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## EXPERIMENTAL INFECTION AND SEROLOGIC SURVEY FOR SELECTED PARAMYXOVIRUSES IN RED-WINGED BLACKBIRDS (*Agelaius phoeniceus*)<sup>□</sup>

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**Abstract:** Red-winged blackbirds (*Agelaius phoeniceus*) were experimentally exposed to three avian paramyxoviruses: turkey parainfluenza virus, Yucaipa virus, and two strains of Newcastle disease virus. Aerosol exposure resulted in infection but exposure in food or drinking water rarely or never did. Tracheal swabs contained virus for up to eight days post exposure, cloacal swabs were negative. Transmission to contact birds occurred infrequently. Antibody response was of low titer and short duration. No hemagglutination inhibition activity against these viruses was found in 387 sera collected from red-winged blackbirds and tricolored blackbirds (*Agelaius tricolor*) trapped in six states.

### INTRODUCTION

From the evidence of virus isolation and serologic studies, the paramyxovirus of Newcastle disease clearly is present in wild waterfowl in North America.<sup>3,5,12,13,15,16,18,21</sup> Little is known about how transmission occurs in waterfowl or how widespread the virus is in other wild bird populations. However, antibodies to the virus have been demonstrated in wild turkeys (*Meleagris gallopavo*)<sup>8</sup> and a red-tailed hawk (*Buteo jamaicensis*),<sup>10</sup> and virus has been isolated from some other species.<sup>7,9,13</sup> Paramyxoviruses, other than Newcastle disease virus, have been isolated from exotic captive passeriforms in United States importation quarantine stations,<sup>14</sup> in Africa,<sup>4</sup> Ireland,<sup>11</sup> and in the Netherlands.<sup>19</sup> Whether these infections are acquired in captivity or are present in the wild populations is not known. The recent isolation of a Yucaipa like virus from a wild bird, a weaver finch (*Amadina fasciata*), in Senegal, Africa<sup>6</sup> is the first evidence of the presence of another paramyxovirus in a free-flying avian species. Resolution of the epidemiology of paramyxoviruses or even

interpretation of data from serologic surveys of wild birds is made difficult by a lack of information on the susceptibility, duration of infection, viral shedding, or immune response in these species.

The potential transmissibility of an infection spread by aerosol or fecal droppings would appear to be much greater in highly gregarious birds that breed or roost as dense populations. An example is the highly abundant red-winged blackbird (*Agelaius phoeniceus*) which spends over half the year in large wandering flocks. An opportunity was provided to test the susceptibility of the red-winged blackbird to several avian paramyxoviruses isolated in North America and to collect and to test sera from different red-winged blackbird populations for antibodies to these viruses.

### MATERIALS AND METHODS

#### Serologic survey

Red-winged blackbirds and tricolored blackbirds (*Agelaius tricolor*) were trapped and bled by the U.S. Fish and Wildlife Service at eight locations (Table

<sup>□</sup> This work was supported by a contract with the U.S. Fish and Wildlife Service. 14-15-0008-840 USFWS.

1). The serum was separated from the blood, frozen and shipped to the Department of Veterinary Science, University of Wisconsin. Included were sera obtained from live-trapped birds collected in the Denver area for experimental studies. All sera were maintained at -20°C until tested.

### Viruses

LaSota and Roakin strains of Newcastle disease virus (NDV), Yucaipa (Yucaipa/chicken/California/60)<sup>1</sup> and turkey parainfluenza (PI/turkey/Wisconsin/68) produce mild respiratory infections in chickens and turkeys. All were isolated in the United States from domestic fowl. Virus stocks were made by inoculating 9 to 10 day old embryonated chicken eggs intraallantoically and harvesting the allantoic fluid after 2 to 3 days of incubation. Virus stocks were maintained at -70°C and titers, determined by inoculating serial 10-fold dilutions into embryonated eggs, were expressed in 50% end points as embryo infectious dose (EID<sub>50</sub>).<sup>20</sup>

### Experimental infections

Red-winged blackbirds ranged in age from juveniles to adults and the majority were males. For exposure to LaSota and Roakin Newcastle disease strains and

turkey parainfluenza virus, the birds were divided into three groups of approximately 20 each, from which 4 to 6 individuals were removed to serve as contact controls, and returned 24 h after their cagemates had been exposed. Each group of birds was exposed to Newcastle disease or turkey parainfluenza viruses either by aerosol, food or drinking water. Exposure to Yucaipa virus was by aerosol only.

