July 1, 1993

LCMR Final Status Report - Summary - Research

I. **Effect of Avian Influenza Virus on Growth and Reproduction Parameters in Mallard Ducks** - Wildlife 31

Program Manager: David A. Halvorson, Professor
University of Minnesota
Dept of Veterinary Pathobiology
301 Veterinary Science Building
1971 Commonwealth Avenue
St. Paul, MN 55108
612-625-5292

A. M.L. 91, Ch 254, Art. 1, Sec. 14, Subd: 9(q)
Appropriation: $16,000
Balance: $ -0-

Effect of Avian Flu Virus in Mallard Ducks: T h i s appropriation is to the University of Minnesota, Department of Veterinary Pathobiology, to research the effects of Avian Influenza Virus on Mallard ducks.

B. Not Applicable

C. Not Applicable

II. Narrative

Wild waterfowl populations are thought to comprise the major reservoir of Avian Influenza Viruses (AIV) in nature. These viruses have been shown to cause morbidity and mortality in domestic poultry populations but very little work has been done on elucidating the pathogenic effects of these viruses in waterfowl. The goal of this study is to assess the tissue trophism characteristics of AIV in Mallard ducklings as well as its effect on reproduction in Mallard hens.

III. Objectives

A. **Analysis of tissue trophism patterns of AIV infection in juvenile Mallard ducks**

A.1. Narrative: Influenza type A viruses have been shown to be widespread in duck populations throughout the world but are not typically associated with overt disease in these birds. Recent studies, however, have shown consistent patterns of pneumonic changes in infected ducks. The goal of this objective is the assessment of the tissue trophism patterns of several waterfowl-origin type A influenza viruses in young Mallard ducks.

A.2. Procedures: 120 Mallard eggs will be obtained from a game farm and hatched in our isolation facility. These ducklings will be reared in isolation to 6 weeks of age. Serum samples and cloacal swabs will be collected prior to inoculation with ten different influenza viruses (10 duckling per virus plus 20 control birds). The birds will be inoculated intravenously with 0.2 ml of undiluted second passage infected allantoic fluid. Control birds will be sham inoculated. At 3 and 5 days post-inoculation serum samples will be taken and the birds will be euthanized and necropsied. Tracheal and cloacal swabs as well as selected tissues will be collected. The tissues will be divided and either frozen at -70° C for virus titration and frozen sections or placed into 10% phosphate buffered formalin for routine histopathological processing and staining with hematoxylin and eosin. Frozen sections will be analyzed for the presence of viral antigens by indirect fluorescent microscopy using a mouse monoclonal antibody specific for the nucleoprotein of type A influenza viruses. Serum samples will be analyzed for the presence of antibodies to AIV using the hemagglutination inhibition and agar gel precipitation tests.

A.3. Budget:

	LCMR Funds	Matching Funds
a. Amount Budgeted:	$3,600	$ -0-
b. Balance:	$ -0-	$ -0-

A.4. Timeline for Products/Tasks:

	Jul91	Jan92	Jun92	Jan93	Jun93
Run Experiment	*******				
Analyze Data		*******			
Final Report			*****		

A.5. Status: Ten avian type A influenza viruses consisting of seven waterfowl-origin, one pheasant-origin, and two turkey-origin viruses were evaluated for their pathogenicity potential after intravenous inoculation in mallard ducks (*Anas platyrhynchos*). The replicative abilities and tissue trophism properties of each virus isolate were examined. The waterfowl-origin viruses were isolated from a large number of tissues, but damage was minimal. Lymphoid depletion of the spleen and bursa was the most significant and consistent lesion noted. The remaining isolates were recovered from only a small percentage of tissues and their effects were minimal. Previous inoculation of turkeys and chickens with some of these isolates resulted in severe renal damage in those species, while in ducks, microscopic lesions seen in the

kidney were very mild. This suggests that influenza viruses are capable of having negative effects on the immune system of ducks, but do not appear to cause significant lesions in other tissues. The results of this study were accepted for publication in Avian Diseases as a manuscript entitled "Comparative evaluation of tissue trophism characteristics in turkeys and mallard ducks after intravenous inoculation of type A influenza viruses."

