EFFECT OF INTRAVENOUS INOCULATION OF AVIAN INFLUENZA VIRUS ON REPRODUCTION AND GROWTH IN MALLARD DUCKS

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ABSTRACT: An avian influenza virus isolate, A/Mallard/Ohio/184/86 (H5N1), was evaluated for its effects on reproduction in isolation-reared adult mallard ducks (*Anas platyrhynchos*) and growth rate in juvenile mallards after intravenous inoculation. There was a significant decrease in egg production in the experimental group during the first week after inoculation, but it returned to the normal production level during the second week. No effect was seen on egg weight, shape, or fertility. Ducklings receiving this influenza virus isolate did not differ from controls in their rate of growth.

Key words: Type A influenza, avian influenza, mallard duck, *Anas platyrhynchos*, egg production, growth rate.

INTRODUCTION

Avian influenza viruses (AIVs) generally are considered to be well adapted to their waterfowl hosts and therefore nonpathogenic in these birds; however, few researchers have examined the effect of AIV infection on waterfowl themselves. Clinical influenza has not been reported in wild, free flying ducks in North America; however, morbidity and mortality, primarily due to respiratory disease, in domestic ducks has been attributed to influenza virus infection (Tanyi et al., 1975; Ronohardjo, 1986). Growth retardation or stunting in domestic ducks has been reported following influenza virus infection (Ronohardjo, 1986) and we previously reported a depressed growth rate in ducklings inoculated intracranially with two influenza isolates in an intracranial pathogenicity index (ICPI) test (Laudert et al., 1993b). Birds are considered to be the primary reservoir of influenza viruses with the reported isolation of influenza viruses from 12 orders and 88 species of free-living birds (Stallknecht and Shane, 1988). Most reported isolations are from the orders Anseriformes and Charadriiformes, and all 14 hemagglutinin (H) and nine neuraminidase (N) subtypes have been isolated from waterfowl or gulls (Webster and Kawaoka, 1988; Kawaoka et al., 1989). Among the influenza subtypes found in ducks, one of those considered most pathogenic in poultry (H5) is found at very low frequencies in wild ducks (Webster et al., 1992; Sharp et al., 1993). These authors suggest that the duck may not be a reservoir for H5. However, sampling trapped ducks biases the sample toward healthy, more aggressive individuals so our detection of H5 in 13% of the isolates from sentinel ducks in 1981 may be a more realistic representation of the situation in nature (Halvorson et al., 1985). Thus we suggest that the H5 subtype could be more pathogenic than others in ducks as well as poultry. Disease caused by influenza virus infection could result in an adverse outcome for both the individual bird (death) and the population as a whole (decreased reproductive success). Our objectives were to evaluate the effect of intravenous inoculation of an avian influenza virus isolate of the H5 subtype on the reproductive abilities of adult mallard hens and drakes, and the growth rate of juvenile mallard ducklings. The intravenous route of challenge was selected to provide the virus with the best opportunity to demostrate pathogenic potential.

MATERIALS AND METHODS

The AIV isolate used in this study, A/Mallard/Ohio/184/86 (H5N1), was recovered from a cloacal swab of a hunter-killed mallard (*Anas platyrhynchos*) shot along the south shore of

TABLE 1. Percent egg production, mean egg weight, percent infertile eggs and early dead embryos in avian influenza virus-infected and non-infected control mallard ducks.

Parameter and treatment	Pre-infection	1 to 7 days post-infection	8 to 14 days post-infection
Egg production			
Infected	249 (71)[a]	207 (59)[b]	218 (62)
Control	247 (71)	242 (69)	216 (61)
Egg weight (g)			
Infected	—	50 (207)[c]	49 (218)
Control	—	50 (242)	49 (216)
Infertile eggs and early dead embryos			
Infected	—	20/187 (11)[d]	13/209 (6)
Control	—	23/226 (10)	17/206 (8)

[a] Number of eggs from 50 hens for the period (%).
[b] Significantly ($P < 0.05$) different from control.
[c] Number of eggs in parenthesis.
[d] Number of infertile eggs and early dead embryos/number of total eggs incubated (%).

Lake Erie (USA) during 1986 (Slemons et al., 1991). We used this isolate because of its great tissue distribution pattern and viral replication in mallard ducklings in comparison to several other avian influenza virus isolates (Laudert et al., 1993a).