Aerosol exposure was accomplished using a nebulizer<sup>21</sup> either attached to an aerosol device<sup>2</sup> or to a hand operated rubber bulb to administer virus to birds enclosed in a plastic covered box. To prepare infected drinking water, virus was diluted in distilled water with 2.5 g skim milk powder added per liter of water as a stabilizer. Virus was added to feed by soaking the feed mixture (millet, milo and cracked corn) for 30 min in a dilution of the virus and then allowing it to drain. Food or water containing virus was placed before the birds for 9 to 12 h. Although no attempt was made to determine the amount of food consumed, water usage was estimated from the amount remaining when removed at the end of the day with an allowance for evaporation. Virus dilutions used as inocula were titrated in chicken embryos. Titers of virus dilutions used in the

TABLE 1. Locations of Red-winged blackbirds and Tricolored blackbirds from which sera were collected for testing in the hemagglutination inhibition test.

Location	Species	Number of sera	Date
Davis, California	Tricolored blackbird	9	July 1974
Davis, California	Red-winged blackbird	8	July 1974
Colusa, California	Red-winged blackbird	6	July 1974
Horicon, Wisconsin	Red-winged blackbird	17	Aug-Sept 1974
Brighton, Colorado	Red-winged blackbird	30	Sept 1974
Columbia, South Dakota	Red-winged blackbird	17	Sept 1974
Patuxant, Maryland	Red-winged blackbird	19	Oct 1974
Gainesville, Florida	Red-winged blackbird	27	Oct 1974
Denver, Colorado	Red-winged blackbird	254	1974-1976
Total		387	

<sup>21</sup> No. 166 Pyrex nebulizer, Vaponefin Company, Upper Darby, Pennsylvania.

exposure are recorded in Table 2. No attempt was made to titrate virus in food and water during exposure period.

Tracheal and cloacal swabs were obtained on alternating days post exposure (PE) starting at day 2 and continuing 8 to 10 days. Wires folded double and twisted to form a loop<sup>17</sup> were used to obtain swab samples. To reduce stress caused by this procedure, only half of the birds were swabbed on any one day. Swabs were eluted in brain heart infusion broth (BHIB) with 5% fetal calf serum and antibiotics. Samples were maintained at -20 C until inoculated into embryos for virus isolation.

Two birds from each group exposed to turkey parainfluenza were killed on days

2, 4, 6 and 8 PE. Swabs and samples of lung, liver, proventriculus, spleen, blood, kidney, small intestine, cecal tonsils and brain were taken from these birds for virus isolation. The tissues were ground in glass tissue grinders with BHIB and antibiotics and inoculated into embryos.<sup>20</sup>

All birds were bled from the jugular vein before exposure and at 3 and 6 weeks PE. Serum was separated and maintained at -20 C until tested in micro-hemagglutination inhibition (HI) test.<sup>20</sup> After heating at 56 C for 30 min, the serum was serially diluted and allowed to react with 4 hemagglutinating units of virus. Antibody levels were expressed as the reciprocal of the dilution of sera at

TABLE 2. Summary of virus isolations from tracheal swabs and positive HI tests from sera of red-winged blackbirds after exposure to several avian paramyxoviruses.

Virus	Exposure Route	Titer <sup>a</sup>	Total # Birds	Virus/# <sup>b</sup> Birds	Serology <sup>c</sup>
Turkey Parainfluenza	Aerosol	10 <sup>8.6</sup> EID <sub>50</sub> /ml	15	7/8	7/7
	Contact		4	NT	0/4
	Food	10 <sup>3.4</sup> EID <sub>50</sub> /ml	15	0/8	0/7
	Contact		4	NT	0/4
	Water	10 <sup>3.5</sup> EID <sub>50</sub> /ml	15	0/8	0/7
	(drinking) Contact		4	NT	0/4
LaSota (NDV)	Aerosol	10 <sup>9</sup> EID <sub>50</sub> /ml	13	5/10	10/13
	Contact		6	0/5	0/6
	Food	10 <sup>7</sup> EID <sub>50</sub> /ml	12	0/10	0/12
	Contact		6	0/4	0/6
	Water	10 <sup>7</sup> EID <sub>50</sub> /ml	12	2/10	1/12
	Contact		6	1/5	0/6
Roakin (NDV)	Aerosol	10 <sup>8.5</sup> EID <sub>50</sub> /ml	14	3/10	6/12
	Contact		6	1/5	0/5
	Food	10 <sup>8.5</sup> EID <sub>50</sub> /ml	14	0/12	0/14
	Contact		6	0/5	0/6
	Water	10 <sup>7</sup> EID <sub>50</sub> /ml	14	0/11	1/14
	Contact		6	0/5	0/6
Yucaipa	Aerosol	10 <sup>9.3</sup> EID <sub>50</sub> /ml	13	NT	8/12
	Contact		4	NT	1/4