A.6. Benefits: A thorough understanding of exactly which tissues AIV is infecting is an essential first step in trying to further understand the pathogenesis of this organism in ducks.

B. Evaluation of the effect of AIV infection on the growth rate and susceptibility to secondary infections in Mallard ducklings

B.1. Narrative: Because AIV does not usually cause a high degree of mortality in ducks very little work has been done on characterizing its potential for producing subclinical detrimental effects on the health and longevity of these birds. We propose to examine the effect of AIV infection on the growth rate of Mallard ducklings and on their resistance to secondary infections in the respiratory tract.

B.2. Procedure: 110 isolation reared ducklings will be infected at 2 weeks of age with a selected Avian Influenza Virus. 50 infected birds and 50 uninfected birds will be weighed weekly for 10 weeks. 60 AIV infected birds plus 60 uninfected control birds will be inoculated with Escherichia coli intratracheally 3 days after being infected with AIV. 10 birds from each group will be euthanized at 6 different time intervals post-infection (6,12,24,48,86 and 172 hours PI). The respiratory tracts will be harvested and analyzed for the presence of E. coli using standard bacterial plate counts.

B.3. Budget:

	LCMR Funds	Matching Funds
a. Amount Budgeted:	$3,100	$ -0-
b. Balance:	$ -0-	$ -0-

B.4. Timeline for Products/Tasks:

	Jul91	Jan92	Jun92	Jan93	Jun93
Start Project		*****			
Analyze Data			*****		
Final Report					*****

B.5. Status: An avian influenza virus isolate, A/Mallard/Ohio/184/86 (H5N1), was evaluated for its effects on growth rate in isolation reared juvenile mallard ducklings after intravenous inoculation. Ducklings receiving this influenza virus isolate did not differ from controls in their rate of growth. Bacterial isolation of experimentally inoculated *Escherichia coli* was repeatedly unsuccessful. The result of this study was accepted for publication in the Journal of Wildlife Diseases as part of a manuscript entitled "Effect of avian influenza virus infection on reproduction and growth in mallard ducks."

B.6. Benefits: Assessing the impact of AIV infection on the juvenile population of these birds is also an essential aspect of understanding the manifestations of this disease in wild waterfowl. Knowing that AIV is causing subclinical disease would impact on wild waterfowl management decisions, particularly decisions concerning large concentrations of waterfowl.

C. Evaluation of the effects of AIV infection on the reproductive efficiency of adult Mallard hens

C.1. Narrative: Previous studies done in domestic poultry have shown that AIV is capable of causing marked decreases in egg production in infected chickens and turkeys. We also know that it is associated with immunosuppression and increased rates of infection with secondary organisms in these birds. Because AIV infection has been shown to have different degrees of pathogenicity in different hosts, we feel it would be worthwhile to evaluate its effect on egg production and egg hatchability in Mallard ducks.

C.2. Procedures: 100 female Mallard ducks will be reared in isolation to 6 months of age at which time half of the birds will be inoculated with a selected strain of Avian Influenza Virus, the rest remaining as uninoculated controls. Uninfected drakes will be added to each group. Egg production parameters such as number and size will be monitored. The eggs will then be incubated until hatching and the duckling's health will be assessed.

