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# A SURVEY OF NORTH AMERICAN MIGRATORY WATERFOWL FOR DUCK PLAGUE (DUCK VIRUS ENTERITIS) VIRUS

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ABSTRACT: A survey of migratory waterfowl for duck plague (DP) virus was conducted in the Mississippi and Central flyways during 1982 and in the Atlantic and Pacific flyways during 1983. Cloacal and pharyngeal swabs were collected from 3,169 migratory waterfowl in these four flyways, principally mallards (*Anas platyrhynchos* L.), black ducks (*Anas rubripes* Brewster), and pintails (*Anas acuta* L.). In addition 1,033 birds were sampled from areas of recurrent DP outbreaks among nonmigratory and captive waterfowl, and 590 from Lake Andes National Wildlife Refuge, the site of the only known major DP outbreak in migratory waterfowl. Duck plague virus was not found in any of the samples. Results support the hypothesis that DP is not established in North American migratory waterfowl as an enzootic disease.

## INTRODUCTION

The first known outbreak of duck plague (DP), or duck virus enteritis, in North America occurred in the white pekin duck industry on Long Island, New York, in 1967 (Leibovitz and Hwang, 1968). The suspected modes for introduction of DP to North America included importation of infected birds, trans-Atlantic migration of wild waterfowl from enzootic areas, and DP virus-contaminated equipment or personnel from enzootic areas in Europe (Leibovitz and Hwang, 1968; Newcomb, 1968). During 1967–1971, DP outbreaks occurred in commercial, avicultural, and other captive and feral waterfowl flocks in New York, Maryland, and Pennsylvania (U.S. Animal Health Association, 1973; Hwang et al., 1975). Except for losses of several hundred wild waterfowl on Flanders Bay, New York, during 1967 (Leibovitz, 1968), outbreaks were not known to occur among free-flying migratory waterfowl.

In January 1973, the first major outbreak of DP in migratory waterfowl occurred at Lake Andes National Wildlife Refuge (NWR) in South Dakota, where over 40,000 of 100,000 mallards wintering at the refuge died (Friend and Pearson, 1973). Since 1973, DP outbreaks have occurred sporadically in domestic and captive-reared waterfowl and nonmigratory waterfowl associated with parks and zoos. In contrast, involvement of migratory waterfowl has been limited to small numbers of birds associated with the aforementioned outbreaks (Hwang et al., 1975; Hanson and Willis, 1976; Jacobsen et al., 1976; National Wildlife Health Laboratory (NWHL), unpubl. data).

The status of DP in migratory waterfowl is not known despite the apparent absence of mortality from DP in migratory populations since the 1973 Lake Andes outbreak. Recently, controversy has arisen concerning the status of DP. If DP is not enzootic in migratory waterfowl, then the disease could potentially become established and cause extensive mortality, similar to the 1973 outbreak at Lake Andes NWR. On the other hand, some believe DP is introduced by migratory waterfowl into domestic and captive waterfowl populations. Determination of the status of DP in migratory waterfowl is thus important for establishing management policies pertaining to its prevention and control, both in migratory and captive waterfowl.

The purpose of this study was to survey

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migratory waterfowl populations in the Atlantic, Mississippi, Central, and Pacific flyways for presence of DP virus. We present results of surveys conducted during 1982 and 1983.

## MATERIALS AND METHODS

*Field sampling:* The presence of DP virus was determined by analysis of cloacal and pharyngeal swabs taken from mallards in each of the four flyways, from pintails in the Pacific flyway, and from black ducks in the Mississippi and Atlantic flyways. These species were chosen as index species for each flyway in order to improve the chances for detecting DP virus. The selection of these species was based on: (1) evidence of involvement in previous DP outbreaks, (2) relative susceptibility to DP virus mortality, (3) ability to serve as carriers of DP virus, (4) species abundance and distribution, and (5) behavioral characteristics conducive to virus transmission. Data from mallards and black ducks were combined and considered as one sample group because of their close phylogenetic relationship (Heusmann, 1974); and because in areas where they were selected as index species, they occupy similar niches and commonly hybridize.

