WATERFOWL IN THE CENTRAL FLYWAY

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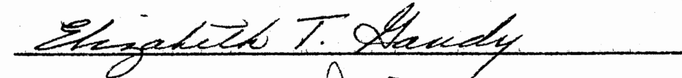

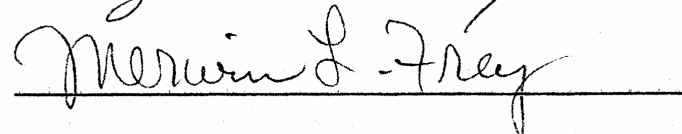

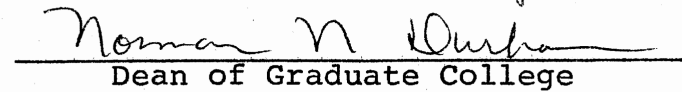

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PREFACE

Recent detection of influenza A and Newcastle disease viruses, in migratory waterfowl, has sparked interest in the presence of these viruses and their possible transmission to domestic flocks. The present study was designed to investigate the presence of myxoviruses in wild waterfowl wintering in and migrating through Oklahoma. In addition, the potential for transmission of these viruses between semi-domestic and wild waterfowl was studied.

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The author would like to express his gratitude to the members of his committee who guided him in this study. He would like to express thanks to Dr. Alan Kocan, his major adviser, for his guidance and counseling. The writer wishes to express thanks to Dr. Merwin Frey and Dr. Elizabeth Gaudy for counseling during the research.

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CHAPTER I

INTRODUCTION

Influenza A, an orthomyxovirus, and Newcastle disease virus, a paramyxovirus, are etiologic agents for a variety of disease conditions in domestic and wild fowl. The clinical course of infection for these viruses ranges from a mild to fatal respiratory condition with occasional involvement of muscle tissue for influenza A and a mild respiratory disorder which may progress to central nervous system disorders and death in Newcastle disease.

Evidence for infection of migratory waterfowl with Newcastle disease virus and influenza A previously have been based on serological data. Recent studies have focused on detection of virus in migratory waterfowl from the Atlantic, Pacific and Mississippi Flyways and have led to considerable concern over the importance of these findings. Information concerning the presence of these viral agents in wintering waterfowl populations, populations in the Central Flyway and populations in Oklahoma is not available.

A large number of waterfowl migrate through the states located along the Central Flyway. More waterfowl, in fact, migrate along the Central Flyway than migrate along the other three flyways.(1) Oklahoma, one of the states in the Central

Flyway, receives large concentrations of wintering waterfowl. This accumulation of waterfowl may provide conditions suitable for the transmission and spread of various infectious diseases.

Since diseases contracted by waterfowl on wintering grounds may influence their subsequent production levels as well as affect the health and reproduction of other free-flying wild and domestic fowl, it becomes important to monitor waterfowl populations for the presence of infectious diseases.

The purpose of this study was to investigate the occurrence of Newcastle disease and influenza A in migratory waterfowl in Oklahoma by virus detection and serological studies. Close attention was paid to seasonal variations in virus prevalence. Potential transmission of viruses between semi-domestic waterfowl and migratory waterfowl was also investigated.

CHAPTER II

LITERATURE SURVEY

The presence of influenza A and Newcastle disease virus in domestic and wild fowl has been demonstrated in many countries.[2, 3, 4, 5] These viruses, as well as antibody to these viruses, have also been detected in numerous species of free-flying wild and domestic birds in the United States. Antibodies to influenza A have been demonstrated in fowl sera by hemagglutination inhibition and gel-diffusion tests.[6, 7, 8] Newcastle disease virus antibodies have also been detected by means of the hemagglutination inhibition test.