C.3. Budget:

	LCMR Funds	Matching Funds
a. Amount Budgeted:	$ 9,300	$ -0-
b. Balance:	$ -0-	$ -0-

C.4. Timeline for Products/Tasks:

	Jul91	Jan92	Jun92	Jan93	Jun93
Hatch Ducklings	*******				
Inoculate Hens		*****			
Assess Data			*********		
Final Report					*****

C.5. Status: After intravenous inoculation with an avian influenza virus isolate, A/Mallard/Ohio/184/86 (H5N1), isolated reared adult mallard ducks were evaluated for effects on reproduction. There was a significant decrease in egg production in the experimental group during the first week after inoculation, but it returned to normal production level during the second week. No effect was seen on egg weight, shape, or fertility. Due to the variability in pathogenicity exhibited by influenza viruese, it is likely that other influenza viruses exist in nature which may be even more pathogenic in waterfowl than the non-pathogenic isolated examined in this study. However, more work is needed using other AIV subtypes and a more natural route of infection to better assess the potential problem for waterfowl populations.

C.6. Benefits: Assessing the effect of AIV on the reproductive performance of Mallard duck populations provides valuable insight into the complex relationship which exists between disease and reproductive efficiency. This will provide valuable information to waterfowl managers.

IV. Evaluation:

The results obtained from each of the foregoing experiments will be analyzed statistically using conventional statistical methods and will be evaluated using a P value < 0.05. Each of the experiments performed will consist of comparisons between an infected group and an uninfected control group.

V. Context:

A. While numerous papers have been published documenting the presence of Avian Influenza Virus in wild waterfowl populations, very little has been done in regard to assessing the effect this virus has on the waterfowl themselves. In the past, most investigators have looked for evidence of viral replication but have not focused on the histopathological characteristics of influenza viruses in Mallards. Recently, however, investigators have shown evidence of pathological lesions in lung tissues of ducks infected with influenza viruses. Other investigators have documented renal pathology in chickens resulting from influenza virus infection. We feel that it is important, therefore, to more carefully evaluate the pathologic potential these viruses have in Mallard ducks.

B. The work proposed here will supply much needed baseline information on the pathogenesis of these viruses in Mallard ducks. The effect of influenza viruses on egg production, hatchability and duckling livability needs to be addressed.

C. The Avian Health Research Group at the University of Minnesota has been involved in Avian Influenza (AI) research since it was first detected in domestic turkeys in 1966. Subsequently, new introductions of AI have been detected every year in the Minnesota poultry industry. Research on AI at the university has addressed several issues over the past 23 years: diagnostic procedures, epidemiology, pathogenesis, control procedures, and vaccines. Results of this research (as well as the research by others) showed that wild waterfowl are the natural reservoir of AI virus, that the virus is commonly found in juvenile waterfowl, and that the virus most commonly enters the poultry industry during the period from August to November. We have also demonstrated that AI virus causes mortality, lack of weight gain, increased susceptibility to disease, and reduced egg production in chickens and turkeys.

D. Not Applicable.

E. Biennial Budget System Program Title and Budget: Not available at this time.

VI. Qualifications:

1. Program Manager:

Dr. David A. Halvorson
Professor, Department of Veterinary Pathobiology
University of Minnesota

D.V.M., University of Minnesota, 1967

Dr. Halvorson's primary expertise is in the area of avian health. He has worked on a wide variety of avian diseases including Avian Influenza Virus for the past 23 years. Dr. Halvorson's primary role will be to supervise the overall project objectives.

2. Major Cooperators:

A) Dr. Elizabeth A. Laudert
Graduate Assistant, Department of Veterinary Pathobiology, University of Minnesota

D.V.M., Colorado State University, 1987

Dr. Laudert's primary interests and expertise are in the area of virology and wildlife diseases. She has previously worked on projects involving the detection of viral diseases in arctic foxes. Her PhD thesis project involved the detection of AIV in surface waters as a means of defining the mechanism of transmission of AIV from wild waterfowl to domestic turkeys. Dr. Laudert's role will be as primary participant in all three objectives of the project.

B) Dr. Vaithianathan Sivanandan
Associate Professor, Department of Veterinary Pathobiology, University of Minnesota

Ph.D., University of Minnesota, 1979
M.S., University of Minnesota, 1976
B.V.Sc., University of Ceylon, 1965

Dr. Sivanandan's primary interests lie in the area of control of avian respiratory diseases. He has been involved in avian diseases for the past 17 years. His primary role will be participation in objective B.

