EXPERIMENTAL RABIES IN A GREAT HORNED OWL

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Abstract: A great horned owl (*Bubo virginianus*) was fed the carcass of an experimentally infected rabid skunk. The bird developed antibody titer to rabies, detected by passive haemagglutination, 27 days after oral inoculation by ingestion. The owl suppressed the infection until corticosteroid administration, after which a maximum antibody titer was attained. Evidence of active rabies viral infection was seen by fluorescent antibody staining of oral swabs, corneal impression smears and histologic tissue smears, by suckling mouse inoculation of oral swab washings, and by transmission electron microcopy. No clinical signs of rabies virus infection were observed.

INTRODUCTION

In an epidemiologic survey for antibodies against rabies virus in the sera from 343 birds, positive passive hemagglutination (PHA) titers were observed in 23. Four of 15 sera collected from great horned owls (*Bubo virginianus*) had rabies antibodies.[9]

Ingestion of prey or carrion containing rabies virus was considered to be a potential source of the virus for stimulation of the antibody response in the 15 PHA positive predatory birds.[9] Previous studies have indicated that rabid mammals, rather than other birds, are the major source of the virus to birds.[6] Transmission of rabies virus to rodents,[4,12] rabbits,[7] and skunks[2] by the oral route has been experimentally documented. It also has been suggested that rabies in foxes possibly associated with caves in Tennessee may occur because of foxes scavenging infected bats.[2] Baer, *et al.*[1] successfully immunized foxes with the ERA strain of rabies virus by the oral route.

Since avian species generally are not regarded as involved in naturally transmitted clinical rabies, an attempt was made to identify a potential means of inducing the rabies viral antibodies in nature. This report describes the induction of rabies virus infection in a great horned owl.

MATERIALS AND METHODS

A great horned owl was captured due to an injury that necessitated amputation of the left wing. The bird was held in captivity for several months before initiation of the experiment and was fed horsemeat supplemented with vitamins and minerals.

A spotted skunk (*Spilogale putorius*) was inoculated in the right rear leg with 0.5 ml of a 20% (W/V) pooled brain suspension (in phosphate-buffered saline, pH 7.2, ionic strength 0.17) from three striped skunks (*Mephitis mephitis*) which were positive for rabies by fluorescent rabies antibody staining (FA).[5] The spotted skunk had been captured in a live-trap and was descented 15 months prior to this study. Pre-inoculation serum obtained from the skunk by cardiac puncture was negative for rabies viral antibodies by the passive haemagglutination (PHA) test.[8] After 14 days a second blood sample was obtained by cardiac puncture which was negative for rabies viral antibodies by PHA titration. Fifteen

days post-inoculation the animal began to show signs of furious rabies. Clinical signs of the disease, including aggression, extensive salivation and inappetence continued until death on day 17. One-half of the skunk's brain was removed for confirmation of rabies by FA. Intensive fluorescent staining was observed. The remainder of the skunk carcass was skinned and divided into four portions. Each section was placed in a plastic bag and frozen at —20 C.

Before inoculation by ingestion, food was withheld from the owl for 48 hrs. The bird was then offered the head portion of the skunk with the remaining one-half of the brain intact. On subsequent days the remaining portions of the skunk carcass were thawed at room temperature for three hrs and fed to the owl. Later it was fed a diet of chicken and occasionally laboratory mice.

Serial blood sampling by jugular venipuncture was initiated on post-inoculation (PI) day 27. Coincidental with blood sampling the pharynx was swabbed with cotton-tipped applicators. The swabs were placed in test tubes containing 1 ml of commercial tissue culture medium as an eluting fluid. Smears of the medium were stained using FA and 0.03 ml of medium was injected intracranially into each of 10 to 20 two-day-old suckling mice. The suckling mice were observed for 21 days following inoculation. Brains of the mice which died after inoculation were stained with FA to confirm the presence of rabies virus.

Two mg of dexamethasone (DMS) were administered intramuscularly on PI day 66 and daily for 10 days thereafter. Blood sampling and oral swabbing were continued during DMS treatment.

Corneal impression smears[10] were made and stained with FA on PI day 75, the last day of DMS administration and regularly thereafter on bleeding days through necropsy.

Twenty-two days after administration of DMS, the owl was killed by intravenous barbiturate overdose and necropsied. At necropsy various tissues were collected for examination by routine histopathological procedures and transmission electron microscopy (EM). Tissue smears were made and examined by FA for evidence of rabies viral infection.

RESULTS

The head portion of the skunk was swallowed by the owl, as evidenced by the appearance of skull fragments in a pellet regurgitated by the bird. Sharp edges were observed on some of the fragments, probably at least partially the result of fracturing the bone to get access to brain tissue for confirmatory diagnosis of rabies prior to offering the skunk to the owl.