Detection of influenza A and Newcastle disease virus in migratory waterfowl has been accomplished in several flyways in the United States and their presence has sparked much interest and concern.[6, 9, 10, 11, 12, 13, 14, 15, 17] Influenza A and Newcastle disease viruses have been detected in Wisconsin, Michigan, Minnesota and Arkansas in the Mississippi Flyway.[13, 14, 16, 17] These viruses have also been detected in California in the Pacific Flyway.[8, 15, 18]

In spite of the evidence supporting the presence of these viruses in free-flying waterfowl, the exact implications of their presence have not been determined. These

viruses have, however, been implicated in epidemics in domestic fowl operations. Turkey flocks in Wisconsin, Michigan, Minnesota, California, and Oregon have suffered the effects of influenza A as have chicken flocks in Alabama.[19, 20, 21, 22, 23] In California an outbreak of velogenic viscerotropic Newcastle disease occurred in poultry flocks and many semi-domestic and exotic birds during 1972 and 1973.[12]

The transmission of influenza A and Newcastle disease virus between domestic flocks and wild waterfowl has not been demonstrated but it is recognized as a potential problem.[21, 22, 24]

CHAPTER III

MATERIALS AND METHODS

Procurement of Waterfowl Biological Samples

Trapping Wild Waterfowl

Wild waterfowl were trapped with the aid of a rocket net, at a Soil Conservation Service Impoundment, (one hundred surface hectares) which is known as Ham's Lake and is located in Payne County, Oklahoma. The migratory waterfowl were trapped over a period of one year beginning in the spring of 1975 and concluding in the early spring of 1976. Various species of ducks were captured including blue-winged teal (Anas discors), green-winged teal (Anas crecca carolinensis), mallard (Anas platyrhynchos), pintail (Anas acuta), wood duck (Aix sponsa) and American widgeon (Anas Americana). One Canada goose (Branta Canadensis) and one snow goose (Anser c. caerulescens) were also trapped.

Semi-domestic Non-flying Waterfowl

Mallard ducks used for study of virus transmission between wild populations and a flightless population were obtained from the Max McGraw Wildlife Foundation. Three

groups of these ducks were provided: wild incubator hatched, McGraw, and a first generation cross between wild incubator hatched mallard ducks and McGraw mallard ducks.

Virus

Influenza A (Turkey/Wisconsin/68) virus was obtained from Dr. Dick Slemons of the Department of Veterinary Science at The University of Wisconsin. Newcastle disease virus (Texas GB strain) was provided by the Department of Veterinary Parasitology, Microbiology and Public Health at the College of Veterinary Medicine at Oklahoma State University.

Antisera

Newcastle disease virus antiserum was produced in Cornish cross chickens obtained from a certified pathogen-free flock from Arbor Acres Farms in Springdale, Arkansas. The chickens were vaccinated at five weeks of age with a live modified virus vaccine.

They were challenged by intramuscular injection and intratracheal inoculation with a virulent Newcastle disease virus nine days after vaccination. The inoculum was five-tenths milliliter of a 1 to 100 dilution of allantoic fluid with a hemagglutination titer of 2560. The chickens were again challenged seventeen days later in order to produce a hyperimmune serum. Fourteen days after the second challenge the chickens were killed and their serum was harvested and frozen until needed. Influenza A antiserum was obtained

from Dr. Dick Slemons at The University of Wisconsin, and from Dr, Robert H. Fennell, Head Diagnostic Products, at Microbiological Associates, Walkersville, Maryland. These antisera were specific for type A ribonucleoprotein. In addition, antiserum was produced to influenza A (Turkey/ Wisconsin/68) in chickens also obtained from Arbor Acres Farms. The chickens were inoculated intratracheally at eight weeks of age. They were then challenged at sixteen and twenty-two days after inoculation. Twenty-one days after the second challenge the chickens were killed and their serum was harvested and stored at minus four degrees Centigrade until needed.

Rearing and Treatment of McGraw Obtained Mallard Ducks

The mallard ducks provided by McGraw were pinioned as ducklings and raised in a specific pathogen-free environment until their release onto the study impoundment in late summer of 1975.

Prior to release of the pinioned mallard ducks cloacal and tracheal swab samples were obtained from twenty percent of the ducks for attempts at viral detection. Blood samples were taken from all the ducks for serologic studies. In addition, each duck was banded with a numbered leg band for individual bird identification. These mallard ducks were periodically captured and blood and swab samples were taken.

