

SEROLOGICAL INVESTIGATION OF CAPTIVE AND FREE LIVING RAPTORS

by

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Abstract

Interest in the role of raptors in zoonotic diseases and concern over the disease agents that can be introduced into raptor colonies by birds from the wild prompted a serological investigation of birds available to the University of California at Davis. Seventy-one raptors of 14 species were tested for antibodies against two or more of the following disease agents: *Toxoplasma gondii*, *Coxiella burnetii* (Q Fever), Newcastle disease virus, adenovirus, reovirus, and infectious bursal disease virus. The techniques used included the indirect hemagglutination, microagglutination, hemagglutination inhibition, and agar gel precipitin tests. Collectively, 30% (21/71) of the birds were seropositive for Q fever rickettsiae antibodies, 8% (6/71) were seropositive for *Toxoplasma* antibodies, and one of 70 had antibodies against infectious bursal disease virus. Antibodies against Newcastle disease virus, adenovirus or reovirus were not detected. The species of raptors that had antibodies against toxoplasmosis were Red-tailed Hawk (*Buteo jamaicensis*) 23% (3/13), Red-shouldered Hawk (*Buteo lineatus*) (1/1), Great Horned Owl (*Bubo virginianus*) (1/10), and Turkey Vultures (*Cathartes aura*) (1/14). The raptors seropositive for *C. burnetii* were Golden Eagle (*Aquila chrysaetos*) 100% (6/6), Red-tailed Hawk 69% (9/13), Vultures 29% (4/14), Harris' Hawk (*Parabuteo unicinctus*) 14% (1/7) and Great Horned Owl 10% (1/10). A Red-tailed Hawk was seropositive for infectious bursal disease virus.

Introduction

Each year thousands of hawks, eagles, and owls are injured in accidents or shot by careless or ignorant hunters (Snelling 1975, Wisecarver and Bogue 1974, Fuller et al. 1974). Of these the vast majority are left to die; however, a few are taken to raptor centers or veterinary hospitals where they can be treated. While the loss of a wing or a foot predisposes the birds to a life of captivity, others recover from their injuries. These few are reeducated to forage for their food and are released into the wild.

Since raptors are protected by the migratory bird treaty, they are usually unavailable for the study of infectious diseases. Consequently, little is known about their exposures to agents responsible for zoonotic diseases or diseases common to domestic birds. These disease agents include such entities as *Tosoplasma gondii*, *Coxiella burnetii*, Newcastle disease virus, avian adenovirus, reovirus and infectious bursal disease virus. A brief description and reason for our interest in these infections of wild birds follows:

Toxoplasma gondii is the protozoan organism that causes toxoplasmosis in a wide variety of birds and mammals. Wild and domestic birds frequently harbor *T. gondii* cysts in their body tissues with no clinical evidence of illness; however, deaths among captive exotic birds and illnesses in domestic fowl have been reported (Ratcliff and Worth 1951, Jacobs and Melton 1966). Birds of prey are of special interest since *T. gondii* can be transferred through carnivores feeding on infected animals.

Coxiella burnetii is the rickettsia responsible for Q fever in man. While infection does not appear to cause clinical illness in birds, the organism can invade and persist in spleen and kidney tissues where it can serve as a source of infection (Bell 1971).

Newcastle disease is a contagious viral disease, primarily of avian species, but it can also cause conjunctivitis in man. The pathogenicity of the agent in birds depends upon the characteristics of the virus strain, and infections can range from minor to fatal. Lesions observed in wild bird species include exudative airsacculitis, petechial hemorrhages in the epicardium, enlarged liver, and catarrhal pneumonia (Palmer and Trainer 1971). However, recent reports of Newcastle disease in birds of prey indicate that gross pathological changes tend to be slight, variable, and nonspecific (Chu et al. 1976). The disease is highly infectious and can be transmitted via exudates, excreta, and eggs. Wild birds may harbor the virus for 30 days or more and are therefore capable of introducing the disease into raptor centers or among domestic poultry.

Avian adenovirus. Since its initial isolation from Bobwhite Quail in 1949 (Olson 1950), avian adenovirus has been implicated in diseases of chickens, turkeys, and captive game birds (Blalock et al. 1975, Dubose 1972, Fadly and Winterfield 1973).