Experiment 1: One hundred female and twenty-five male mallard ducks were obtained from a game farm (Wild Wings of Oneka, Hugo, Minnesota, USA) as day-old ducklings. They were reared separately in isolation on 12 hr of light (12L:12D) until 7 mo of age. They then were divided among four isolation rooms (25 females and six males per room) and exposed to artificial light for 16 hr each day (16L:8D). Approximately 2 m of linear nest box space and straw bedding were provided in each room. The ducks were given feed (University of Minnesota Feed Mill, Rosemont, Minnesota), water, and crushed oyster shell *ad libitum*. Eggs were collected twice daily for the next 2 wk and refrigerated at 6 C. Two weeks after the onset of egg production, hens and drakes in two rooms were inoculated intravenously with infectious allantoic fluid containing 1.25 × 10⁵ embryo infectious dose 50% (EID_{50}) of AIV. Ducks in the other two rooms were inoculated similarly with sterile saline and served as controls. After the ducks were infected, eggs were collected separately for 2 wk from all rooms daily and refrigerated at 6 C. At the end of each week, all eggs collected were counted, washed, labeled, and weighed. They were incubated at 37 C in a standard poultry egg incubator (Jamesway 252B,

James Manufacturing Co. Inc., Fort Atkinson, Wisconsin, USA) for 7 days. Following the incubation period, the eggs from each week were candled and categorized as being fertile and alive, infertile, or dead (fertile, but had died during incubation). Serum was collected from ten birds in each group on day 0 and 15 post-inoculation and antibody to AIV ribonucleoprotein detected by agar gel preciptin (AGP) test (Beard, 1970).

Experiment 2: Fifty-three isolation-reared, 2-wk-old mallard ducklings were randomly divided into control and experimental groups (27 and 26 birds, respectively) and weighed (day 0). Birds in the experimental groups were intravenously inoculated with allantoic fluid containing 1.1 × 10⁶ EID_{50} of AIV. Control group birds were intravenously inoculated with an equal amount of sterile saline. All birds were weighed 7 and 15 days later. Serum was collected from 10 birds in each group for AIV antibody analysis at all sampling periods. Antibody to AIV ribonucleoprotein was detected using the AGP test (Beard, 1970).

Two sample *t*-tests were used to detect significant differences between treatment and control group means (Zar, 1984). All P-values ≤0.05 were considered significant.

RESULTS

Experiment 1: The AIV infected group had a significantly ($P < 0.05$) reduced egg production during the first week post-inoculation (Table 1). Clinical disease was not seen in either group. The mean egg weights were not significantly different, and no misshapen or abnormal eggs were observed. The percentages of fertile, infertile and early dead eggs produced by each group were not significantly different. Serum samples collected from birds in both groups on day 0 were negative for AIV antibodies. All samples collected on day 15 from the experimental group were positive for AIV antibodies while the control group remained negative.

Experiment 2: No significant differences were detected in the mean body weights of birds in each group at any of the three sampling times. Serum samples collected from birds in both groups on day 0 were negative for AIV antibodies. All samples collected days 7 and 15 from the experimental group were positive for AIV

antibody while the control group remained negative.

DISCUSSION

The pathogenicity of different isolates of avian influenza viruses varies considerably depending upon host, subtype and individual isolate differences (Alexander, 1987). While almost all isolates are considered apathogenic, mildly to highly pathogenic isolates have caused considerable economic losses to the poultry industry (U.S. Department of Agriculture, 1985). Influenza virus infections also have been associated with decreased egg production in chickens (Halvorson et al., 1980), turkeys (*Meleagridis gallopavo*) (Samberg et al., 1982) and Japanese quail (*Coturnix coturnix japonica*) (Rinaldi et al., 1972). Infections in domestic birds also have been associated with increased numbers of abnormal eggs in turkeys (Mohan et al., 1981), increased proportion of infertile eggs in chickens (Alexander and Stuart, 1982) and decreased hatchability in Japanese quail (Rinaldi et al., 1972). The effect of influenza infection on the reproductive ability of waterfowl has not been examined previously.

With the virus isolate used in the present study, egg production was adversely affected after intravenous inoculation of influenza virus into adult mallards, but egg weight, shape, and fertility were not affected. Growth rate in ducklings inoculated at 2 wk of age also was unaffected. Due to the variability in pathogenicity exhibited by influenza viruses, it is likely that other influenza viruses exist in nature which may be even more pathogenic in waterfowl than the isolate examined in this study. However, more work is needed using other AIV subtypes and a natural route of infection to better assess the pathogenic potential for waterfowl.

ACKNOWLEDGMENTS

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