Biological Samples

Swabbing for Detection of Virus

Swab samples of the trachea of captured waterfowl were made using sterile aluminum shafted nasopharyngeal Calgiswabs. Cloacal swab samples were made with standard sterile cotton-tipped applicator sticks. The swabs were placed in a cold two milliliter solution of tryptose phosphate broth with five-tenths percent gelatin and antibiotics in four inch screwcapped glass tubes.[25]

The following antibiotics were employed in high concentrations to kill bacteria and fungi: penicillin - ten thousand units per milliliter; streptomycin - ten milligrams per milliliter, and amphotericin B - twenty-five hundredths milligrams per milliliter. The tubes were immediately placed on ice, then transported to the laboratory where they were frozen at minus seventy degrees Centigrade.[6]

Collection of Blood for Antibody Detection

All waterfowl captured, including both wild waterfowl and McGraw provided mallard ducks, were bled by peripheral venipuncture with a three cubic centimeter syringe and a twenty gauge one inch needle. One to three cubic centimeters of blood was obtained depending on the size of the duck. The blood was allowed to clot in the syringe and the serum was separated from the clot by use of an International clinical centrifuge. The serum was placed in labeled individual

vials and was stored at minus four degrees Centigrade until antibody detection studies could be made.

Virus Detection

Inoculation and Harvest of Embryonated Eggs

Swab samples were thawed at room temperature and then placed in a refrigerator at four degrees Centigrade for one and one-half hours.[25] Three nine- to twelve-day old embryonated chicken eggs were then each injected with three-tenths milliliter of a swab sample solution. Two-tenths milliliter were injected into the chorioallantoic cavity of each egg while one-tenth milliliter was injected into the amniotic sac of each egg. Inoculated eggs were candled twice daily to detect embryo death. Allantoic and amniotic fluids were collected from each egg when death was detected or at four days after inoculation.[6]

Hemagglutination (HA) Tests

In order to detect the presence of hemagglutinating agents, a microtiter HA technique (utilizing a multi-microdiluter with twenty-five thousandths milliliter capacity capillary transfer pipettes, an automatic, nonelectric, pipette and disposable microtiter plates) was performed.[26]

The HA test was run on each of the samples of fluid harvested. A two-fold dilution of the fluid was made starting with a 1 in 2 dilution and running through a 1 in 256

dilution. Five-tenths percent chicken erythrocytes in buffered saline were used in the test.

Allantoic fluids containing hemagglutinating agents were tested for bacterial and fungal contamination by streaking on heart infusion blood agar and IPPLO agar plates and by inoculation of thiol broth. These media were then incubated in both aerobic and anaerobic atmospheres.

Detection of Influenza A and Newcastle Disease Viruses

Hemagglutination Inhibition Test (HIT) for Newcastle Disease Virus

All hemagglutinating viruses detected were run in a microtiter beta HIT against anti-Newcastle disease virus serum to determine if the viruses were Newcastle disease virus.[26, 27] Virus dilutions from a 1 in 2 dilution to a 1 in 1024 dilution were made. Five-tenths percent chicken red blood cells and four HA units of the hemagglutinating virus were utilized.[28]

Micro-immunodiffusion for Detection of Influenza A Viruses

Hemagglutinating viruses that were not inhibited in the Newcastle disease virus HIT were run in a micro-immunodiffusion test.[7, 29, 30, 31] Standard plastic petri dishes, fifteen by ninety millimeters, were used. Noble agar nine-tenths

percent, eight and five-tenths percent NaCl, one-hundredth percent phenol and trypan blue dye were used to prepare the gel-diffusion agar. The antigen for the test was prepared by ultracentrifugation of the allantoic fluid of embryonated eggs inoculated with the viruses. The virus concentrate provided by ultracentrifugation was then frozen and thawed three times to disrupt the virus particle and expose the matrix proteins and the ribonucleoproteins. The disrupted virus concentrate was run in the micro-immunodiffusion test with known influenza A antiserum and controls (saline and normal chicken serum) to determine if the viruses were influenza A virus.

