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EXPERIMENTAL AVIAN PMV-2 INFECTION IN A DOMESTICATED WILD HOST: DAILY BEHAVIOR AND EFFECT ON ACTIVITY LEVELS

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ABSTRACT: Paramyxovirus type 2 (PMV-2) isolated from wild birds is often considered nonpathogenic, but nothing is known about its effects on overall behavior and fitness of free-flying birds. Domestically bred, African cut-throat finches (*Amadina fasciata*), a species from which PMV-2 has been isolated in the wild, were inoculated with a Central American field strain of PMV-2. Patterns of behavior were examined before and after viral challenge to quantify inapparent, sublethal effects of the disease. Infected birds demonstrated a significant decrease in activity ($P = 0.01$) followed by an apparent recovery period. Antibody titers confirmed infection in inoculated birds and indicated that sentinel birds did not become infected.

Key words: Paramyxovirus type 2, African cut-throat finch, *Amadina fasciata*, Yucaipa, behavior modification, ecology, avian, captive study.

INTRODUCTION

Disease associated with avian paramyxovirus type 2 (PMV-2) (Yucaipa-like) isolates ranges from inapparent disease to sudden death in cage birds, and from nonfatal respiratory disease to 90% mortality in domestic chickens and turkeys (Alexander et al., 1982; Alexander, 1982, 1986). Furthermore, isolations of PMV-2 from wild, free-flying birds are usually obtained from apparently healthy individuals (Nymadawa et al., 1977; Fleury, 1978; Fleury and Alexander, 1979; Tumova et al., 1979; Alexander, 1980; Kida et al., 1982; Lipkind et al., 1982; Ottis and Bachmann, 1983; Mbugua and Karstad, 1985). It is possible that inapparent PMV-2 infections alter the activity and behavior of their hosts rather than cause obvious clinical signs. We designed a model to test this hypothesis using computer-modulated, continuous recording equipment. This study is a preliminary attempt to quantify the effects of a finch-strain PMV-2 infection on the behavior of a natural host, and to gain further knowledge about paramyxovirus infections in wild birds.

MATERIALS AND METHODS

Experimental animals

Domestically bred, African cut-throat finches (*Amadina fasciata*) were obtained from local pet stores for experimental inoculation. This species is one from which a Yucaipa-like, PMV-2 virus has been previously isolated (Fleury, 1978; Fleury and Alexander, 1979; Alexander, 1982). All purchased birds tested negative for virus in cloacal swabs and were negative for antibody to the experimental and Yucaipa viruses.

Finches were caged individually several weeks prior to inoculation for adjustment to their surroundings. Birds were housed in isolation facilities on a photoperiod of 12 hr light, from 0700 to 1900 hours, and 12 hr dark. Food was continuously available and consisted of a variety of millet and canary seeds (NuPet® Finch Food from Fur, Fin, and Feather, Madison, Wisconsin 53707, USA), charcoal grit and oystershell kept in an open feeder, hung from the cage wall close to the floor. Cuttle bone and a water dish hung in a similar manner, with the latter supplemented with Avitron® vitamins (Lambert Kay, Cranbury, New Jersey 08562, USA). All maintenance and procedural tasks were performed by the same individual, to insure minimal disturbance.

Equipment

Steel bird cages (39 × 31 × 29 cm; Fig. 1) were modified to electronically record individual bird movements. A central aluminum extension from the cagetop suspended the floor so that it did not contact the cage walls but hung freely. The aluminum extension fit into a large spring and microswitch attached on top of the cage. Each mechanism was adjusted for individual bird mass, to sense and record every contact that a bird made with the floor. One activity event was recorded at every moment a bird changed position. No perches were available except for the sides of the food and water dishes.

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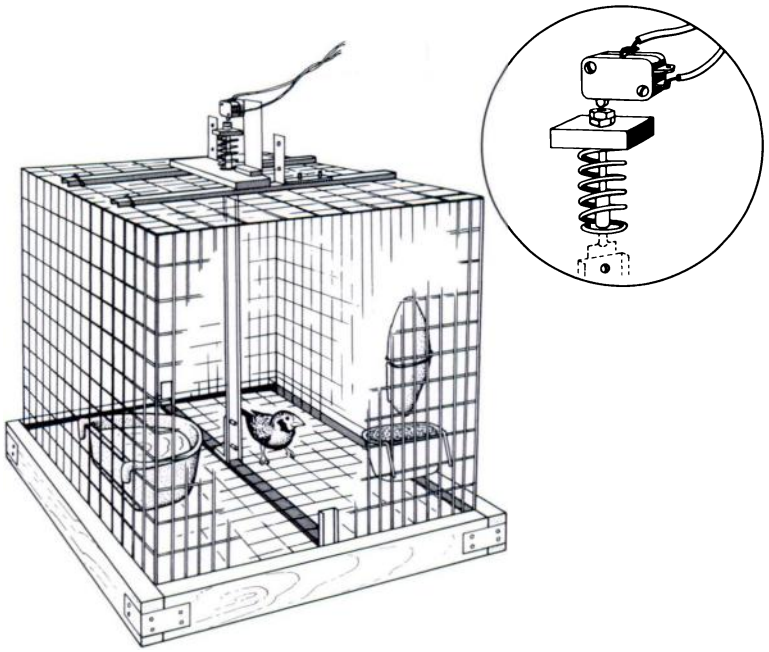