The results of FA staining of pharyngeal swabs and corneal impression smears, suckling mouse inoculations and PHA serologic analysis are summarized in Table 1.

A blood sample obtained from the owl on PI day 27 showed sero-conversion by the PHA procedure. The antibody titer declined over the next six weeks and returned to negative by the PI day 73. A positive PHA antibody titer was observed again on the third week following corticosteroid treatment.

Pharyngeal rabies viral antigen was detected by FA 52 days following oral inoculation. Rabies virus and viral antigen were detected in the pharynx by mouse inoculation and/or FA before, during and after DMS treatment. Rabies virus was detected by FA in brain smears of all suckling mice which died following intracerebral inoculation with pharyngeal swab suspensions.

Corneal impression smears were FA-positive for rabies viral antigen on PI day 86 (12 days after the last of the 10 daily injections by dexamethasone). An impression smear of the right cornea at necropsy was questionally positive for the antigen.

Of 28 tissues and organ samples examined by FA, rabies viral antigen was demonstrated only in cerebellum, brain stem, left nictitating membrane, and palpebral conjunctiva. Limited transmission EM studies demonstrated rabies virus in cerebellum, brain stem, and left

TABLE 1. Laboratory results on great horned owl specimens.

Date	Day Post Inoculation	Serologic Titer (PHA)	Pharyngeal Swabs FA[a]	Pharyngeal Swabs Suckling Mice[b]	Corneal Impressions Rt.	Corneal Impressions Lt.
2-21-75[c]		—	ND	ND	ND	ND
3-20-75	27	+8	ND	ND	ND	ND
3-31-75	38	+8	—	1/15	ND	ND
4-7-75	45	+4	—	0/12	ND	ND
4-14-75	52	+4	+	0/15	ND	ND
4-21-75	59	+2	+	4/17	ND	ND
4-28-75[d]	66	+2	+	0/14	ND	ND
5-5-75[d]	73	—	—	4/17	ND	ND
5-7-75[d]	75	ND[e]	ND	ND	—	—
5-12-75	80	+2	—	1/15	—	—
5-19-75	87	+2	—	0/10	+	+
5-29-75[f]	97	+32	—	1/16	±	—

[a] Samples on dates 5-12, 5-19 and 5-29 represent direct smears of pharyngeal swabs.
[b] Number of deaths/Total number tested.
[c] Pre-inoculation sample.
[d] Administration of dexamethasone; 2 mg IM per day for ten consecutive days.
[e] Not Done.
[f] Date owl was killed.

nictitating membrane. Mouse inoculations were not used and thus the FA results were not confirmed. There was no gross or microscopic histopathologic evidence of rabies viral infection. Renal coccidiosis, an infection of no known clinical significance was diagnosed histologically.

DISCUSSION

Although birds are susceptible to infection with rabies virus, occurrence of the naturally transmitted clinical disease in avian species is apparently rare.[11] Rapid production of CNS-bound antibody, as well as serum antibodies, occurred in fowl inoculated intracranially with several strains of rabies virus and was proposed as the reason for the self-limiting nature of the disease in those birds.[11] These antibodies do not interrupt proliferation of the intracellular virus but rather interfere with its exit from the cells and thus prevent further spread.

In a survey of sera from wildlife for antibodies against several infectious agents, 23 of 343 (6.7%) birds of various species were observed to have low titers of antibodies reacting with antigens of rabies virus in the passive haemagglutination test.[8] Fifteen of these 23 seropositive birds and 4.4% of the total 343 birds tested were birds of prey. One factor that could contribute to the greater prevalence of humoral antibody among these species is the greater susceptibility of birds of prey to infection with rabies virus.[18] More likely, however, it reflects the greater opportunity for exposure of these predatory species to rabies virus in their prey or scavenger carrion.

The natural diet of the great horned owl includes a wide variety of birds and mammals, including other birds of prey and skunks.[3] Rabies in the skunk population is recognized as a serious problem in many areas of the Midwest.

The induction of rabies antibody activity in the great horned owl indicates that the ingestion is an effective route of infection of birds with rabies virus. This route of infection by rabies viral antigens may account for antibody as detected in serum from birds examined in a previous study.[9]

Typical clinical signs of rabies virus infection were never observed in the owl. Slight increase in irritability and reluctance to sit on its perch two weeks prior to necropsy were the only behavioral changes noted.

Although birds are not generally regarded as being epidemiologically important in studies of rabies, the demonstration of pharyngeal rabies virus by FA and by suckling mouse inoculation indicates the great horned owls and possibly other birds could potentially initiate a rabies viral infection. Use of serological survey of owls and other raptors and scavengers (e.g., crows) to monitor presence of rabies in their prey and scavenged species is a possible epidemiological tool for monitoring the prevalence of rabies virus.

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