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THE INFLUENCE OF SEVEN ENVIRONMENTAL AND PHYSIOLOGICAL FACTORS ON DUCK PLAGUE VIRUS SHEDDING BY CARRIER MALLARDS

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ABSTRACT: Duck plague (DP) carrier mallards (*Anas platyrhynchos*) were subjected to seven environmental and physiological conditions in an attempt to stimulate DP virus shedding. The conditions were: food quality, social interaction, reproductive state, time dependency of food and water, noise, exercise, and sex of bird. Cloacal and oral swabs were taken daily for 10 days and assayed for DP virus content. The stimulated carrier ducks shed DP virus intermittently in amounts up to 10^6 ffus/swab/day (the highest 10-fold dilution still showing specific fluorescence). Unstimulated DP carrier ducks shed only up to 10^3 ffus/swab/day. Reproductive state and exercise were the only two factors that acted in concert to stimulate the shedding of virus in oral secretions.

INTRODUCTION

Duck plague (DP) (a herpesvirus infection of anseriformes) causes an acute highly fatal disease that can result in serious losses in commercial duck production (Walker et al., 1970) and wild waterfowl (Friend and Pearson, 1973). Duck plague also produces a persistent infection in apparently healthy birds that shed small amounts of duck plague virus (DPV) intermittently for up to 5 yr (Burgess et al., 1979).

Recurrent disease is common among herpesviruses with recrudescent shedding of virus over long periods of time (Klein, 1976). Viral shedding can be stimulated by naturally occurring stressors (Openshaw et al., 1979) or elicited by corticosteroids (Sheffy and Davies, 1972). Several physiological and environmental factors have been shown to alter the immune response, i.e., nutrition (Hudson et al., 1974), social stress (Gross, 1972; Mohamed, 1980), food and water deprivation (Spalatin and Hanson, 1974), hormone levels (Hudson et al., 1974), and noise (Monjan and Collector, 1977). In previous studies we have shown that within a group of DP carrier ducks, the intermittent virus shedding was occasionally synchronous, suggesting that some factor was acting on the birds to stimulate DP virus shedding (Burgess et al., 1979).

It is not known if the small amount of DPV shed by some carrier birds is sufficient for transmission to occur. The objective of this experiment was to determine if DP carrier mallards

could be stimulated to shed large amounts of virus and to attempt to determine what factor or combination of factors effect viral shedding. Seven environmental and physiologic conditions were chosen (food quality, social interaction, reproductive state, time dependency of food and water, noise, exercise, and sex of bird) in an attempt to simulate natural stressors.

MATERIALS AND METHODS

Birds

Six-mo-old mallard ducks free of DPV were obtained from McGraw Wildlife Foundation (Dundee, Illinois 60118, USA).

Virus infection

The mallards were infected with the LA-SD-73 isolate of DPV by contact exposure with other mallards known to be persistently infected with this DP virus isolate. LA-SD-73 DP virus could be detected by immunofluorescence (IF) from cloacal swabs taken after 2 mo of contact with the LA-SD-73 DP virus carrier birds. The birds were then placed in individual cages for 1 mo. The mallards were considered to be persistently infected with LS-SD-73 when the virus was detected by IF at the end of the month.

Housing

The birds were housed unconfined in an isolation room until they were used in the experiment. When the birds were used in the experiment they were housed in individual $14 \times 21 \times 18$ -inch metal cages enclosed on the three sides, top and bottom and with an open wire front.

Diet

The balanced diet consisted of 16.9% protein, 0.67% phosphorus and 0.8% calcium (ground corn, oats, wheat middlings, alfalfa meal, soybean meal, meat scraps, calcium carbonate, dicalcium phosphate, iodized salt, vitamin premix).

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TABLE 1. Environmental and physiologic factors used in the semi-iterative design.

N*	Variable	Level		Center replicate
		-	+	
1	Food quality	All corn	Balanced	½ corn + ½ balanced
2	Social interaction	One bird	Three resident + one test bird for 15 min daily	Two resident + one test bird for 15 min daily
3	Reproductive state	No gonadal development (8 hr light daily)	Gonads developed (20 hr light daily)	Regressing gonads (20 hr light followed by 8 hr light daily)
4	Time dependency of food and water availability	Ad lib.	Given one day, unavailable two days	Given on alternate days
5	Noise	No gunshot	22 blank gunshot once daily	22 blank gunshot every other day
6	Exercise	None	Treadmill ¼ mile/hr/15 min/day	Treadmill every other day
7	Sex	Male	Female	Male and female

* N = variable level.

