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EVIDENCE OF EXPOSURE OF WATERFOWL AND OTHER AQUATIC BIRDS TO THE HEMAGGLUTINATING DUCK ADENOVIRUS IDENTICAL TO EDS-76 VIRUS

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ABSTRACT: Serum and fecal samples from 12 species of aquatic birds were studied for evidence of exposure to a hemagglutinating duck adenovirus (DAV). DAV is serologically indistinguishable from egg-drop syndrome-76 virus. A total of 285 serum samples were tested by the hemagglutination-inhibition (HI) test. Forty-two percent of the birds had HI antibodies, with titers ranging from 8 to 256. Wild ducks showed the highest frequency of antibodies (56%) while in coots and grebes, antibody was less frequent, 33% and 26%, respectively. Attempted virus isolations from 79 fecal samples were unsuccessful. The data support the hypothesis that DAV is indigenous in wild duck populations and suggest that infection and viremia are limited in time and occur at a very early age.

INTRODUCTION

Egg-drop syndrome-76 (EDS-76), a disease of laying hens, is characterized by a drop in egg production accompanied by misshapen and soft shelled eggs. It was first described by Van Eck et al. (1976) in Holland and has since been recognized throughout Europe (McFerran et al., 1977; Badstue and Schmidt, 1978; Baxendale, 1978; Picault, 1978; Muelemans et al., 1979) and in Japan (Yamaguchi et al., 1981). From these outbreaks, several strains of a hemagglutinating adenovirus have been isolated which cannot be distinguished serologically. The isolates most commonly studied are V127 (McFerran et al., 1977) and BC-14 (Baxendale, 1978). In the United States, clinical EDS-76 is not known to exist. However, hemagglutinating adenoviruses serologically indistinguishable from the European strains have been isolated from clinically normal ducks (Villegas et al., 1979) and a clinically normal chicken (Schloer et al., 1978). One of these isolates, Duck Adenovirus (DAV) has been designated as the prototype of the EDS-76 virus in the United States (American Type Culture, 1983) and has been studied extensively in our laboratory (Piela, 1981; Gulka et al., 1982; Walker et al., 1982).

Serological studies of chicken flocks have indicated the presence of antibodies only on farms experiencing drops in egg production and virtual absence on farms with normal production (McFerran et al., 1977; Yamaguchi et al., 1981). Surveys of commercial duck flocks have shown a high frequency of antibody to the EDS-76 virus in the absence of any disease symptoms (Baxendale, 1978; Calnek, 1978; Malkinson and Weisman, 1980; Schloer, 1980). This has led to the suggestion that the virus is in fact a duck virus (Calnek, 1978).

Workers in Europe and Israel have found evidence of antibody to the EDS-76 virus in several species of wild waterfowl and other wild birds (Kaleta et al., 1980; Malkinson and Weisman, 1980; Bartha et al., 1982). In the United States surveys of wild ducks and other waterfowl have been limited to one study. Schloer (1980) showed very low prevalence of antibody in mallards (*Anas platyrhynchos*) and Canada geese (*Branta canadensis*). To date no other wild species of waterfowl have been examined.

The present study was undertaken to

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determine the prevalence of DAV (EDS-76 virus) in a variety of migratory waterfowl and other aquatic birds. Serum samples were tested for antibodies to DAV and virus isolation was attempted from fecal samples.

MATERIALS AND METHODS

Aquatic birds

Samples were obtained from wild ducks and other aquatic birds during ecological studies by the Department of Energy's Savannah River Ecology Laboratory, in Aiken, South Carolina. Between March 1979 and February 1981, samples from 12 species were collected. Ages and sexes were determined according to the method of Kortright (1953).

Virus isolation

Seventy-nine fecal samples from 10 species were used in this study. Each sample was diluted in 3 ml of phosphate-buffered saline (PBS) containing 500 U penicillin, 500 μg streptomycin, and 120 U mycostatin/ml. The fecal suspension was centrifuged at 1,500 RPM (383 *g*) for 10 min, and 0.2 ml of supernatant from each sample was inoculated into the chorioallantoic sac (CAS) of three 11-day-old embryonating Pekin duck eggs. Eggs were obtained from a commercial farm in Massachusetts. The allantoamniotic fluid (AAF) was harvested at 6 days postinoculation (PI) and tested for hemagglutination (HA) activity. AAF harvested from each sample was re-inoculated into three 11-day-old embryonating duck eggs. Samples remaining negative after being passaged three times were considered free of DAV.