Detection of Antibody in Duck Sera to Newcastle Disease Virus or Influenza A Virus

Hemagglutination Inhibition Test (HIT) for Detection of Antibody to Newcastle Disease Virus

Detection of Newcastle disease virus antibodies in the sera collected from wild and semi-domestic (McGraw) ducks was attempted by microtiter HIT.[26, 27] Chicken erythrocytes (five-tenths percent suspension in saline) and four HA units of Newcastle disease virus were used in the test.[28] Duck sera were tested in dilutions of 1 in 2 to 1 in 256 for hemagglutination inhibition activity. Newcastle disease virus antiserum, normal serum and saline were run as controls.

Micro-immunodiffusion for Detection of Influenza A Antibodies

Detection of influenza A antibodies in the duck sera was attempted by micro-immunodiffusion according to a procedure provided by the Oklahoma Department of Public Health.[29] A one-eighth inch plexiglass template with a seventeen - seven well pattern purchased from L. L. Pellet Company, 8843 Larchwood Drive, Dallas, Texas 75238 was utilized. The antigen for this test, influenza A (Turkey/Wisconsin/68), was grown in one hundred nine- to twelve-day old embryonated eggs and was concentrated by ultracentrifugation. The virus was disrupted to expose the type-specific matrix protein and ribonucleoprotein antigens. Reference antiserum and saline controls were run along with the duck serum samples.

Demonstration of McGraw Ducks Susceptibility to Influenza A Virus

Three of each of the McGraw, wild-incubator hatched, and first generation cross ducks were inoculated with influenza A (Turkey/Wisconsin/68). The ducks were inoculated intratracheally and intranasally with five-tenths milliliter of a 1 in 100 dilution of allantoic fluid having a HA titer of 2560. They were then placed in an isolation cage equipped with a three-tenths micron filter. They were observed daily for clinical signs and were bled for antibody detection studies before inoculation and at four and twelve days after

inoculation. The ducks were killed at twelve days post inoculation and examined for lesions.

CHAPTER IV

RESULTS

McGraw Mallard Ducks Susceptibility to Influenza A

Antibody Production

Sera taken prior to inoculation of McGraw ducks with influenza A (Turkey/Wisconsin/68) tested negative for influenza A antibodies in both the hemagglutination inhibition test and the micro-immunodiffusion test. Sera collected twenty-one days after initial intranasal and intratracheal inoculation of these ducks inhibited hemagglutination by influenza A. These sera had hemagglutination titers varying from 80 to 320. Sera collected on the twenty-ninth day after inoculation also inhibited hemagglutination by influenza A with hemagglutination inhibition titers from 40 to 320. The micro-immunodiffusion agar test used for sera collected at twenty-one and twenty-nine days following inoculation likewise resulted in the detection of antibodies to influenza A.

Clinical Signs

Mild depression was noted in many of the McGraw mallard ducks four to thirteen days after inoculation with influenza

A (Turkey/Wisconsin/68). Two of the susceptibility study ducks showed small red lesions on the epithelial lining of the trachea when necropsied at twenty-nine days post inoculation. The remainder of these ducks had no visible lesions.

Prerelease Swabs and Serum Samples

Serum samples obtained from the McGraw mallard ducks prior to their release onto Ham's Lake were tested for antibodies to influenza A and Newcastle disease virus. The microimmunodiffusion test was used to attempt to detect antibodies to influenza A. The hemagglutination inhibition test was used to test for antibodies to Newcastle disease virus. Antibodies to these two viruses were not detected in the prerelease McGraw ducks sera by these methods. In addition, attempts to detect these viruses in the McGraw ducks prior to their release were unsuccessful.

Detection and Identification of Hemagglutinating Viruses

Hemagglutinating Viruses

A total of seventeen hemagglutinating agents were obtained from three hundred forty-eight wild and sixty-three recaptured McGraw waterfowl. Four of these hemagglutinating agents were detected in the trachea and thirteen were detected in the cloaca of the ducks. One hemagglutinating agent was obtained from both the trachea and the cloaca of a duck. The

hemagglutinating agents were bacteriologically sterile and grew rapidly at thirty-seven degrees Centrigrade in the allantoic cavity of chicken embryos. None of these hemagglutinating viruses, when tested in the hemagglutination inhibition test against anti-Newcastle disease virus serum, were found to be Newcastle disease virus.

