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The Common Crow as a sentinel species of rabies in wildlife populations

Joseph Martin Schaefer
Iowa State University

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THE COMMON CROW AS A SENTINEL SPECIES OF RABIES IN WILDLIFE POPULATIONS

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The Common Crow as a sentinel species of rabies in wildlife populations

by

Joseph Martin Schaefer

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GENERAL INTRODUCTION

Since the development of effective rabies vaccines for dogs and cats (Habel, 1973), the source of most rabies in the United States has shifted from pet animals to wildlife species (CDC, 1980). However, it is not known whether the increase in laboratory diagnosis of sylvatic rabies should be attributed to an actual increase in the occurrence of the disease or to a greater awareness of, and more testing for it. There is, at present, no unbiased, convenient, and economically feasible means available to determine the true prevalence of rabies in wildlife.

Wild animals generally are submitted to diagnostic laboratories for confirmation of the presence of the viral agent only if they are suspected of being rabid and if there has been potential human or domestic animal exposure. Because of this, the percentage of submitted animals that are rabies-positive probably is greater than the true prevalence in wildlife populations (Tabel et al., 1974).

A survey of trapped mammals tested for rabies may give a conservative estimate of incidence because rabies-infected individuals may become less susceptible to trapping (Verts, 1967; Niemeyer, 1973). Also, any rabid animals that die are not sampled.

Much effort has been exerted in recent years to develop rabies vaccines (Debbie, 1974; Winkler et al., 1975; Winkler and Baer, 1976; Dubreuil et al., 1979; Black and Lawson, 1980) and techniques for administering vaccines (Glosser et al., 1970; Wandeler et al., 1974; MacInnes and Johnston, 1975; Frost and Kiefert, 1978; Bogel et al., 1981) to wild mammal populations with the ultimate goal of eradicating rabies. Without a reliable

technique for measuring the effectiveness of vaccination programs, efforts to control and eradicate rabies have been severely hampered.

Although birds generally are considered resistant to clinical rabies infections (Kraus and Clairmont, 1900; Marie, 1904; von Lote, 1904; Schneider and Burtsher, 1967), Gough and Jorgenson (1976) found that some wild predatory and scavenger birds possessed rabies antibody titers. Jorgenson et al. (1976) reported that a Great Horned Owl (*Bubo virginianus*) developed an immune response after ingesting a rabid Spotted Skunk (*Spilogale putorius*). Because of this pathogenic relationship between rabies virus and avian hosts, it seems logical that a reliable index of the prevalence of this disease in wildlife populations might be established by periodically surveying bird species that feed on rabies-susceptible mammals. A serosurvey of birds would not have the biases inherent in surveys of mammals.

The feasibility of using the Common Crow (*Corvus brachyrhynchos*) as a sentinel species of rabies in wildlife was investigated in this study. This species is an abundant year-round resident in many areas of the United States (Goodwin, 1976). The crow also eats carrion (Barrows and Schwarz, 1895; Crook, 1936; Platt, 1956; Plott and Sherrod, 1974) and, thus, may be expected to ingest rabid carcasses.

The objectives of this study were to determine: 1) if rabies virus can be orally transmitted to crows; 2) the viability of rabies virus in brains and salivary glands of rabid Striped Skunk carcasses; 3) daily crow movement patterns; 4) the importance of rabies-susceptible animals in the crow's diet; and 5) if a significant positive correlation exists between

the prevalence of rabies antibodies in wild crows and rabies antigens in wild Striped Skunks.

SECTION I.

ORAL TRANSMISSION OF RABIES VIRUS TO THE COMMON CROW (*CORVUS BRACHYRHYNCHOS*)

INTRODUCTION

Natural rabies infection of avian species was described as early as the late 1800s (Schneider and Burtscher, 1967), but reports of the disease in birds have been much rarer than in mammals. Experimental transmission of rabies to birds usually has resulted in subclinical, latent, and nonlethal infections (Kraus and Clairmont, 1900; Marie, 1904; von Lote, 1904; Schneider and Burtscher, 1967). Schneider and Burtscher (1967) proposed that rapid production of central nervous system and serum antibodies causes birds to be highly resistant to rabies.