Virus detection

Cloacal and oral esophageal swabs were eluted in 2 ml of diluent and examined by immunofluorescence (IF) for the presence of DP virus as previously described (Burgess et al., 1979). Tenfold serial dilutions of the supernatants were made, the highest dilution having specific DPV positive fluorescence (1 fluorescent focus unit or ffu) was considered the end point. Immunofluorescence was used as a detection because the virus causes cytopathic effect inconsistently.

Cell culture

Mallard embryo fibroblast cells were used for IF and neutralization tests. Cells were prepared from 14-day-old DPV-free embryos as described by Rozzo and Burke (1973).

Neutralization tests

Neutralization tests were done using the constant virus varying serum method as described previously (Burgess et al., 1979) using approximately 100 plaque forming units of LA-SD-73 DP virus and 80% plaque reduction.

Experimental design

A fractional factorial design was used to explore simultaneously the effects of the seven variables on DP virus shedding by carrier mallards. Fractional factorial designs require fewer experiments to estimate main effects of the variables and permit screening of large numbers of variables (Box et al., 1978; Porter and Busch, 1978). The environmental and physiologic factors and the levels of each are shown in Table 1. Each factor was assigned a plus, minus, and a center replicate value. Because space and logistical factors did not permit simultaneous testing of all runs, the design was broken into eight blocks of two runs (Table 2). The runs lasted for 10 days each, and were done two blocks at a time from May through December. Cloacal and oral esophageal swabs

were taken daily for each of the 10 days. The amount of virus shed each day and the total virus shed for 10 days (\bar{x} of $n = 2$) were determined by endpoint dilution of the IF. The blocks of the design were randomized into four sets. Two birds were used in each run (32 total plus 8 center replicates). Testing over several months also enabled estimation of uncontrolled variables (such as seasonality) that might occur during the year. The estimation of effects of uncontrolled variables was done by the use of center replicates, which consisted of four male and four female mallards persistently infected with DP virus that received the center replicate values each day for 10 days. Two ducks, a male and female, were repeated with each set of two blocks as center replicates. Since the same center replicate values were used each time, any variation in the amount of virus shed over the four center replicate runs would indicate an uncontrolled variable acting on virus shedding. The center replicates were only compared to other center replicates, not to test birds or control birds. The center replicate levels were arbitrarily picked and were not used to estimate effects of the conditions per se but only to determine if any untested factor was influencing the shedding of DP virus over time.

This design resolved the main effects of variables 2, 3, 4, 5, and 7, and the two-factor interaction of 3, 6 and 6, 7. Two control mallards (1 male, 1 female) persistently infected with virus were swabbed daily for 10 days. These birds had a balanced diet and water daily, 8 hr light, and were housed individually. These birds were used to determine if daily handling had an effect on DP virus shedding. The amount of virus shed by the control birds was compared to the amount shed by the test birds.

RESULTS

No single factor or two-factor interaction had an effect on DP virus shedding during the 10-day test period. The interaction of reproductive

TABLE 2. Total virus shedding by duck plague virus carrier mallards subjected to environmental stressors according to blocking patterns for semi-iterative design. The variable number and levels refer to those given in Table 1.

Block	Dates conducted	Variable number							Run number	$\bar{x} \pm \text{SE log virus shed}$	
		1	2	3	4	5	6	7		Cloacal	Oral
1	17–26 May	+	–	–	–	+	+	+	2	22.5 \pm SE	21.5 \pm SE
		–	+	+	+	–	–	–	15	21.5	16.5
2	6–15 Dec	+	+	–	–	–	+	–	4	12.5	13.0
		–	–	+	+	+	–	+	13	18.5	13.5
3	18–27 July	+	–	+	–	+	+	–	6	29.5	25.0
		–	+	–	+	–	–	+	11	25.5	30.0
4	17–26 May	+	+	+	–	–	+	+	8	16.5	18.5
		–	–	–	+	+	–	–	9	23.0	18.0
5	6–15 Dec	–	+	+	–	+	–	–	7	14.3	13.7
		+	–	–	+	–	+	+	10	13.5	13.5
6	8–17 June	–	–	+	–	–	–	+	5	22.5	21.5
		+	+	–	+	+	+	–	12	25.0	19.5
7	8–17 June	–	+	–	–	+	–	+	3	27.5	15.0
		+	–	+	+	–	+	–	14	24.0	22.5
8	18–27 July	–	–	–	–	–	–	–	1	34.0	31.0
		+	+	+	+	+	+	+	16	26.5	32.5

* \bar{x} = Total virus shed for the 10-day period; mean of two birds.

state and gonadal development augmented oral DP virus shedding on day 9. The effect was determined by adding or subtracting the \log_{10} of virus shed for each run according to the variable sign, i.e., +8, –2.5, +1, etc. (Box et al., 1978; Porter and Busch, 1978). The total was divided by the number of blocked runs (8) to give an estimate of the effect and this was compared to the pooled variance of the runs and the center replicates with a $P = 0.02$.