VII. Reporting requirements:

Semiannual status reports will be submitted not later than January 1, 1992, July 1, 1992, January 1, 1993 and a final status report by June 30, 1993.

1991 RESEARCH PROJECT ABSTRACT
FOR THE PERIOD ENDING JUNE 30, 1993
This project was supported by MN Future Resources Fund (MS 116P.13)

TITLE: Effect of Avian influenza virus on growth and reproduction parameters in Mallard ducks
PROGRAM MANAGER: Dr. David A. Halvorson
ORGANIZATION: University of Minnesota
LEGAL CITATION: M.L. 1991, Chp. 254, Art. 1, Sec. 14, Subd. 9(q)
APPROP. AMOUNT: $16,000

STATEMENT OF OBJECTIVES

To evaluate the pathogenicity potential of avian influenza viruses in mallard ducks (*Anas platyrhynchos*) as well as to investigate their effect on growth rate in juvenile ducks and reproduction in adult ducks.

RESULTS

Obj. A - Ten avian type A influenza viruses consisting of seven waterfowl-origin, one pheasant-origin, and two turkey-origin viruses were evaluated for their pathogenicity potential after intravenous inoculation in mallard ducks (*Anas platyrhynchos*). The replicative abilities and tissue trophism properties of each virus isolate were examined. The waterfowl-origin viruses were isolated from a large number of tissues, but damage was minimal. Lymphoid depletion of the spleen and bursa was the most significant and consistent lesion noted. The remaining isolates were recovered from only a small percentage of tissues and their effects were minimal. Previous inoculation of turkeys and chickens with some of these isolates resulted in severe renal damage in those species, while in ducks, microscopic lesions seen in the kidney were very mild. This suggests that influenza viruses are capable of having negative effects on the immune system of ducks, but do not appear to cause significant lesions in other tissues.

Obj. B - An avian influenza virus isolate, A/Mallard/Ohio/184/86 (H5N1), was evaluated for its effects on growth rate in isolation reared juvenile mallard ducklings after intravenous inoculation. Ducklings receiving this influenza virus isolate did not differ from controls in their rate of growth. Bacterial isolation of experimentally inoculated *Escherichia coli* was repeatedly unsuccessful.

Obj. C - After intravenous inoculation with an avian influenza virus isolate, A/Mallard/Ohio/184/86 (H5N1), isolated reared adult mallard ducks were evaluated for effects on reproduction. There was a significant decrease in egg production in the experimental group during the first week after inoculation, but it returned to normal production level during the second week. No effect was seen on egg weight, shape, or fertility. Due to the variability in pathogenicity exhibited by influenza viruese, it is likely that other influenza viruses exist in nature which may be even more pathogenic in waterfowl than the non-pathogenic isolated examined in this study. However, more work is needed using other AIV subtypes and a more natural route of infection to better assess the potential problem for waterfowl populations.

PROJECT RESULTS USE AND DISSEMINATION

The results Obj. A were accepted for publication in Avian Diseases as a manuscript entitled "Comparative evaluation of tissue trophism characteristics in turkeys and mallard ducks after intravenous inoculation of type A influenza viruses," for publication in 1993. They were also presented as a poster at the 1992 American Veterinary Medical Association/American Association of Avian Pathologists (AVMA/AAAP) annual meeting in Boston, MA, August 2-6, 1992.

The results of Obj. B and C were accepted for publication in the Journal of Wildlife Diseases as a manuscript entitled "Effect of avian influenza virus infection on reproduction and growth in mallard ducks," for publication in 1993. These results will also be presented as an oral paper at the 1993 AVMA/AAAP annual meeting in Minneapolis, MN, July 17-21, 1993.

The results from all objectives were also submitted by Elizabeth Laudert as partial fulfillment of the requirements for the PhD degree, University of Minnesota, 1993.