Serum testing

Blood samples from 285 birds (12 species) were allowed to clot, the serum decanted and frozen at −20 C until tested. Serum was heat inactivated at 56 C for 30 min prior to testing for antibody in the hemagglutination-inhibition test.

Hemagglutination (HA) and hemagglutination-inhibition (HI) tests

HA and HI tests were performed by standard microtiter method (Palmer et al., 1975) using 0.025-ml volumes and 0.8% chicken erythrocytes. DAV, obtained from B. W. Calnek (Cornell University, Ithaca, New York 14850, USA) was propagated in embryonating Pekin duck eggs. AAF, diluted to contain four HA units, was used in the HI test. Sera were tested at a starting dilution of 1:8 and titers were expressed as the reciprocal of the highest serum dilution showing 100% inhibition of HA.

RESULTS

Virus isolation

Fecal samples included 15 from grebes, one from a coot, and the remaining 63 from ducks. All were negative for HA even after three passages in duck embryos.

Serology

HI tests indicated 121 positive samples out of 285 (42%), with titers ranging from 8 to 256 (Table 1). Grebes (Podicipediformes) had the lowest frequency of HI antibodies at 26%, followed by marsh birds (Gruiformes) at 33% of the birds sampled. Waterfowl (Anseriformes) showed the highest percentage, with 56% of the birds possessing antibody to DAV. When grouped by age, 36% of the birds less than 1 yr old and 43% of those greater than 1 yr possessed antibody. By chi-square, this difference was not significant. When grouped by sex, females showed a higher proportion of positive samples than males, 62% (58/93) to 36% (49/137). By the chi-square test, these results were significant at the 0.05 level.

DISCUSSION

The results indicate frequent occurrence of antibody to DAV in populations of wild waterfowl and other aquatic birds. Schloer (1980) reported 11/298 positive mallard sera and 11/74 positive Canada geese sera by the HI test. Since the present study only tested three mallards and no Canada geese, direct comparisons cannot be made. The prevalence of antibody found in the present study is in agreement with the 59% found in wild ducks (no species given) by Bartha et al. (1982) in Hungary. Kaleta et al. (1980) found positive sera from one of four storks (*Ciconia* sp.), one of two swans (*Cygnus cygnus*) and one of 18 geese (*Anser anser*) in West Germany. Again no direct comparisons

TABLE 1. Hemagglutination-inhibition titers of antibody to DAV, arranged by species. Data indicate the number of samples with each titer and the ratio of positive samples (≥8) to total number tested.

Scientific name	Common name	<8	8	16	32	64	128	256	Ratio*
Podicipediformes									
Podiceps auritus	Horned grebe	48	5	1	2	10	4	1	23/71
Podilymbus podiceps	Pied-billed grebe	21	0	0	2	0	0	0	2/23
Gruiformes									
Fulica americana	American coot	26	2	6	2	1	1	1	13/39
Anseriformes									
Oxyura jamaicensis	Ruddy duck	34	10	10	4	3	1	1	29/63
Aythya collaris	Ring-necked duck	11	2	2	2	9	1	1	17/28
Aix sponsa	Wood duck	11	3	2	2	6	1	0	14/25
Bucephala albeola	Bufflehead	5	2	5	1	2	1	0	11/16
Aythya affinis	Lesser scaup	2	0	1	1	3	2	0	7/9
Mergus spp.	Merganser	0	4	0	0	0	0	0	4/4
Anas platyrhynchos	Mallard	0	1	0	0	1	1	0	3/3
Anas strepera	Gadwall	0	2	0	0	1	0	0	3/3
Anas clypeata	Northern shoveler	0	0	0	0	1	0	0	1/1

* Ratio of positive sera to total number tested.

can be made since none of these species were tested in the present study. However, it is evident that antibody to the virus is common.

The highest occurrence of antibody appeared in the birds most closely related to domestic ducks, the Anseriformes. These data support the hypothesis that the virus is indigenous in duck populations (McFerran, 1977; Calnek, 1978), and indicate that wild ducks are as susceptible as domestic ducks. In addition, a variety of other aquatic birds exhibited significant serological evidence of exposure to DAV.

