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# SEROLOGICAL SURVEY FOR RABIES ANTIBODIES IN RAPTORS FROM CALIFORNIA

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ABSTRACT: Fifty-three newly captive birds of prey were tested serologically for neutralizing antibodies against rabies virus, using a fluorescent focus inhibition test. No significant antibody titers were detected with this sensitive and specific technique in any of these birds. This study supports the contention that free-ranging birds of prey are of limited importance in the epidemiology of rabies.

*Key words:* Raptors, rabies, neutralizing antibodies, fluorescent focus inhibition test, serological survey.

## INTRODUCTION

The role of avian species in the epidemiology of rabies is unclear. It has long been thought that wild birds are neither a reservoir for rabies virus nor transmitters of the disease. However, in 1884 domestic fowl were experimentally infected with rabies virus (Gibier, 1884), and subsequently various species of domestic and wild birds have been infected (Kraus and Clairmont, 1900; Von Lote, 1904; Remlinger and Bailly, 1929; Reilly, 1968; Blancou and Samudio, 1979; Schaefer, 1983). Birds are not highly susceptible and usually recover after showing few or no signs of illness (Kraus and Clairmont, 1900; Von Lote, 1904; Jorgenson and Gough, 1976; Blancou and Samudio, 1979). Von Lote (1904) felt that raptors were more susceptible than either chickens or pigeons. Infection has been achieved by direct virus inoculation and by oral ingestion of rabid prey (Correa-Giron and Sulkin, 1970; Bell and Moore, 1971; Jorgenson and Gough, 1976; Blancou and Samudio, 1979). Skunks, bats, raccoons and foxes are susceptible to rabies and are often infected with the virus (Pacer et al., 1985). These mammals are included in the diet of raptors that consume large prey or carrion (Reilly, 1968), and could serve as potential sources of oral infection in free-ranging birds.

Naturally-occurring rabies disease in wild raptors has not been reported. Two studies have stated that rabies antibodies were present in wild caught birds and presented evidence that rabies infection had occurred. Gough and Jorgenson (1976) in Iowa tested 65 raptors using a passive hemagglutination technique and reported positive antibody titers in 23% of the birds. Using the same technique, Albers (1983) reported positive antibody titers in raptors from Florida. Further research by Gough and Jorgenson (1976) showed seroconversion of a great horned owl after experimental infection following ingestion of rabid prey. However, there is conflicting evidence. Trimarchi, Stafford and Becker in 1983 tested 43 raptors from New York and found them all to be negative for neutralizing antibody (unpubl., pers. comm.). Rosatte (1985) in Alberta, Canada tested five owls which were all negative as well. Confirmed cases of rabies in mammals have been found in all of these areas (Gough and Jorgenson, 1976; Albers, 1983; Rosatte, 1985; Trimarchi, pers. comm.).

California has a greater number of reported cases of rabies than Florida (Pacer et al., 1985), and rabies is endemic in 90% of California counties (1987 records, Veterinary Public Health Unit, Infectious Disease Section, California State Department of Health Services, Berkeley, California 94704, USA). If infection of raptors is a significant event and results in some nonfatal, immunizing infections, then rabies

antibodies should be demonstrable in raptors from California.

## MATERIALS AND METHODS

### Study areas

Five wildlife rehabilitation centers (University of California at Davis Raptor Center, Davis, California 95616, USA; Marin Wildlife Center, San Rafael, California 94915, USA; Alexander Lindsey Junior Museum, 1901 First Avenue, Walnut Creek, California 95496, USA; Wildlife Rescue, Inc., 4000 Middlefield Road, Palo Alto, California 94303, USA; Five Mile Creek Raptor Center, 1851 West Lincoln Road, Stockton, California 95207, USA) and the Zoological Medicine Service at the University of California Veterinary Medical Teaching Hospital (Davis, California 95616, USA) served as sources of birds. In general, birds are brought to these facilities because they have been found injured or in a weakened condition. Birds came from a total of 17 different counties, mainly in northern California. All of the counties are confirmed rabies areas, and three out of the four counties with the highest numbers of confirmed cases in 1985 were represented in our study. Many individual birds have large foraging ranges, so the exposure of these birds to rabies-positive prey may have been greater.

### Serum selections

Large raptors and scavengers were chosen, since they would be most likely to have fed on animals such as skunks and bats, which comprise the majority of species involved in rabies cases in California. Rabies virus has been shown to remain viable in carcasses for ≥2 wk (Schaefer, 1983). Between August 1985 and August 1986, 53 blood samples were taken from the following species: red-tailed hawk (*Buteo jamaicencis*, 24), golden eagle (*Aquila chrysaetos*, seven), bald eagle (*Haliaeetus leucocephalus*, three), great horned owl (*Bubo virginianus*, 14), and turkey vulture (*Cathartes aura*, five). Only birds that had been in captivity for <6 mo were sampled, since it has been shown that antibody titers may decrease over time (Jorgenson and Gough, 1976; Schaefer, 1983). One-third of the birds had been in captivity for <2 wk, and over one-half had been in captivity for <2 mo. A 2-ml blood sample was drawn from the brachial vein of each bird, allowed to clot, and serum was separated and frozen at −20 C until tested, up to 8 mo later.