Birds were sampled on wintering grounds in the Central and Mississippi flyways during January–March 1982, and in the Atlantic and Pacific flyways during January–June 1983. Waterfowl were live-trapped in conjunction with state and federal waterfowl banding programs or were found freshly dead (<24 hr) during epizootics due to other causes. A sample size of 459 birds from each index species within each flyway was required to be 90% confident that DP virus would be detected if it was being shed in 0.5% or more of the population.

Waterfowl were also sampled, using the same criteria, in specific areas where DP has been a recurrent disease problem in nonmigratory and captive waterfowl (U.S. Animal Health Association, 1973; Montgomery et al., 1981; NWHL, unpubl. data). Such "enzootic areas" (for DP in nonmigratory and captive waterfowl) included Long Island, New York, and the Eastern Shore of Maryland (Atlantic flyway); and the Sacramento area of California (Pacific flyway). In the Atlantic flyway, mallards and black ducks were sampled in enzootic areas. In the Pacific flyway, waterfowl sampled in enzootic areas included nonmigratory and feral waterfowl located at city park settings and exotic waterfowl at the Sacramento Zoo because of the unavailability of migratory mallards and pintails at these areas. A sample of 590 mallards was also obtained during February 1982 at Lake Andes NWR, South Dakota, at the site of the 1973 DP outbreak.

*Duck plague virus isolation and identification:* Cloacal and pharyngeal swabs from each bird sampled were combined in a tube of viral transport medium consisting of Hanks' balanced salt solution with 0.5% gelatin containing 1,500 IU of penicillin, 1,500 μg of streptomycin, 100 μg of gentamicin, and 50 IU of nystatin per ml. Samples were sealed in Whirl Pac bags and frozen on dry ice for shipment back to the NWHL, where they were held at −70 C until assayed. In 1982, a 10% duplicate random sample was collected and sent to the National Veterinary Services Laboratory (NVSL), Ames, Iowa, as a quality control on NWHL isolation methods.

Samples were thawed and centrifuged at 200 *g* for 15 min; then 0.2 ml of supernatant was inoculated onto a muscovy embryo (MSDE-F) monolayer (Rovozzo and Burke, 1973) in 24-well (2-cm²) plates. The plates were incubated at 40 C for 14 days; medium was changed at 7 days and examined every 48 hr for viral cytopathic effect (CPE). If no CPE was observed, the cell layer was subjected to a freeze-thaw, and the cell suspension was inoculated onto a MSDE-F monolayer in 2-cm²-chambered slides. As an internal NWHL control, these specimens were also blind passed to 96-well plates (1 cm²/well) containing a MSDE-F monolayer. After 7 days of incubation and observation, the slides were stained with a fluorescein-conjugated anti-DP virus reference serum made in sheep (NVSL, Ames, Iowa; E. C. Burgess, Univ. of Wisconsin–Madison) and viewed with an incident light ultra-violet microscope. If CPE was observed, a serum neutralization test (Tokumaru, 1969) using reference DP virus antisera (E. C. Burgess, Univ. of Wisconsin) was used to determine if the viral isolate was DP virus. Appropriate cell culture, serum, and antigen controls were incorporated into these assays.

In addition, birds were examined for the presence of lesions resembling sublingual erosions associated with some birds persistently infected with DP (Burgess et al., 1979). Such birds were killed, frozen, and submitted to the NWHL for DP virus isolation and histopathologic studies.