Adenovirus has been the reported cause of a variety of clinical syndromes including fatal respiratory disease of Bobwhite Quail, inclusion body hepatitis of chickens, hemorrhagic enteritis of turkeys, and marble spleen disease of pheasants (Bickford 1974, Lini et al. 1973, Olson 1950; Winterfield et al. 1973). Also miscellaneous syndromes such as mild respiratory disease, egg production declines, and egg-shell-quality problems in chickens and turkeys have been described. Although recent evidence suggests that inapparent adenovirus infections are very common in domestic fowl (Boyle 1973, Cook 1970, Green et al. 1976, McMillan 1976), there is very little known about its potential pathogenicity in other avian species.

Avian reovirus. The reoviruses are common animal viruses, having been isolated from the feces and respiratory tracts of man and a variety of domestic and wild animals, including chimpanzees, monkeys, cattle, dogs, (Deshmukh and Pomeroy 1969, Lou and Wenner 1963), mice and birds (Macrae 1962, Rosen 1962). The avian reoviruses are ubiquitous among poultry populations and have been reported to cause cloacal pasting in young chicks, respiratory infections in chickens, enteritis in turkeys, and viral arthritis in chickens (Olson 1975). The incidence and effects of reovirus infections in other avian species are unknown.

Infectious bursal disease virus. IBDV causes an acute disease in susceptible 3- to 6-

week-old chickens, characterized by severe depression, ruffled feathers, trembling, and incoordination (Cosgrove 1962). Infection of susceptible chickens less than 2 weeks of age is usually inapparent (Hitchner 1971) but results in immunosuppression (Cho 1970), leaving affected birds more susceptible to other diseases (Faragher et al. 1972; Faragher, Allan et al. 1972, Wyeth 1975). The incidence and effects of IBDV infection in other avian species are unknown.

This report deals with serotesting of wild birds that were sampled in the wild or were brought to the Veterinary Medical Teaching Hospital (VMTH) for treatment or were maintained at the Raptor Center at the University of California, Davis.

Materials and Methods

Birds. A total of 71 raptors of 14 species were serotested. Sixteen of the birds were blood sampled during their initial examination upon admittance to the VMTH for treatment. Most of them were admitted for gunshot wounds of the wing. Thirty-two of the birds were from among the 200 being maintained at the UCD Raptor Center. They are birds that have been permanently impaired and are unable to survive in the wild or that have recovered from their injuries and are being retrained for release. The birds are fed newly hatched chickens and laboratory mice.

The vultures were captured in the wild in cooperation with the Department of Fish and Game. The serum samples were originally collected for the testing of antibodies against botulinum toxins (Ohishi et al. 1977). The remaining serum samples were submitted by persons licensed to raise raptors.

Most of the birds originated from central California with a few from as far south as Santa Cruz County or as far north as Butte County. The birds ranged from less than one to eleven years of age, and others of unknown age had been in captivity since 1974.

Serology. From 0.5 to 2.0 ml of blood was drawn from the wing vein using a 3 ml syringe and 25 g needle. The blood vial was laid on its side to maximize the clotting surface of the blood and increase the serum yielded. The serum was transferred to small vials and kept frozen until tested. Not all tests were done on each sample because of insufficient quantity.

The indirect hemagglutination (IHA) test was used to examine the sera for antibodies against *T. gondii*. One drop (0.025 ml) of serum was added to the first well of a microtiter plate, and doubling dilutions of serum from 1:2 to 1:4096 were made. The serum was tested at 1:64 using commercially available antigen.¹ An agglutination reaction of 2+ was considered positive, and the serum was tested to the end point. The 1:32 serum dilution was used for the nonsensitized cell control.

The 1:2 to 1:16 serum dilution was tested for antibodies against *C. burnetti* using the microagglutination (MA) method (Fiset et al. 1969). The antigen prepared from Nine Mile strain of *C. burnetti* in phase I was grown in the yolk sac of embryonated hens' eggs. The antigen was transformed from phase I to phase II activity using trichloroacetic acid and stained with hematoxylin.

The serum was tested for antibodies against Newcastle disease virus by the micro hemagglutination inhibition test (HI) (Lancaster 1966). Eight units of viral suspension and 0.05 ml of a chicken red blood cell suspension were used.