### Serological testing

Sera were tested at the Viral and Rickettsial Disease Laboratory (VRDL; California State Department of Health Services, Berkeley, California 94704, USA) by the fluorescent focus inhibition test for neutralizing antibody against rabies virus (Lennette and Emmons, 1972), utilized for 18 yr in the VRDL to determine rabies neutralizing antibody titers of immunized individuals at occupational risk of rabies exposure. It has been extensively applied also to the analysis of sera from canines, equines and skunks, and has been found to be a sensitive and specific assay (Lennette and Emmons, 1972; Emmons, unpubl.). The test involves combining heat-inactivated (56 C, 30 min) test serum dilutions with 100–300 rabies fluorescent foci/50 of the Flury-LEP strain of rabies virus. Test controls include uninfected cell controls, back titration of the rabies virus challenge dose, negative rabies serum and titration of positive rabies antibody serum (titer 1:128). Reaction mixtures are incubated for 90 min at 35–37 C. Test mixtures and controls are inoculated onto monolayers of BHK 0853 cell cultures on glass slides which are then incubated 96 hr in a $CO_2$ incubator at 34–35 C. Slide cultures are then acetone-fixed (10 min, 25 C) and tested for rabies antigen foci by direct immunofluorescence staining, utilizing fluorescein isothiocyanate-conjugated immune hamster serum prepared in the VRDL. The rabies virus neutralizing antibody titer is the reciprocal of the highest serum dilution which inhibits the development of fluorescent foci in the respective cell cultures (Lennette and Emmons, 1972).

## RESULTS

Our results are summarized in Table 1. Of the 53 samples collected during a 1-yr period, 51 (96%) had titers less than 1:4, and the two (4%) remaining samples had titers less than 1:8 (the test could not be interpreted at the 1:4 dilution because of toxicity). Thus, there was no evidence of rabies neutralizing antibody found in these birds.

## DISCUSSION

Demonstration of specific antibodies to rabies virus in serum of man or animals is considered definitive evidence of natural infection or parenteral introduction of rabies virus or vaccine. A highly sensitive and specific method is desirable for determining rabies antibody titers. The mouse neutralization test is more cumbersome and requires a narrow range of virus challenge dose. The fluorescent focus inhibition test

TABLE 1. Avian species tested and results of the fluorescent focus inhibition test for neutralizing antibodies against rabies virus.

| Species | Number of birds tested (total) | Antibody titers | |
|---|---|---|---|
| | | <1:4 | <1:8[a] |
| Red-tailed hawk (*Buteo jamaicensis*) | 24 | 23 | 1 |
| Golden eagle (*Aquila chrysaetos*) | 7 | 7 | 0 |
| Bald eagle (*Haliaeetus leucocephalus*) | 3 | 3 | 0 |
| Great horned owl (*Bubo virginianus*) | 14 | 13 | 1 |
| Turkey vulture (*Cathartes aura*) | 5 | 5 | 0 |
| Totals | 53 | 51 | 2 |

[a] Sera could not be evaluated at a titer of 1:4.

for neutralizing antibody has largely replaced this method, since it is equally sensitive and more convenient (Lennette and Emmons, 1972; Thomas, 1975). In any test, problems with specificity may occur due to natural virus inhibitors in serum or cytotoxic effects of certain test sera, particularly in sera at low dilutions. Therefore, it is difficult to assess the significance of antibody titers at dilutions of $\leq$1:4.

In Gough and Jorgenson's (1976) study, rabies antibody titers of $\geq$1:2 were considered significant. However, in Albers' (1983) study, even though Gough tested the samples, only titers of $\geq$1:16 were considered indicative of rabies virus exposure. Using the latter criterion, only one bird in the Gough and Jorgenson (1976) study had a significant titer, rather than the 23% reported. In Albers' (1983) study, only four of approximately 62 birds tested (the actual number was unclear) had a titer of $\geq$1:16. These studies utilized a passive hemagglutination test (Gough and Dierks, 1971; Gough and Jorgenson, 1976; Albers, 1983). To our knowledge, this test has not been extensively compared with a neutralization test like the one used in our study, and it may not correlate with neutralizing antibody or with recovery from a non-fatal rabies infection. However, this point should be further studied.

The suggestion that the presence of rabies antibody is evidence that natural infection of avian predators has occurred rests on the assumption that some birds could undergo a non-fatal infection and that antibody would persist long enough and in sufficient titer to be detected. Our survey failed to detect specific neutralizing antibody in 53 raptors, but the survey was time-limited and such birds are difficult to collect in large numbers. Also, we were not able to determine the age of the birds accurately. A larger survey, including many older birds which probably have a greater chance for exposure to rabid animals, might detect evidence of what may be a very rare event. Further serologic studies should use the most specific and sensitive methods available and should explore the significance of various types of antibody response to rabies infection. Additional experimental infection models also might provide better understanding of the problem.

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