The DP carrier mallards were stimulated to shed up to 10^8 fluorescent focus units (ffus) of virus per swab per day while unstressed control carrier mallards shed up to 10^3 ffus/swab/day. Fifteen of 32 birds shed over 10^6 ffus/swab/day on at least one of the 10 days. Oral shedding by the carrier birds did not correlate with cloacal shedding.

The viral shedding by the stressed and unstressed control birds was intermittent over the 10-day period but the stressed birds shed more virus. Figure 1 is an example of the data accrued, using virus shedding by birds in run 6 as compared to the unstressed control birds. The overall mean for shedding for all the treated birds for the 10 days was significantly greater than the mean of virus shed for 10 days by the control birds ($P < 0.001$).

The results of DP virus shedding by the center replicate birds over the year's time period

are shown on Figure 2. Center replicate birds shed greater amounts of DPV in the feces in May than in June, July or December.

None of the DP carrier ducks had detectable DPV neutralizing antibodies either pre- or post-treatment with the seven factors.

DISCUSSION

Duck plague carrier birds can be stimulated to shed high levels of DP virus. The treated birds shed quantities of virus that were up to 10^5 -fold higher per day than the quantity shed by untreated control DP carriers in this or previous experiments (Burgess et al., 1979). The stimulated birds also shed significantly greater total quantities for the 10 days than did unstimulated control carrier DP birds.

No single factor tested had an effect on DP virus shedding. The only factors that interacted were reproduction and exercise, which had an augmenting effect on oral shedding of DP virus on day 9. Some unknown combination of factors did stimulate the high levels of both oral and cloacal DPV shedding on other days.

The cloacal shedding of large quantities of DP virus by the center replicate birds in May but not June, July and December indicates the influence of seasonal effects of a condition or combination of conditions other than one of those being tested. The stimulated DPV carriers

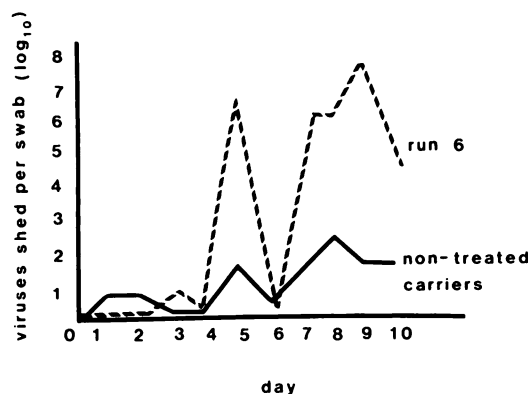


FIGURE 1. Duck plague virus shed by environmentally stressed (treated) and untreated mallards in one experiment (run 6).

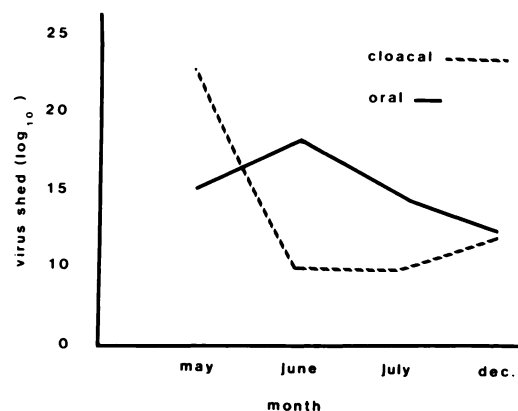


FIGURE 2. Seasonal changes in oral and cloacal duck plague virus shedding by carrier mallards (center replicate group, two birds per point).

shed DP virus intermittently as did the untreated control DPV carriers. The intermittent DPV shedding would indicate an unknown factor acting upon the birds, also.