¹International Biological Laboratories Inc., P.O. Box 1247, Rockville, Maryland 20850.

Avian adenovirus antigen was prepared in specific pathogen-free (SPF) embryonating eggs by CAM inoculation with the CELO adenovirus strain (Woernle 1966, Yates 1975). Chicken sera from an adenovirus-antibody-positive flock were pooled and used as a positive control.

Avian reovirus antigen was prepared in chicken embryo kidney (CEK) cells (Olson 1975). Positive control sera was obtained from hyperimmunized 8-week-old SPF chickens.

Infectious bursal disease virus was propagated in three-week-old SPF chickens (Hirai and Shimakura 1972) via intranasal inoculation. The infected bursea were harvested, homogenized, and centrifuged, with the resulting supernate used as IBDV antigen. Positive control sera was obtained from hyperimmunized eight-week-old SPF chickens.

Agar gel precipitin plates (Miles Laboratories) were prepared, using 1.25% agarose (Sigma Chemical Co.) in barbital buffer (pH 7.8) with 8.0% NaCl and 0.01% sodium azide. Antigen was placed in the center well of a 6-well cluster, with 2 positive control sera. The plates were tightly covered and incubated overnight at room temperature then were examined in indirect light.

Results

Of the 71 birds tested, 6 (8%) were seropositive to toxoplasmosis, 21 (30%) had agglutinating antibodies against the Q fever rickettsiae, and one of 70 was reactive for antibodies to IBDV. None of the birds tested were seropositive for Newcastle disease virus, adenovirus or reovirus (table 1).

The highest prevalence of antibodies against *T. gondii* (23%) was among Red-tailed Hawks. The prevalence among the Red-shouldered Hawks is probably similar, but too few of the species were tested to estimate with certainty. In all cases the birds tested had low titers that did not exceed 1:64.

The antibody prevalence for *C. burnetii* was high among Golden Eagles (100%) Red-tailed Hawks (69%), and vultures (29%) (table 1). The median antibody titer for eagles and Vultures was 1:4 whereas the median titers among Red-tailed Hawks was 1:8 with two hawks having titers \geq 1:16.

Discussion

In a recent survey, serological evidence of exposure to *T. gondii* was found among 3.5% of 401 wild birds that were tested (Franti et al. 1976). The prevalence of this parasite among raptors appears to be more than twice that of nonraptors, probably reflecting the raptor habit of feeding on small rodents. Since the prevalence of *T. gondii* is approximately 2% among the species of rodents that raptors commonly feed upon, this mode of exposure seems likely in view of the large number of rodents consumed by these species of birds. Birds are probably an important intermediate host for *T. gondii* in nature since they are a primary food source for wild carnivores, including bobcats, a definitive host of this parasite.

Previous surveys of antibodies against *C. burnetii* among wild birds in association with livestock indicated a prevalence of 13% among birds on a sheep range and 38% among birds on a dairy farm (Enright, Longhurst et al. 1971). The highest prevalence (33% to 67%) was among species of birds that ate carrion (crows, ravens, and turkey vultures). Raptors tested here also appeared to be exposed to *C. burnetii* because of their feeding habits. Approximately 5% of the seed-eating birds and from 2% to 31%

of the small mammals and rodents that are a food supply of raptorial birds have serological evidence of being infected with *C. burnetii* (Enright, Franti et al. 1971).

The significance of individual antibody titers is obscure because little is known concerning zoonotic diseases of raptors. If raptors respond to *C. burnetii* infection the way mammals do, the following should apply: agglutinating antibodies are detectable