## RESULTS

Tests for DP virus were carried out on cloacal and pharyngeal swabs from 3,169 migratory waterfowl in all four flyways,

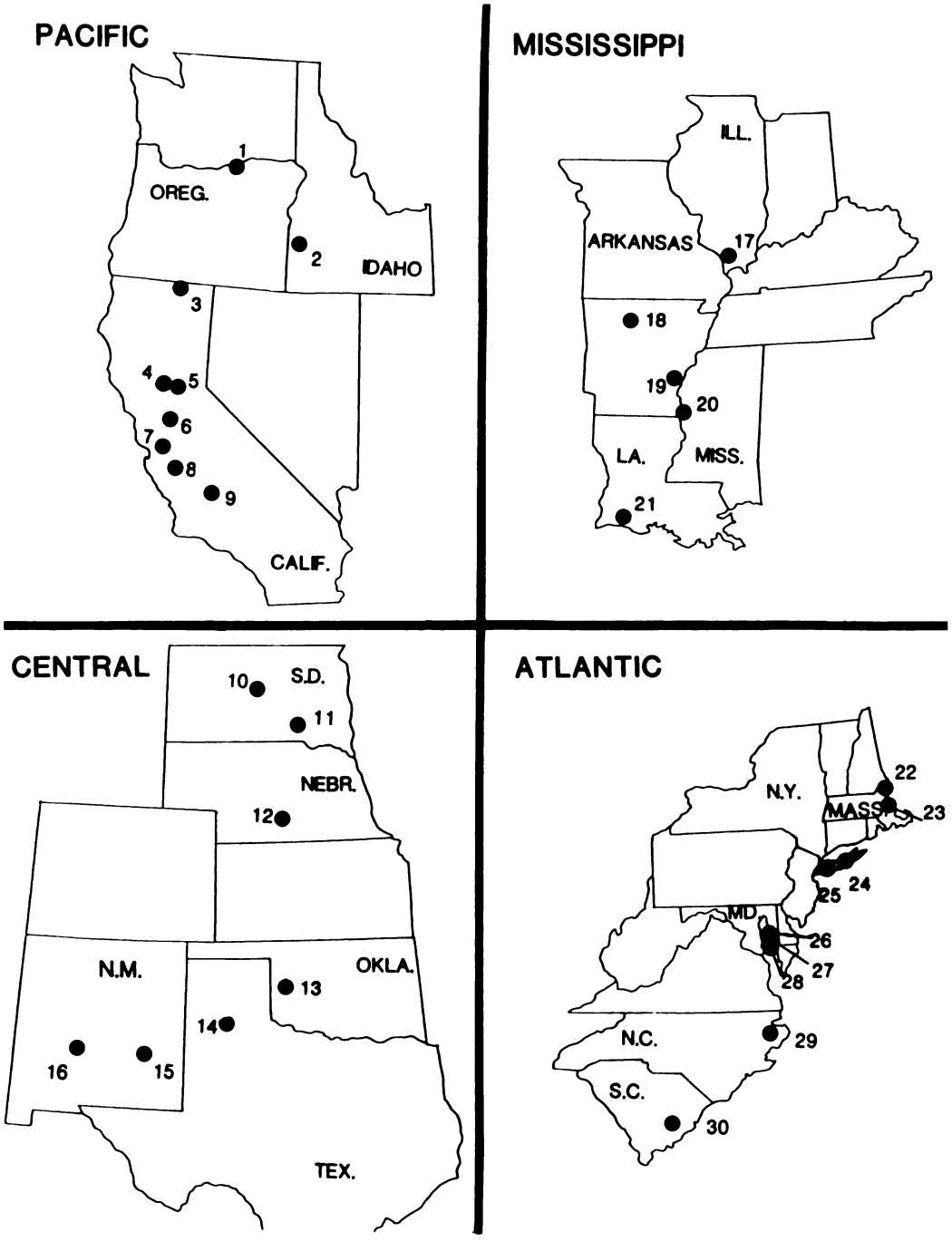


FIGURE 1. Duck plague survey collection sites in the four North American flyways. Refer to Table 1 for sample locations, numbers, species sampled, and sampling dates corresponding to site numbers.