NT = Not tested

<sup>a</sup>Virus titer of fluid used in exposure

<sup>b</sup>Number of birds from which virus was isolated/number of birds swabbed

<sup>c</sup>Number of sera positive for hemagglutination inhibition antibody/number of sera tested

which there was complete inhibition of hemagglutination.

## RESULTS

### Serologic Survey

No evidence of hemagglutination inhibition antibody against NDV, turkey parainfluenza and Yucaipa were found in the 387 sera collected from the red-winged and tricolored blackbirds trapped in 8 locations in 6 states.

### Experimental infections

Results of experimental infections have been summarized in Table 2. LaSota NDV was isolated from tracheal swabs from 2 birds in the aerosol exposure group on second day PE, and from 3 birds on PE day 5. Virus was not detected in swabs on PE day 8. In the drinking water exposure group, 2 birds exposed to the virus were positive (virus isolated from tracheal swabs) on PE day 2, and one of the contact controls was positive on PE day 5. No virus was isolated from swabs in the food exposure group. Pre-exposure sera contained no HI antibodies against NDV. At 3 weeks PE, 10 of 13 aerosol inoculated birds were serologically positive, as was one of the 12 water exposed birds. None of the 12 food-exposed birds developed HI antibodies. The range of HI titers was 0 to 16. Only one bird exposed by aerosol still had detectable HI antibody at 6 weeks.

Roakin NDV was isolated from tracheal swabs of one bird in the aerosol exposure group on day 2 PE, 2 birds on day 6 and in one contact control on day 10. One bird died 5 days following exposure and virus was isolated from the lung tissue only. Interestingly, the gross appearance of the internal organs suggested visceral gout. Six of the 12 aerosol exposed birds were positive for HI antibody at 3 weeks; the range of titers was 0 to 16. None of the contact birds had HI antibody. There was no detectable antibody by 6 weeks PE in any

of the birds. No swabs were positive for virus in the food or water exposed groups, and only one bird in the water exposed group had NDV HI antibody at 3 weeks PE.

In the turkey parainfluenza experiment, the 8 birds from each group that were swabbed PE were also used for the pathogenesis study. In the aerosol exposed group, one tracheal swab was positive on day 6 PE and 1 tracheal swab was positive on day 8. In the tissues examined, virus was recovered only in the lung in 7 of 8 birds tested and in the esophagus of one bird on day 8. Virus was not isolated from any birds that had been exposed to the virus by food or water. All birds in the aerosol exposure group developed HI antibody. The range of titers was 0 to 32. Antibody persisted for at least 9 weeks in 4 of the 7 birds that became serologically positive. None of the birds in the food and water exposure groups or any contact birds developed HI antibody.

Aerosol exposure to Yucaipa resulted in detectable HI antibody at 3 weeks in 8 of the 13 birds exposed. Titers ranged from 0 to 8. One contact control also became serologically positive. No attempts were made to isolate virus. By 6 weeks PE, only 2 birds had detectable antibody.

## DISCUSSION

Red-winged blackbirds were susceptible to the paramyxoviruses used in this study but detected infection was limited to the respiratory tract. Infection of few birds following drinking water exposure probably was the result of breathing virus aerosolized by splashing.

Virus isolated from tracheal swabs of 5 birds that did not develop detectable antibody suggests the local immune response restricts virus replication to the respiratory tract, eliminating the virus infection before there is an opportunity for substantial replication, viremia and

stimulation of high level of circulating antibody. Only the turkey parainfluenza produced an antibody response that lasted longer than six weeks.

The paucity of virus isolations from the tracheal swabs and complete absence from cloacal swabs indicates that only limited shedding of virus occurs during infection. It is improbable that this species could become a carrier of these viruses.

The information obtained from these experimental infections may be useful in interpreting and evaluating data from serologic surveys. The ephemeral nature of the antibody response to these viruses, the low titers of antibodies produced, the formidable task of obtaining an adequate sample of a population that is so large and which at the same time is short-lived, makes serologic surveys for paramyxoviruses in this species impractical.

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