Table 1. Raptors Tested for Antibodies Against Various Diseases

Scientific Name	Common Name	Toxoplasmosis	Q Fever	Newcastle disease	Adenovirus	Reovirus	Infectious bursal disease
Cathartidae							
<i>Catartes aura</i>	Turkey Vulture	1/14*	4/14	ND	0/14	0/14	0/14
Elaninae							
<i>Elanus leucurus</i>	White-tailed Kite	0/3	0/3	0/3	0/3	0/3	0/3
Accipitrinae							
<i>Accipiter cooperii</i>	Cooper's Hawk	0/1	0/1	0/1	0/1	0/1	0/1
Buteoninae							
<i>Buteo jamaicensis</i>	Red-tailed Hawk	3/13	9/13	0/11	0/11	0/11	1/12
<i>Buteo lineatus</i>	Red-shouldered Hawk	1/1	0/1	0/1	0/1	0/1	0/1
<i>Buteo regalis</i>	Ferruginous Hawk	0/1	0/1	0/1	0/1	0/1	0/1
<i>Parabuteo unicinctus</i>	Harris' Hawk	0/7	1/7	ND	0/7	0/7	0/7
<i>Aquila chrysaetos</i>	Golden Eagle	0/6	6/6	0/6	0/6	0/5	0/6
Circinae							
<i>Circus cyaneus</i>	Marsh Hawk	0/2	0/2	0/2	0/2	0/1	0/2
Falconinae							
<i>Falco mexicanus</i>	Prairie Falcon	0/2	0/2	0/2	0/2	0/2	0/2
<i>Falco sparverius</i>	American Kestrel	0/3	0/3	0/3	0/3	0/2	0/3
Tytonidae							
<i>Tyto alba</i>	Barn Owl	0/7	0/7	0/7	0/7	0/7	0/7
Strigidae							
<i>Otus asio</i>	Screech Owl	0/1	0/1	0/1	0/1	0/1	0/1
<i>Bubo virginianus</i>	Great Horned Owl	1/10	1/10	0/10	0/9	0/9	0/10
Totals		6/71	21/71	0/48	0/68	0/64	1/70
Percent Positive		8	30	0	0	0	1

*Number positive / number tested

ND Not done

by 10 days after the host is infected, the antibody response reaches a peak by 20 days and begins to decline slowly thereafter (Fiset and Ormsbee 1968). Therefore, the low titers found among birds in this study probably reflect long past exposures. However, since both *C. burnetii* and *T. gondii* can cause chronic infections, the organisms can sometimes be recovered from hosts with little or no antibody titer.

Newcastle disease among raptors appears to be sporadic and is often linked with birds in captivity that are fed carcasses of dead poultry or game birds. Since Newcastle disease appears to have an affinity for colonial birds that frequent the environment of commercial flocks, the disease may be much less common among solitary living raptors. Nevertheless, surveillance and possibly a vaccination program should be maintained to guard against introducing the disease into raptor centers.

Interpretation of nonreactive agar gel precipitin (AGP) tests requires some caution. Precipitin antibody of low serum concentration may not be detected because of the relative insensitivity of the AGP test. Also, the AGP test may not detect virus-neutralizing antibodies. However, on the basis of previous experience with similar serologic surveys, the results reported here indicate that there probably have not been recent infections with any of the test viruses. The one exception was a single weakly positive reactor to IBDV. A larger sample size or experimental susceptibility tests would be necessary to assess the significance of this observation. However, this positive reaction suggests that raptors may be susceptible to IBDV infection.

The agar gel precipitin test has proved to be a valuable surveillance and diagnostic aid in the health management of domestic poultry. Its potential importance in the health management of captive avian species increases as more birds are placed in rehabilitation and breeding facilities. This simple test can be used to produce a serologic profile of existing captive birds, to screen new arrivals for inapparent infections, or to aid in the diagnosis of a disease outbreak. The AGP test could also be used to evaluate the effectiveness of vaccination programs in cases where experimental challenge of birds is unwarranted. A battery of AGP tests for potential pathogens including Newcastle disease virus, avian pox, and herpesvirus would provide valuable information for the health management of captive birds of prey.

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ANNOUNCEMENT

THE HAWK TRUST

Readers of *Raptor Research* may know relatively little about the work and aims of the Hawk Trust. It is hoped, in this and subsequent issues, to report some of the trust's activities.

The Hawk Trust is a British charitable organization which is concerned at the downward trend of many of our raptor populations. The aims of the trust are to carry out research into bird of prey breeding, ecology, and treatment of disease, as well as providing wardens at vulnerable breeding sites of rare species in conjunction with other conservation bodies.

A particularly successful event organized by the trust in 1977 was its Open Day on October 8. Despite very wet weather, nearly 100 people attended, amongst them several overseas visitors who had earlier been at the Oxford Conference. In addition to seeing the aviaries and learning something about the trust's work, the visitors were



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