Hemagglutinating Viruses Detected During a One-Year Period

The viruses were detected in both wild and McGraw provided waterfowl in the spring of 1976 and the fall and winter of 1976-1977. Table I shows the months during which the detections were made and the number of waterfowl captured and sampled during a particular month. This table includes captured McGraw mallard ducks as well as wild waterfowl. Table II lists the percentage of the total number of viruses obtained during each month.

Viruses Obtained from Various Waterfowl Species

Viruses were obtained from several species of wild waterfowl. Table III shows the number of each species of wild waterfowl captured, when they were captured, and the number of viruses per species.

TABLE I

HEMAGGLUTINATING VIRUSES DETECTED DURING A ONE-YEAR PERIOD

Month	Number of Waterfowl Captured	Number of HA Viruses Detected
March (1976)	108	0
April (1976)	103	1
May (1976)	19	0
June (1976)	0	0
July (1976)	0	0
August (1976)	0	0
September (1976)	0	0
October (1976)	41	5
November (1976)	26	6
December (1976)	21	2
January (1977)	32	2
February (1977)	60	1

TABLE II

PERCENT OF VIRUSES DETECTED EACH MONTH

Month*	Percent of Total Viruses Detected
April (1976)	5.8
October (1976)	29.4
November (1976)	35.2
December (1976)	11.7
January (1977)	11.7
February (1977)	5.8

*No viruses were detected during months not shown in the table.

TABLE III

VIRUSES DETECTED IN EACH SPECIES OF WILD WATERFOWL CAPTURED

Waterfowl Species	Number Captured	No. of Viruses Detected	% of Species Carrying Virus	Time of Year Captured
Mallard	91	10	10.9	Oct., Nov., Dec., Jan., Feb., Mar.
American Widgeon	22	1	4.5	Oct.
Pintail	4	1	25.0	Nov., Feb.
Blue-winged Teal	98	0	-	Mar., Apr., May
Green-winged Teal	126	1	0.79	Mar., Apr., May
Wood Duck	5	0	-	Apr., May
Canada Goose	1	0	-	Dec.
Snow Goose	1	0	-	Dec.

Viruses Obtained from McGraw Mallard Ducks

Several of the viruses were obtained from McGraw mallard ducks during the fall and winter of 1976-1977. Table IV shows the number of McGraw ducks sampled, when they were sampled, and the number of viruses obtained.

TABLE IV

VIRUSES FROM THE McGRAW MALLARD DUCKS

Month*	No. of Ducks Sampled	Viruses Detected
October (1976)	18	4
November (1976)	1	0
December (1976)	4	0
January (1977)	0	0
February (1977)	38	0

*The McGraw mallard ducks were released onto the impoundment in late July and early September and so were not available for sampling prior to that time.

Identification of Viruses

The seventeen viruses obtained were tested by means of a microimmunodiffusion test against anti-influenza A ribonucleoprotein serum. Fourteen of these viruses showed lines of identity with influenza A (Turkey/Wisconsin/68) against

the type A-specific antiserum used. Three of these viruses remain unidentified. Table V shows the time of year detected, the species of waterfowl in which they were detected and the number of influenza A viruses detected.

TABLE V

INFLUENZA A VIRUSES

Date of Virus Detection	Species of Waterfowl	Number of Influenza A Viruses
April 14, 1976	Green-winged Teal	1
October 22, 1976	McGraw Mallard	2
October 22, 1976	Mallard	1
October 31, 1976	McGraw Mallard	2
November 10, 1976	American Widgeon	1
November 10, 1976	Mallard	1
November 10, 1976	Pintail	1
November 28, 1976	Mallard	3
December 8, 1976	Mallard	1
December 22, 1976	Mallard	1

Detection of Antibody in Waterfowl Sera To Newcastle Disease Virus and Influenza A Virus

Detection of Newcastle Disease Virus Antibodies by the Hemagglutination Inhibition Test

Waterfowl sera were tested in the microtiter HIT for Newcastle disease virus antibodies. Data obtained on the presence of antibodies to Newcastle disease virus in the waterfowl sera are summarized in Table VI. It lists the species of waterfowl, the date of capture, the number of positive reactors and the percent of reactors.