FIGURE 1. Diagram of an experimental cage and microswitch used to record finch activity. Cut-throat finch (*Amadina fasciata*) is perched on the floor that hangs freely from cagetop. Microswitch is in the open position.

Electronic impulses from the microswitches were received and stored by a Commodore SX-64 personal microcomputer. Post-acclimation activity of the finches was continuously monitored and summarized into 30 min records, from 14 days prior to inoculation until completion of the experiment.

Inoculation of birds

Two groups of finches were inoculated. One group consisted of seven adult females, the other group of three females and four males alternately placed in a row of neighboring cages. Each finch served as its own control for pretreatment versus post-treatment analysis. Two finches were given sterile phosphate buffered saline instead of virus inoculum to serve as sentinels for virus transmission. They were randomly selected with the criterion that they were surrounded by caged, virus-inoculated finches, and not at the end of a row.

Virus stock came from PMV-2 Costa Rican field isolate N-35 (Goodman and Hanson, 1988) aliquots #852 (for Group 1), and #864 (for Group 2). The aliquots had been passaged three times in 10-day-old embryonating chicken eggs prior to use. The inocula contained $\times 10^{9.3}$ and $\times 10^{8.9}$ embryo infectious dose 50% (EID_{50}) per 0.1 ml. A total of 0.1 ml was administered by a combination of nasal, oral and eye droplets and intraperitoneal injection.

Virus isolation and serology

Dacron swabs (1 to 2 mm width, Spectrum Laboratories, Inc., Houston, Texas 77032, USA) were used for all sample collections. Cloacal samples were collected on days 3, 7, and 12 postinoculation (PI), and placed in 1 ml of media containing Earle's lactalbumin hydrolysate, 2% fetal calf serum, potassium penicillin G, streptomycin sulfate, and sodium bicarbonate. They were immediately inoculated into chicken eggs for attempted virus isolation. This method has been used routinely to isolate PMV-2 from birds (Tumova et al., 1979; Ottis and Bachmann, 1983; Mbugua and Karstad, 1985; Goodman and Hanson, 1988). Tissue swabs were collected at necropsy on day 26 PI. Birds were bled from the jugular on days 21 and 26 PI and their sera tested for antibody. Hemagglutination (HA) and hemagglutination-inhibition (HI) tests were performed in V-bottom microtiter plates using two-fold dilutions, 0.025 ml aliquots and 1% chicken red blood cells. Antigen for the HI test was prepared using 4 HA units of virus. Observation from a hidden area was conducted daily for signs of respiratory and nervous disorder.

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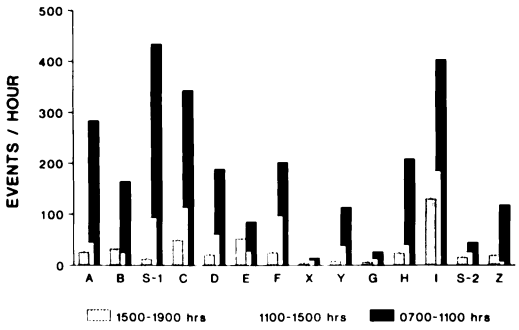


FIGURE 2. Daily pattern of finch activity before inoculation with PMV-2 or saline. Levels represent mean counts of activity for each hour during each time period. Letters represent individual birds. S-1 and S-2 are sentinels for virus transmission.

Data analysis

Statistical analyses of pre-inoculation versus post-inoculation finch activity were conducted using two-way analysis of variance (ANOVA, SAS Institute, Inc., Cary, North Carolina 27511, USA) and the Chi-square tests. Furthermore, in order to test for interference by time-dependent variables, a multivariate repeated measures analysis was done (SAS).

RESULTS AND DISCUSSION

Virus isolation and serology

Virus was not isolated from cloacal or tissue swabs. No attempt was made to detect viremia, in order that the finches' activity and overall behavior could be monitored with minimal disturbance. All virus-inoculated finches from the female group produced HI antibody to Costa Rican PMV-2, at titers from 1:32 to 1:128 on days 21 and 26 PI, indicating that infection had occurred. Two males and one female from the mixed group produced antibody with HI titers of 1:32 on day 26 PI. Sentinel finches did not produce antibody. Lack of virus recovery indicated a low viral replication, or problems attributable to the swab method. Bankowski and Corstvet (1961), Bradshaw and Jensen (1979) and Lipkind et al. (1982) reported isolation of Yucaipa virus from tracheal swabs, a method impossible to use on small passerines without excessive trauma. Lack of virus and antibody production in the sentinels suggested that the virus was not produced in a quantity capable of causing aerosol transmission in small groups. One male and two female finches (X, Y, and Z) had negative antibody titers of $\leq 1:8$ and were considered uninfected.