None of the treated ducks died during or after the experiment. They were apparently healthy, shed large amounts of virus, and had no detectable serum neutralizing antibodies. If this situation occurs in wild or domestic flocks, DP carriers could go unrecognized. It is possible that DP carriers could exist in a flock for long periods of time, shed small amounts of virus and pose little risk to susceptible birds until stressed by some combination of factors at a given season, that would stimulate them to shed large amounts of DPV, thus contributing to a DP outbreak. This may have been the cause of repeated DP outbreaks at the same location (i.e., Fine Arts Museum, San Francisco, Calif. in April 1972 and 1974; Sacramento, Calif., May 1974 and February 1977; Islip, N.Y., May 1967 and December 1967 [Spieker, 1977]).

Our objective of determining if DP carriers could be stimulated to shed large quantities of DPV was achieved. These stimulated DP carrier birds would pose an increased potential risk to uninfected birds as much more DPV would be shed in the environment. It is still apparent that certain environmental or physiologic conditions can increase shedding. Other factors are likely to be involved in stimulation of shedding also. Waterfowl management practices should take these stressors into account when they have been more clearly defined.

Management of birds should focus on limit-

ing the amount of virus shed by DP carriers and the chance of susceptibles coming in contact with the virus. This would include minimizing crowding of birds for long periods of time as occurs during migration. In domestic flocks minimizing contact between breeding birds and juvenile birds plus a yearly turnover of the entire flock would be advisable.

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BOOK REVIEW . . .

Noninfectious Diseases of Wildlife, G. L. Hoff and J. W. Davis, eds. Iowa State University Press, Ames, Iowa, USA. 1982. 174 pp. \$19.95 US (clothbound).

In the preface to the book the editors indicate that this first edition represents an experimental effort to bring together some of the diffuse knowledge on noninfectious diseases of free-ranging and captive wildlife. They have succeeded by describing some of the noninfectious disease syndromes, and in general those covered were well written and provide a good reference source.

This volume is best reviewed by discussing omissions of noninfectious disease syndromes. Specific deficiency diseases were discussed under nutritional and metabolic diseases, but the malnutrition/starvation complex was not covered. This syndrome has a greater impact on wildlife populations than any other disease condition and its omission was a major oversight. A chapter was devoted to tooth anomalies, but anomalies of other systems were not covered. Some lesser omissions include: dystocia (difficult birth); rumen overload; bloat; indigestion (gastritis and enteritis of noninfectious origin); urea poisoning; dermatosis of noninfectious origin; physiologic monitoring of condition and nutritive status using blood, hair, urine, milk, marrow and other tissues; atherosclerosis in caribou and reindeer (*Rangifer tarandus*); capture myopathy in muskox (*Ovibos moschatus*); hair element analyses for monitoring toxic element accumulation; and chronic wasting disease (CWD) in elk (*Cervus elaphus*). The editors did not imply that the book would cover all noninfectious diseases and therefore, some omissions were anticipated. The omissions listed above are based upon one person's experience and are not necessarily complete, but demonstrate the potential for future editions.

It is most difficult to organize a treatise on noninfectious diseases, and particularly with a multiple authored volume. This volume suffers somewhat from that problem. There is a good deal of overlap in the chapters on shock and stress, particularly on background and pathogenesis. However both are excellent chapters and stand well alone. Hypothermia and

hyperthermia are discussed in three chapters (Shock, Stress and Disease of the Cardiovascular System, and Physical Injury). These topics deserve a chapter or section on their own. Nutritional diseases were discussed under birds, mammals, and reptiles. Perhaps, this should have been the system used throughout the text.

The major organizational problem is that disease problems of free-ranging and captive animals were not adequately differentiated. Some discussions focus on both and/or one or the other, but the text would be more useful if there was separate coverage. The book could be divided into two parts; captive and free-ranging. Under each of these sections, diseases could be discussed under birds, mammals and reptiles. Further classification under each of these could be considered. This arrangement would make the book much more useful and give it a semblance of organization.

The last major problem with the book is the lack of discussion in many of the diseases covered of the significance of the disease on populations. It is important that this be covered to avoid misdirecting the reader as to the relative importance of the disease. It may be important to an individual animal, but insignificant to the population. This brings forth another reason for separating the book into free-ranging and captive sections. To a zoo keeper the impact of a disease may be altogether different from that of a wildlife biologist.

The book is a start in recognizing the importance of noninfectious diseases in wildlife. As persons involved in wildlife diseases become more oriented to preventive medicine and place clinical medicine in a proper perspective, noninfectious wildlife diseases will receive greater emphasis. The editors and authors should be commended for their efforts. They should go forward with the concept and build from this pioneering experiment.

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