The Detection of Antibody to Influenza A by Immunodiffusion

Waterfowl sera were tested by micro-immunodiffusion against influenza A antigen. Test sera, which contained antibodies to influenza A, produced lines of identity with influenza A type-specific antiserum. These data are summarized in Table VII. The table shows the species of waterfowl, the date of capture, the number of reactors, and the percent of reactors.

TABLE VI

HEMAGGLUTINATION INHIBITING NEWCASTLE DISEASE VIRUS ANTIBODIES IN CAPTURED WATERFOWL

Species	Date of Capture	No. of Reactors	% of Reactors
McGraw Mallard	Oct., Nov., Dec., Feb.	0/63	-
Mallard	Oct., Nov., Dec., Jan., Feb., Mar.	0/95	-
American Widgeon	Oct.	1/17	5.8
Pintail	Nov., Feb.	0/4	-
Blue-winged Teal	Mar., Apr., May	6/93	6.5
Green-winged Teal	Mar., Apr., May	0/107	-
Wood Duck	Apr., May	0/5	-
Canada Goose	Dec.	0/1	-
Snow Goose	Dec.	0/1	-

TABLE VII

INFLUENZA A ANTIBODY IN CAPTURED WATERFOWL

Species	Date of Capture	No. of Reactors	% of Reactors
McGraw Mallard	Oct., Nov., Dec., Feb.	13/63	20.6
Mallard	Oct., Nov., Dec., Jan., Feb., Mar.	20/95	21.0
American Widgeon	Oct.	0/17	-
Pintail	Nov., Feb.	0/4	-
Blue-winged Teal	Mar., Apr., May	5/93	5.4
Green-winged Teal	Mar., Apr., May	4/107	3.7
Wood Duck	Apr., May	0/5	-
Canada Goose	Dec.	0/1	-
Snow Goose	Dec.	0/1	-

CHAPTER V

DISCUSSION

Occurrence of Newcastle Disease Virus and Influenza A Virus in Migratory Waterfowl in Oklahoma

Newcastle Disease Virus

None of the viruses detected during the period of March 1, 1976 to February 28, 1977 were Newcastle disease virus. Seven of three hundred eighty-six serum samples tested by the hemagglutination inhibition test contained antibodies to Newcastle disease virus.

Six of the seven serum samples, which contained antibody to Newcastle disease virus, were collected from blue-winged teal in late April and early May. Teal do not winter in Oklahoma but instead winter in southern Texas and parts of Central and South America.[1] These teal were thus captured on their spring migration back to their northern breeding grounds. Antibodies in their serum may indicate exposure to Newcastle disease virus at the particular wintering grounds that the teal utilized.

The failure to detect Newcastle disease virus and the small number of serum samples containing antibody to Newcastle

disease virus indicate that Newcastle disease virus is not commonly found in migratory waterfowl in Oklahoma.

These findings differ from findings of investigations in the Atlantic and Pacific Flyways. Rosenberger, et. al., detected Newcastle disease virus in Canada geese in the Atlantic Flyway.[24] In other studies of the Atlantic Flyway, a large percentage of serum samples collected contained antibodies to Newcastle disease virus.[13, 15] Antibodies to Newcastle disease virus also were found in a large percent of the serum samples from waterfowl examined in a study by Pearson and McCain in California, which is in the Pacific Flyway. In addition, Newcastle disease viruses were detected in numerous wild fowl during their study.[12]

The differences in the findings of these studies might be explained by the absence of numerous, large poultry flocks in Oklahoma, such as those found in California, Michigan, Minnesota, Arkansas, and other poultry producing states in the Atlantic, Mississippi, and Pacific Flyways. Since domestic fowl are known to be more susceptible to Newcastle disease virus than are wild waterfowl,[32] the inability to detect the virus in wild waterfowl and the lower number of positive serologic reactors may thus be explained by the lack of opportunity for exposure to the domestic source.