TABLE 1. Collection sites and numbers of samples[a] tested for duck plague virus. Enzootic areas refer to areas where duck plague outbreaks have recurred in nonmigratory and captive waterfowl.

| Location | Site no.[b] | Date | No. samples tested | | | |
|---|---|---|---|---|---|---|
| | | | Mallards | Black ducks | Pintails | Other species |
| Pacific flyway | | | | | | |
| Umatilla NWR[c], Oregon | 1 | Jan 83 | 185 | | | |
| Deer Flat NWR, Idaho | 2 | Jan 83 | 88 | | 23 | |
| Tule Lake NWR, California | 3 | Dec 82 | 48 | | 5 | |
| Lower Klamath NWR, California | 3 | Dec 82–Jan 83 | 268 | | 50 | |
| Sacramento NWR, California | 4 | Jan 83 | 44 | | 53 | |
| Delevan NWR, California | 4 | Jan 83 | 47 | | 46 | |
| Colusa NWR, California | 4 | Jan 83 | 67 | | 74 | |
| Sutter NWR, California | 4 | Jan 83 | 54 | | 20 | |
| Grey Lodge WA[d], California | 5 | Jan 83 | 33 | | 33 | |
| Grizzly Island WA, California | 7 | Jan 83 | 42 | | 49 | |
| Mendota WA, California | 8 | Jan 83 | 108 | | 177 | |
| Kern NWR, California | 9 | Jan 83 | 2 | | 44 | |
| Enzootic area | | | | | | |
| Sacramento, California | 6 | Apr–Jun 83 | 273 | | | 56[e] |
| Flyway total (1,890) | | | 1,259 | | 574 | 57 |
| Central flyway | | | | | | |
| Pierre, South Dakota | 10 | Jan 82 | 120 | | | |
| Lake Andes NWR, South Dakota | 11 | Feb 82 | 590 | | | |
| Rainwater Basin, Nebraska | 12 | Jan 82 | 220 | | | |
| Washita NWR, Oklahoma | 13 | Jan 82 | 23 | | | |
| Playa Lakes, Texas | 14 | Feb 82 | 9 | | 3 | |
| Bitter Lake NWR, New Mexico | 15 | Jan 82 | 129 | | | |
| Bosque del Apache NWR, New Mexico | 16 | Jan 82 | 148 | | | |
| Flyway total (1,242) | | | 1,239 | | 3 | |
| Mississippi flyway | | | | | | |
| Union County WA, Illinois | 17 | Jan 82 | 66 | 12[f] | | |
| Horseshoe Lake WA, Illinois | 17 | Jan 82 | 23 | 5 | | |
| Holla Bend NWR, Arkansas | 18 | Jan 82 | 100 | | | |
| White River NWR, Arkansas | 19 | Jan 82 | 20 | | | |
| Yazoo NWR, Mississippi | 20 | Feb 82 | 201 | 1 | | |
| Lacassine NWR, Louisiana | 21 | Jan–Feb 82 | 36 | 1 | 5 | 3[g] |
| Flyway total (473) | | | 446 | 19 | 5 | 3 |
| Atlantic flyway | | | | | | |
| Parker River NWR, Massachusetts | 22 | Jan 83 | | 96 | | |
| Quincy, Massachusetts | 23 | Feb 83 | | 29[f] | | |
| Pungo NWR, North Carolina | 29 | Jan 83 | 104 | 6 | | |
| Santee NWR, South Carolina | 30 | Jan 83 | 243 | 5 | | |
| Enzootic areas | | | | | | |
| Wertheim NWR, New York | 24 | Jan 83 | 5 | 116[f] | | |
| Quogue, New York | 24 | Jan 83 | 10 | | | |
| West Crow Island, New York | 25 | Feb 83 | 31 | 113[f] | | |
| Eastern Neck NWR, Maryland | 26 | Jan 83 | 63 | 76[f] | | 1[g] |
| Eastern Shore, Maryland | 27 | Jan–Feb 83 | 68 | 78 | | |
| Blackwater NWR, Maryland | 28 | Jan–Feb 83 | 89 | 54 | | |
| Flyway total (1,187) | | | 613 | 573 | | 1 |