Daily activity patterns

Figure 2 illustrates the daily levels and pattern of activity of the finches before inoculation. As would be expected from a sample of wild birds, individual birds showed different amounts of daily activity; however, the activity level of all birds peaked each morning from 0700 to 1100 hours, and represented 69% of the mean daily activity. Between 0700 and 1500 hours, 90% of all daily activity occurred. This periodic cycle of activity was consistent for each bird prior to treatment. The circumstance of sentinel birds (S-1, S-2) having opposite activity levels was fortuitous.

Infected birds (A–I), with the exception of bird E, showed a progressive decline in daily activity (Fig. 3) after inoculation, followed by slight recovery towards the end of the experiment. Week 0 indicates the daily mean activity before treatment. It is evident that a decrease in activity occurred, especially between weeks 0 and 1. The average activity at the end of week 1 PI decreased 24%. Activity further decreased to −33% by the end of week 2, and increased only slightly to −30% by the end of week 3. Overall, infected birds A, C, F, H, and I demonstrated a significant difference between the pre- and post-virus inoculation activity at the $P = 0.01$ level, and birds D and G were significant at the 0.05 and 0.06 levels (Chi-square and two way ANOVA). Virus sentinels and bird B did not demonstrate a significant activity change. Analysis by multivariate repeated measures showed a quadratic difference ($P = 0.004$) in the time course of behavior between treated birds A–I (except E) and sentinel birds. Infected birds showed a trend of depressed activity followed by apparent recovery.

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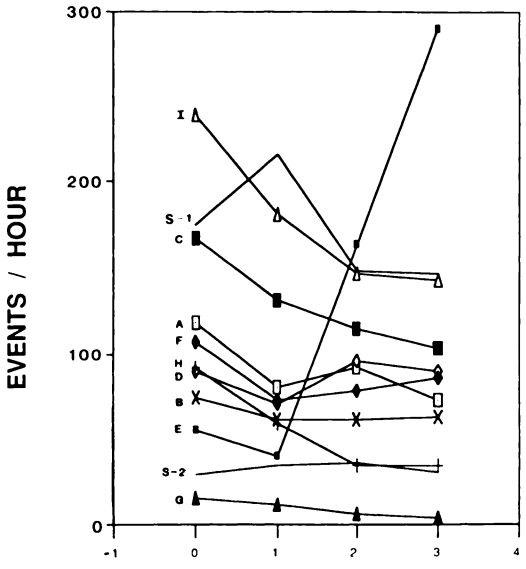


FIGURE 3. Mean hourly activity of infected birds A–I and sentinels before and after inoculation with Costa Rican PMV-2 or saline.

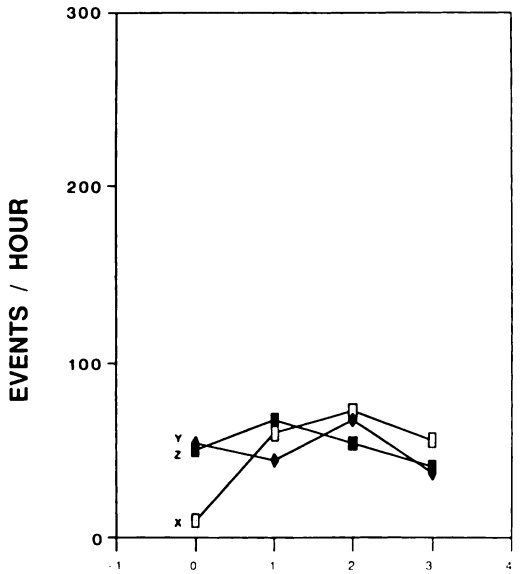


FIGURE 4. Mean hourly activity of inoculated birds X, Y, and Z. No significant change in activity occurred.

Bird E was exceptional in that it showed decreased activity the first week PI, followed by subsequent hyperactivity that lasted until termination of the experiment. It might have died if caged for a longer time period. In pilot studies with uninoculated finches, we observed that some birds increased their activity at an exponential rate prior to death. This phenomenon requires further study. Birds X, Y, and Z, which were not infected following inoculation, showed no significant change in activity (Fig. 4).