Influenza A Virus in Migratory Waterfowl in Oklahoma

Influenza A virus was detected in four species of

waterfowl, including wild and McGraw provided ducks, and a total of thirteen individual ducks out of three hundred eighty-six captured and sampled in Oklahoma. Antibody to influenza A was detected in forty-two of three hundred eighty-six serum samples from the waterfowl.

Considerably more influenza A viruses were detected in waterfowl during the winter months of October, November, and December than in the spring. The chronology of these detections indicates that waterfowl experience a "flu season" during the winter months. Other factors, however, should be considered to explain these differences. One obvious factor could be differences in susceptibility to influenza A of various waterfowl species. Another could be the weather conditions and other environmental stress factors, which influence the prevalence of virus infection at the wintering grounds utilized by different waterfowl species.

Most of the influenza A viruses detected in wild waterfowl were detected in mallard ducks while only one influenza A virus was detected in each of the following species: green-winged teal, American widgeon, and pintail ducks. This appears to indicate that there is a difference in species' susceptibility to influenza A. However, the influenza A detection data might be explained better by examining the availability for capture of the various waterfowl species.

The availability for capture of wild waterfowl in Oklahoma varies according to their migratory behavior. One species of waterfowl may, therefore, predominate in total

numbers present in Oklahoma at any given time. Mallard ducks, in which many viruses were detected, winter in Oklahoma and so were more available for sampling during the winter months. Although they do not winter in Oklahoma, pintail and American widgeon ducks were, also, available for sampling in the winter months. However, fewer of these species were captured and sampled which may account for the lower number of viruses detected. Even though only one virus was detected in each of these species, the detection rate per duck was similar to the detection rate for the mallard ducks. Teal, on the other hand, do not winter in Oklahoma. Instead they are only available for capture in Oklahoma in the spring during their migration to their northern breeding grounds. The rate of detection per duck of these species was low, possibly because they were captured after the "flu season" had ended.

The McGraw mallard ducks, which were released onto the study impoundment in late summer, also might appear to be responsible for the increase in influenza A viruses detected in the fall. After the initial exposure of the McGraw flock to influenza A, presumably from a natural wild source, they could have transmitted the virus to wild waterfowl and thus played a role in the increase in influenza A viruses detected. Still, their presence could not account for the occurrence of influenza A virus since no viruses were detected in them prior to their release. The McGraw mallard ducks simulated a wintering wild waterfowl flock and were exposed to the same environmental conditions as wintering wild waterfowl. The McGraw

flock did not provide any conditions for the spread of influenza A that did not already exist for the wintering wild waterfowl flocks. Thus, these environmental conditions to which both the wild and McGraw ducks were exposed appears to be the cause of this increased virus detection and not the mere presence of the McGraw flock on the impoundment.

More serum samples containing antibodies to influenza A were collected from mallard ducks than any other waterfowl species captured. These samples were collected in the winter months and might represent blood drawn immediately after recovery or during recovery from infection. Antibody levels in these serum samples drawn from mallard ducks would, therefore, be expected to be high. This may be true of all serum samples from birds exposed to influenza A collected during the winter. Other species captured during the winter months would be expected to have high antibody levels in their serum had they been exposed to influenza A.

Serum samples obtained from waterfowl captured in the spring following a winter "flu season" would be expected to have lower antibody titers. This may have been demonstrated in the case of the captured teal. Many teal were captured and sampled in March and April, yet few serum samples contained detectable levels of antibody to influenza A.

Results of any serologic investigation depends on the sensitivity of the procedures used. The serum samples collected for this study were tested for the presence of antibody to influenza A by micro-immunodiffusion. The

mciro-immunodiffusion test was used to determine if antibody to influenza A ribonucleoprotein, a type-specific antigen, was present. Since antibody to influenza A ribonucleoprotein is not detected in waterfowl sera as easily as is antibody to its hemagglutinin or neurminidase spikes, serum containing antibodies to influenza A might go undetected when tested by this method.[6, 31] Therefore, it is possible that some of the serum samples collected contained undetectable levels of antibody to influenza A.