The most common natural dissemination route of rabies virus is a bite from an infected animal that is secreting the virus in its saliva. Aerosol, oral, and transplacental methods of transmission to various mammals also have been documented (Charlton and Casey, 1979; Afshar, 1979). Antibodies reacting with rabies virus have been found in wild predatory birds (Gough and Jorgenson, 1976) and crows (see Section IV, herein) that may have been infected by feeding on rabies-susceptible mammals. In a controlled oral transmission experiment with a Great Horned Owl (*Bubo virginianus*), both antibodies and antigen were detected subsequent to challenge but no clinical signs of rabies were observed (Jorgenson et al., 1976).

This study examined the susceptibility of the Common Crow (*Corvus brachyrhynchos*), a scavenger species, to oral transmission of rabies virus. The specific objectives were to determine 1) if the rabies virus can be orally transmitted to crows, and 2) the effects of crow age and inoculant dosage on serum antibody production and viral replication in orally inoculated crows.

MATERIALS AND METHODS

Thirty-five nestling crows were removed from their nests in central Iowa. These birds were used for the experiments described in Trials 1 and 2. All crows were kept in enclosed buildings allowing free flight and fed both canned and dried dog food.

Trial 1 - fledgling crows

At approximately 3 months of age, 5 crows were fed (with a syringe) 1 ml of Challenge Virus Standard (CVS) rabies infected brains (11th passage; 10% suspension in BHK medium) removed from mice and prepared in a tissue grinder, 4 were fed 1 ml of whole rabid mouse carcass suspension prepared in a Waring blender, and 2 crows were held as controls. The mean titer of the virus in mice brains was $10^{7.2}$ $MICLD_{50}$/ml. The crows were sampled 2 weeks before inoculation and at 4, 6, 8, and 10 weeks post inoculation. Corneal impression smears (CIS) and swabs of the oral-pharyngeal cavity were tested for presence of rabies antigen by the fluorescent antibody (FA) technique (Schneider, 1969; Dean and Abelseth, 1973, respectively). Blood was collected by jugular venipuncture for analysis of antibody production by the passive hemagglutination (PHA) test (Gough and Dierks, 1971). Additional tests to determine the occurrence of nonspecific agglutination in the PHA test were not conducted during Trial 1.

Trial 2 - adult crows

Because the fledgling crow response to oral inoculation with rabid mouse brains was greater than that with rabid mouse carcasses (Table 1), only brains were used as the Trial 2 inocula. Crows were fed different

Table 1. Rabies viral antigens in corneal impression smears (CIS) and oral swabs (OS), and serum antibodies of captive fledgling crows fed 1 ml (mean titer = $10^{7 \cdot 2}$ $MICLD_{50}$/ml) of 10% suspension of CVS rabies samples from mouse brains and carcasses. CIS and OS samples were analyzed with the FA technique. Serum samples were tested by the PHA procedure

Infected tissue in inoculant	Sample tested (test used)	Pre-inoc.	— Weeks post-inoc. — 4	6	8	10	Total positive samples	Total positive birds
Brains (5 crows)	CIS (FA)	0	ND[a]	ND	4[b]	1	5	4
	OS (FA)	0	ND	ND	3	0	3	3
	Total (FA)	0	ND	ND	7	1	8	4[c]
	Serum (PHA)	0	3	3	1	0	7	3
	Totals	0	3	3	8	1	15	5
Carcasses (4 crows)	CIS (FA)	0	ND	ND	1	1	2	1
	OS (FA)	0	ND	ND	2	0	2	2
	Total (FA)	0	ND	ND	3	1	4	2[d]
	Serum (PHA)	0	2	3	2	0	7	3
	Totals	0	2	3	5	1	11	4
Controls (2 crows)	CIS (FA)	0	ND	ND	0	1	1	1
	OS (FA)	0	ND	ND	0	1	1	1
	Total (FA)	0	ND	ND	0	2	2	2
	Serum (PHA)	0	0	0	0	0	0	0
	Totals	0	0	0	0	2	2	2

[a] ND = not done.

[b] All values indicate positive samples.

[c] Two of these were PHA negative throughout the trial.

[d] One of these was PHA negative throughout the trial.

dosage suspensions, in BHK medium of brains removed from rabid mice (mean titer = $10^{7.2}$ $MICLD_{50}$/ml, CVS strain, 13th passage, Table 2). One 2-year-old and three 1-year-old control birds were fed normal mouse brains. The crows were sampled one hour before inoculation and at weekly intervals for 12 weeks. CIS, oral swabs, and serum samples were analyzed using the same techniques described for Trial 1. Sera also were exposed to other viral antigens to determine if PHA reactions were specific. All adult crows were killed at the end of the 12th week, and brain impression smears were examined by FA. Brain tissue samples were tested by the mouse inoculation (MI) procedure (Koprowski 1973) for evidence of rabies virus infection.