The average activity during 0700 to 1100 hours (Fig. 5) reflects the major activity changes of all birds, as it also represents the period of greatest daily activity. Analysis of activity between 1100 to 1500 hours, when 21% of total daily activity normally occurred, showed a continued significant decrease in activity for birds C, D, F, H, and I. During 1500 to 1900 hours, when only 10% of total daily activity normally occurred, birds C, F, G, and I also showed a significant decrease in activity. The decrease in activity throughout the day implies that no time period is free from an effect from PMV-2 infection. Observation several times daily revealed that infected birds would more frequently hide or rest under their water dish, rather than sing and display in a manner observed before infection.

Changes in behavioral parameters, such as in these daily patterns and levels of activity, should be considered when assessing the importance of apparently non-pathogenic infections in birds. Thus far they have not been studied. Matsuoka et al. (1980) found no clinical signs in amaduvade finches (*Estrilda amandava*) experimentally infected with PMV and suggested that the virus might not be virulent to them. Fleury and Faivre (1983) noted that pigeons (*Columba livia*) infected with Yucaipa-like virus showed no clinical signs of disease. Reports of PMV avirulence in non-domestic birds that are based solely on the absence of obvious clinical signs may be incomplete. Behavioral changes and physiological variables such as food consumption, metabolic rate and body

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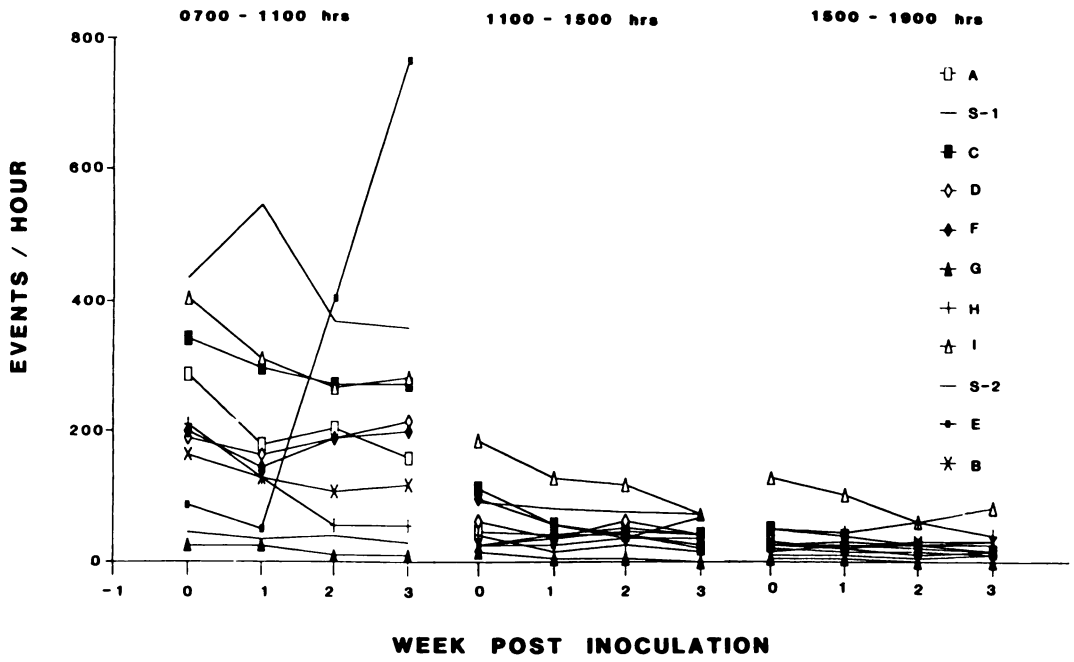


FIGURE 5. Mean levels and daily pattern of finch activity before and after inoculation with Costa Rican PMV-2 or saline. Letters represent individual birds. S-1 and S-2 are sentinels for virus transmission.

temperature all contribute to the health and overall fitness of wild birds.

The results of this study are preliminary in scope, whereas future studies would benefit from both a larger sample size and by increasing the timeframe and number of variables observed. The experimental cut-throat finches challenged with PMV-2 had food continuously available. It is not known whether their food consumption or weight decreased. These factors contribute to daily energetics of the birds and need to be assessed in future work. It is possible that in the field, limited access to food and water or altered environmental conditions during a PMV-2 infection might further alter bird behavior. Long term survival of wild birds depends on sufficient energy reserves to forage extensively for themselves and their young, compete for food, remain alert for predators, tolerate climatic extremes, successfully reproduce, and in some instances undergo the rigors of long-distance migration.

Clinical signs, serology, and histological changes alone may not be the best way to determine the pathogenic effects of apparently non-lethal avian infections. It is especially difficult to make inferences from laboratory studies about the effects disease might have on wild bird populations. Disease in an avian population may not have immediately discernable consequences; however, when viewed in context with the behavioral ecology, physiology, and environment of a particular species, a non-lethal disease might become a contributing factor or ultimate cause of death.

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