It appears that waterfowl wintering in and migrating through Oklahoma carry influenza A virus. Although the migratory waterfowl carry the virus no major epinoritics caused by this virus have been reported in wild waterfowl. The detection of the presence of influenza A virus in, and the detection of antibody to influenza A in serum samples collected from, migratory waterfowl in Oklahoma is consistent with findings of other investigators in the Pacific, Mississippi and Atlantic Flyways. Pomeroy and Easterday detected influenza A virus in migratory waterfowl in the Mississippi Flyway.[6, 10] Siemons and Johnson detected influenza A in waterfowl in the Pacific Flyway[8] and Rosenberger and Krauss detected the virus in the waterfowl in the Atlantic Flyway.[14]

Transmission of Influenza A to McGraw Mallard Ducks

The flock of non-flying or semi-domestic mallard ducks appeared to be free of Newcastle disease virus and influenza

A infection when they were placed onto the study impoundment. The serum samples drawn prior to the release of each McGraw mallard duck would have been expected to contain detectable antibody levels against Newcastle disease virus or influenza A had they been infected. In addition, because of their confined quarters prior to release, any infection in the flock should have been widespread and sampling twenty percent of these ducks would be expected to detect any myxovirus present.

Three months after their release onto Ham's Lake four hemagglutinating viruses were obtained from the McGraw mallard ducks. These viruses proved to influenza A by the agar gel-diffusion test. They were detected in October but subsequent attempts at detection during the following winter months proved fruitless.

Antibody to influenza A was detected in serum from two McGraw ducks captured and sampled in November. Antibodies to influenza A were also detected in ten of thirty-eight McGraw mallard ducks captured and sampled in early February. Lack of data for the McGraw mallard ducks during the months of December and January makes accurate conclusions difficult. Still, this seems to indicate that the McGraw birds were exposed to influenza A and some became infected in the early winter, possibly October. The virus may have spread to other susceptible McGraw ducks and so many of them produced antibodies to influenza A high enough in titer to be detected in the gel-diffusion test.

This indicates there was transmission of influenza A to the McGraw mallard ducks placed on the study impoundment. However, it does not indicate certain transmission of the virus from wild migratory ducks since exposure to other fowl may have occurred. Still, the McGraw ducks were exposed predominantly to migratory waterfowl. This occurred because the wild waterfowl's presence on the impoundment placed them in closer proximity to the semi-domestic ducks. In addition, intermingling of the McGraw ducks and wild waterfowl was commonly observed. Exposure to other birds such as sparrows, crows, etc., was limited by the differences of habitat preferences of ducks and these other birds. The chance for transmission of influenza A virus from wild waterfowl to the McGraw ducks would, therefore, be far greater than the chance for transmission from other sources.

CHAPTER VI

SUMMARY

The main objective of this study was to investigate the presence of Newcastle disease virus and influenza A in migratory waterfowl in Oklahoma by virus detection and serologic tests. In addition, studies of transmission of viruses to a flock of semi-domestic ducks was undertaken.

No Newcastle disease virus was detected in waterfowl which were captured at the study impoundment. Antibody to Newcastle disease virus was detected, however, in seven of three hundred twenty-three serum samples collected from wild waterfowl. No antibody to Newcastle disease virus was detected in sixty-three serum samples collected from recaptured McGraw mallard ducks.

Influenza A viruses were obtained from four species of ducks. These species included the mallard, the pintail, the American widgeon, and the green-winged teal. Antibodies to influenza A were also detected in twenty-nine of the serum samples collected from wild waterfowl. Serum samples from five of ninety-three blue-winged teal, four of one hundred seven green-winged teal, and twenty of ninety-five mallard ducks tested positive for antibodies to influenza A.

Influenza A was also obtained from four McGraw mallard ducks which were released onto Ham's Lake. Thirteen of sixty-three serum samples collected contained detectable levels of antibody to influenza A. These ducks were tested thoroughly prior to their release to determine if they had been exposed to influenza A. Detection of influenza A virus from these ducks showed that transmission of the virus to the McGraw ducks had occurred. The viruses were most likely transmitted to the McGraw flock from wild waterfowl which intermingled freely with them.

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