Table 2. Dosages of rabies suspensions fed to crows in Trial 2

Number of crows			
1 yrs old	2 yrs old	Dosage	Concentration
6	4	1.00 ml	$10^{7.2}$ $MICLD_{50}$/ml
3	4	1.00 ml	$10^{4.2}$ $MICLD_{50}$/ml
3	0	0.25 ml	$10^{4.0}$ $MICLD_{50}$/ml

RESULTS

Trial 1

Four weeks after oral inoculation of 3-month-old crows with rabid mouse brains, 3 of 5 were sero-positive (Table 1). Four weeks later, rabies virus was identified in CIS from 4 and in oral swabs from 3 birds. Four crows fed brains were FA positive, 3 were PHA positive, and all birds were either FA or PHA positive at least once during the trial.

Seroconversion also was found at 4 weeks after 3-month-old crows ingested rabid mouse carcasses (Table 1). Rabies virus was identified in CIS and oral swabs from 2 birds in this group at the same time as it was found in crows that were fed brains. Two crows fed carcasses were FA positive, 3 were PHA positive, and all birds were either FA or PHA positive at least once during the trial. Seroconversion lasted for at least 4 weeks and virus shedding for at least 2 weeks. The highest recorded antibody titer of 32 on the 4th week was from the serum of a crow fed carcasses.

All experimental birds did not react identically. Rabies virus was detected in 3 crows (2 fed brains, 1 fed carcasses) that were sero-negative throughout the experiment, and rabies antigen was not found in 3 crows (1 fed brains, 2 fed carcasses) that became sero-positive during the experiment. Rabies virus was detected in both controls on the 10th week post-inoculation, but these birds remained sero-negative throughout the experiment (Table 1).

Trial 2

Trial 2 was conducted 2 years after Trial 1. The experimental birds used in Trial 2 were older than those in Trial 1, and the inoculant concentration and dosage also varied. One 1-year-old and one 2-year-old crow used in Trial 2 had titers of 8 one hour before inoculation (Table 3). Their subsequent titer levels and serum profiles were similar to those of the other experimental crows. Rabies viral antigens were detected in CIS of 7 experimental and 1 control crow during the preinoculation sampling. This did not seem to influence the results, because only 4 of these 7 experimental birds compared with 7 of the 13 other crows were FA positive during the trial.

Virus secretion was found more often in 1-year- than in 2-year-old birds (Table 3). Eleven positive CIS samples and 1 positive oral swab sample were obtained from 8 and one 1-year-old birds, respectively. Most of these (83%) occurred within the first 6 weeks post-inoculation. Only 3 CIS from 2-year-old birds were positive (3 birds). Antigen shedding persisted only for 1 week in most positive birds, although it continued for 2 weeks in one 1-year-old crow. All crow brains were FA and MI negative on the last day of the experiment.

Specific PHA reactions were found for all 1-year-old and 6 of 8 2-year-old crows during Trial 2 (Table 3). One-year-old birds had greater incidence of positive serum samples (24.3%; N=144) than did 2-year-old birds (11.5%; N=96; $\chi^2 = 5.16$; df = 1, $P < 0.025$). Three important serum testing results for the 1-year-old class were: at least 1 positive serum sample was observed during 11 of the 12 weeks; 9 crows seroconverted by

Table 3. The weekly number of positive samples for rabies viral antigens in corneal impression smears (CIS) and oral swabs (OS), and rabies specific serum antibodies of captive 1- and 2-year-old crows orally inoculated with rabies virus. Crows were fed various dosages and concentrations of CVS rabies suspensions from mouse brains. CIS and OS samples were tested with the FA technique. Serum samples were tested by the PHA procedure