[a] Samples include cloacal and pharyngeal swabs from each bird.
[b] Refer to Figure 1 for location of collection site.
[c] NWR = National Wildlife Refuge (Federally administered).

including four samples from non-index species (Table 1). In addition, swabs were tested from 1,033 migratory, nonmigratory, and captive waterfowl in areas where DP appears enzootic in these latter populations; and 590 mallards were sampled from Lake Andes NWR. Figure 1 shows sampling locations within each flyway. Duck plague virus was not detected in any of the samples tested at NWHL, nor at the NVSL in the 10% duplicate samples they tested during 1982.

Three mallards were trapped at Eastern Neck NWR, Maryland, which had erosions in the buccal cavity. Attempts to isolate DP virus from the oral lesions, livers, spleens, intestines, and esophagus were unsuccessful; histopathologic studies did not show lesions or inclusion bodies typical of DP virus infection.

## DISCUSSION

The apparent absence of DP virus in free-flying waterfowl in this study suggests that the virus was not being shed, or that virus shedding was extremely infrequent (<1 shedder per 500 birds; $P < 0.1$) during the period tested. It is difficult to determine what the lack of evidence for DP virus shedding means relative to whether or not DP is enzootic in North American migratory waterfowl because DP infections can be characterized by periods during which virus shedding is not detected (Burgess et al., 1979). Virus shedding may be increased as a result of certain stressors or combinations of stressors. Burgess and Yuill (1983), for example, found increases in virus shedding related to the combination of reproduction and exercise, as well as to seasonal effects of other unknown factors.

Sampling design used in the present study maximized the likelihood of obtaining virus if it were being shed. Selection of index species, time periods of sampling, and location of sampling sites were based on the maximum potential for transmission and maintenance, and hence detection, of DP virus in migratory waterfowl populations. In addition, samples taken in the vicinity of recurrent outbreaks in captive and nonmigratory waterfowl, and at Lake Andes NWR, increased the likelihood of detecting DP virus if it persisted in migratory waterfowl in enzootic foci. In fact, a DP outbreak occurred among muscovy ducks in a city park near Sacramento, California (NWHL, unpubl. data), during April 1983, shortly after Pacific flyway migratory waterfowl were sampled with negative results. Additional samples (273) collected near Sacramento immediately after the outbreak, including 27 birds from the die-off site, were also negative for DP virus.

Previous attempts to assess the status of DP in North American wild waterfowl have been limited and have not demonstrated the presence of DP virus nor its antibody during non-epizootic periods. During 1967, 305 blood samples were taken from wild waterfowl in New York, Massachusetts, New Hampshire, Delaware, and Maryland in an attempt to evaluate the status of DP after initial outbreaks on commercial white pekin duck farms on Long Island (Dardiri and Hess, 1968). There were no positive (serum neutralization index > 1.75) titers in any of the birds tested. Asplin (1970), however, found significant titers in three of 291 (1.0%) mallards and none of 219 other waterfowl sampled in England during 1968–1969.

Results of this survey support the hy-

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[d] WA = wildlife area (State-administered).

[e] Includes 5 muscovy ducks, 15 white pekin ducks, and 1 grey goose from city parks; and 35 exotic waterfowl species held at the Sacramento Zoo.

[f] Includes mallard × black duck hybrids.

[g] Wigeon (*Anas americana* Gmelin).

pothesis that DP is not enzootic in North American migratory waterfowl. Our data, combined with previous survey findings and the absence of DP mortality in migratory waterfowl, suggest that DP is not currently established in these populations. Since the 1973 Lake Andes NWR outbreak, the occurrence of occasional mortality from DP in migratory waterfowl only in association with ongoing DP mortality among nonmigratory and captive birds indicates their continued susceptibility. It also suggests that observed DP mortality in migratory waterfowl may be a result of contact with infected nonmigratory and captive populations.

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