The data suggest that birds acquire immunity to DAV at an early age, since there was not a significant difference between the two age classes tested. This is in agreement with Schloer (1980) who found that the prevalence of antibody to V127 increased up to 1 yr of age and then remained constant. Only 44% of 7-wk-old ducklings had antibody, whereas 91% of 1-yr-old ducks from the same farm had antibody. Older, breeder ducks had a similar frequency of 90%. The significantly higher prevalence of antibody in the female birds is unexplainable at the present time.

The lack of virus isolations further supports the hypothesis that DAV is an early infection. In chickens, adenovirus isolations are most frequent from very young birds (Khanna, 1966; Yates et al., 1976) and the period of virus excretion after infection has been short (Clemmer, 1972). Thus, the birds sampled in the present study may have been exposed to the virus at a very early age and were no longer actively excreting DAV. DAV has been isolated from 3-wk-old (Baxendale, 1978) and 36-wk-old (Villegas et al., 1979) Pekin ducks. Whether these isolations represent very recent infections or chronic shedding is not known. Actual patterns of virus excretion can only be ascertained through experimental infection in the laboratory. Another possibility for the lack of virus recovery may be the fact that the embryonating eggs used in virus isolation came from a flock serologically positive for DAV. The maternal antibody present in the eggs may have prevented replication of very low levels of virus.

The present study suggests that DAV may be widespread in the wild duck population in the Atlantic flyway. The potential exists for spread to other species. What this means in terms of possible threat to poultry in this country is still unknown, since the route of natural transmission is in need of further clarification. In addition, the American DAV isolate does not cause severe decreases in egg-laying by chickens (USDA, 1979).

The significance of duck adenovirus to wild waterfowl populations is unknown. All isolations of DAV have been from clinically normal ducks (Villegas et al., 1979), however the only manifestation of EDS is a drop in egg production. Egg production on duck farms is not monitored as closely as it is on chicken farms, and therefore the effect of EDS may not be noticed. Whether the virus affects production of eggs in wild waterfowl can only be determined under controlled conditions.

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BOOK REVIEW . . .

How to Write and Publish a Scientific Paper, 2nd Ed., Robert A. Day. ISI Press, 3501 Market St., University City Sciences Center, Philadelphia, Pennsylvania 19104, U.S.A. 1983. 175 pp. 11 tables. 14 illus. Paper $11.95, cloth $17.95 US.

Plainly and simply, if you write scientific papers or teach a technical writing course, get/adopt this book. It is great! This edition is slightly expanded over edition one (three new chapters and other new material), but if your old copy has been used as much as mine, it's time to replace it anyway.

Day's writing is clear, concise, organized, and funny. The 29 short chapters will guide you in virtually every detail of getting your papers published. Here are some chapter highlights: Ch. 1. What is a Scientific Paper? (". . . if the ingredients are properly organized, the paper will virtually write itself"). Ch. 2. How to Prepare the Title (most are too long). Ch. 5. How to Prepare the Abstract (not over 250 words. "Usually, a good Abstract is followed by a good paper . . ."). Ch. 6. How to Write the Introduction (among other rules, "it should state the principal results . . ."). Ch. 7. How to . . . Materials and Methods ("give a copy of your finished manuscript to a colleague and ask if he or she could repeat the experiments"). Ch. 8. Results ("present representative data rather than endlessly *repetitive* data"). Ch. 9. Discussion (heed Day's six injunctions that will keep "the clear stream of the discussion" from ending "in a swampy delta"). Ch. 11. Literature Cited (contains an interesting section on the pros, cons and realities of various citation systems). Ch. 12. How to Design Effective Tables and (Ch. 13) Illustrations ("If the numbers just sit there with no exciting trend in evidence," . . . use the table approach). Ch. 15. Where and How to Submit the Manuscript ("If you submit . . . to a wrong journal, one of three things can happen, all bad"). Ch. 17. The Publishing Process (". . . if you read proof . . . at the same speed that you read scientific papers, you . . . miss 90% of the typographical errors"). Ch. 23. How to Write a Thesis ("Most 200-page theses I have seen are composed of maybe 50 pages of good science"). Ch. 26. Use and Misuse of English (read the "Ten Commandments of Good Writing"). Ch. 27. Avoiding Jargon ("Writers never *use* anything—they utilize").

Many anonymous reviewers have taken me to task, sometimes quite bluntly (seldom with humor), for vagaries in my writing. Thanks to Day, I'm improving. You can too. Buy the book.

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