Age class	Sample tested (test used)	Pre-inoc.	Weeks post-inoculation 1	2	3	4	5	6	7	8	9	10	11	12	Total positive samples	Total positive birds
1 year (12 crows)	CIS (FA)	4	3[a]	2[a]	1	1	1[a]	1[a]	1	0	1[a]	0	0	0	11	8
	OS (FA)	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1
	Total (FA)		3	2	1	1[a]	1	2	1	0	1	0[a,a]	0	0[a]	12	9
	Serum (PHA)	1	3	3	3[a]	3[a]	1	1	3	2	4	8	0	4[a]	35	12
	Totals		6	5	4	4	2	3	4	2	5	8	0	4	47	12
2 year (8 crows)	CIS (FA)	3	0	0	1[b]	0	0	0	0	1[a]	0	0	1	0	3	3
	OS (FA)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total (FA)		0	0	1	0[a]	0[b]	0[b]	0[a]	1[a,b]	0	0	1	0	3	3
	Serum (PHA)	1	0	1	0	3[a]	1[b]	1[b]	2[a]	2	0	1	0	0	11	6
	Totals		0	1	1	3	1	1	2	3	0	1	1	0	14	6
Controls (4 crows)	CIS (FA)	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1
	OS (FA)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total (FA)		0	0	0[a]	0	0	0	0	1	0	0[a]	0	0	1	1
	Serum (PHA)	0	0	0	2[a]	0	0	0	0	0	0	2[a]	0	1	5	3
	Totals		0	0	2	0	0	0	0	1	0	2	0	1	6	3

[a]One FA positive sample at pre-inoculation.

[b]One PHA positive sample at pre-inoculation.

week 4; and most birds (8) were sero-positive on week 10. The 2-year-old class showed a different serum profile: no sero-positive birds were observed during 5 sampling periods; 4 crows seroconverted by week 4; and very few birds were sero-positive each week of sampling.

Testing for nonspecific agglutinins in serum in Trial 2 provided some interesting results. Nonspecific PHA titers greater than 4 were found in nine 1-year-old and six 2-year-old experimental birds (33 samples). No significant difference was detected between the rate of this occurring in 1-year-old (16.0%; N=144) and in 2-year-old crows (10.4%; N=07; $\chi^2 = 1.50$). Approximately 57% of the nonspecific responses of 1-year-old birds (N=23) occurred during weeks 2 to 5 and the rest during weeks 8 to 12. Ninety percent of the nonspecific responses of 2-year-old crows (N=10) occurred during weeks 1 to 4 and only one other was found on week 11.

Unlike Trial 1, 3 of the controls were sero-positive at least once (Table 3), but seroconversion generally occurred later for controls than for challenged birds. Rabies virus antigen was detected in one 2-year-old control on the 8th week.

The same data also were analyzed according to the concentration and dosage of the inoculant (Table 4). Rabies viral antigens were detected in 4 Group 1 birds, 5 Group 2 birds, and 2 Group 3 birds. All of the positive samples from birds inoculated with the lowest dilution (Group 1) were found during the first 6 weeks. Virus shedding in the other 2 groups occurred more randomly but over a longer period of time.

Specific antibody responses were not found for 1 crow in Group 1 and 1 in Group 2 (Table 4). All nine 1-year-old birds that received 1 ml of

Table 4. The weekly number of positive samples for rabies viral antigens in corneal impression smears (CIS) and oral swabs (OS), and rabies specific serum antibodies of captive 1- and 2-year-old crows orally inoculated with rabies virus. Crows were fed various dosages and concentrations of CVS rabies from mouse brain suspensions. CIS and OS samples were analyzed with the FA technique. Serum samples were tested by the PHA procedure

Test group	Sample tested (test used)	Pre-inoc.	Weeks post-inoculation 1	2	3	4	5	6	7	8	9	10	11	12	Total positive samples	Total positive birds
Group 1	CIS (FA)	2	1[a]	1	1[b]	1	1[a]	1[a]	0	0	0	0	0	0	6	4
1 ml of $10^{7.2}$	OS (FA)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$MICLD_{50}$/ml	Total (FA)		1	1	1[a]	1	1[b]	1[b]	0[a]	0[b]	0	0[a]	0	0	6	4
(10 crows)	Serum (PHA)	2	4	3	1[a]	1	2[b]	2[b]	4[a]	2[b]	3	5[a]	0	2	29	9
	Totals		5	4	2	2	3	3	4	2	3	5	0	2	35	9
Group 2	CIS (FA)	3	1	1[a]	1	0	0	0	0	1[a]	0	0	1	0	5	5
1 ml of $10^{4.2}$	OS (FA)	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1
$MICLD_{50}$/ml	Total (FA)		1	1	1	0[a,a]	0	1	0	1[a]	0	0	1	0	6	5
(7 crows)	Serum (PHA)	0	0	0	2	5	0	0	0	2[a]	1	3	0	1	14	6
	Totals		1	1	3	5	0	1	0	3	1	3	1	1	20	6
Group 3	CIS (FA)	2	1	0	0	0	0	0	1	0	1[a]	0	0	0	3	2
0.25 ml of	OS (FA)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$10^{4.0}$	Total (FA)		1	0	0	0	0	0	1	0	1	0[a]	0	0[a]	3	2
$MICLD_{50}$/ml	Serum (PHA)	0	0	0	0	0	0	0	1	0	0	1[a]	0	1[a]	3	3
(3 crows)	Totals		1	0	0	0	0	0	2	0	1	1	0	1	6	3

[a]One FA positive sample at pre-inoculation.

[b]One PHA positive sample at pre-inoculation.

inoculant were sero-positive at least once during weeks 1 to 4 post-inoculation. There was no statistical difference between the incidence of positive serum samples in Group 1 (24.2%; N=120) and in Group 2 (16.7%; N=84; $\chi^2 = 1.66$). The number of positive samples in Group 3 was not large enough for statistical comparisons. At least one serum sample was positive 11 of 12 weeks for the crows in the lowest dilution group with peak periods similar to those for 1-year-old crows (Tables 3 and 4). The humoral immune response of birds in Group 2 began 2 weeks later than that for Group 1 and only was detected in half of the trial weeks. Specific rabies antibodies only were detected once for each bird in Group 3 during the latter part of the trial.

Nonspecific responses were found in 8 Group 1 birds, 6 Group 2 birds, and 1 Group 3 bird. No significant difference was found between the rate of this occurring in Group 1 (15.0%; N=120) and in Group 2 birds (14.3%; N=84; $\chi^2 = 0.02$). The 3 nonspecific reactions found in Group 3 were not enough for statistical comparisons. About 83% of the nonspecific responses (N=18) for birds in Group 1 occurred during weeks 2 to 5 with the other 3 found on weeks 8, 11, and 12. Six nonspecific responses for birds in Group 2 occurred on weeks 1 to 3 and 6 more during weeks 8 to 11. Nonspecific agglutination of samples from 1 bird in Group 3 occurred on weeks 5, 8, and 11.

The highest specific titer (16) was found in serum samples from two 1-year-old crows in Group 1. Specific rabies antibody levels persisted from 1 to 2 weeks in individual birds. None of the experimental birds died or showed overt clinical signs of rabies during either trial.

DISCUSSION

Although this study demonstrated that crows can contract rabies orally, viral antigens were not found in all inoculated birds. Possible explanations for this might be related to the frequency of sampling, site of sampling, or individual differences in refractoriness to infection. Schneider and Burtscher (1967) reported that rabies virus was not detected in the spinal cord, peripheral nerves, internal organs, urine, feces, or saliva following intracerebral inoculation of domestic fowl. Rapid production of large amounts of central nervous system (CNS) bound antibody was believed to restrict and inactivate the virus locally in those birds.

The seroconversion rate in crows was higher than that found in most published oral inoculation studies with mammals. This suggests that crows are more susceptible to rabies virus infection via the oral route than are mammals (Debbie et al., 1972; Charlton and Casey, 1979).

The rapid humoral immune response found in Trial 2 is typical of other orally infected birds (Jorgenson et al., 1976) and mammals (Winkler and Baer, 1976). The short persistence of antibodies is considerably less than that found for mammals (>1 year; Schmidt and Sikes, 1968; Sikes et al., 1971; Black and Lawson, 1980) in response to oral inoculation. The antibody titers of crows also were lower than those reported for mammals following ingestion of inoculants with similar dilutions and dosage (Winkler et al., 1975; Winkler and Baer, 1976). This may be a result of birds depending more on the highly effective CNS-bound antibody which is developed earlier and in greater amounts than is the serum antibody in birds (Schneider and Burtscher, 1967).

The continual presence of serum antibodies and viral antigens in the experimental birds suggests exposure and reinfection from virus shed by infected crows in the same room. This explanation would also account for the infection of control birds.

Gross and Colmano (1971) reported that, in a stressful social environment, chickens were more susceptible to infection with several different viral agents. Trial 2 was conducted from April 1 to July, which is the normal nesting season for crows in Iowa. An increase in the amount of aggression, especially among the 2-year-old birds, was observed before the experiment. It is possible that these captive birds were subject to greater stress that wild birds and that this caused a more severe reaction.

The inverse relationship between age and strength of immune response to various antigens has been reported for other birds (McCorkle and Glick, 1979) and mammals (Makinodan et al., 1976; Segre and Segre, 1976a,b; Albright and Makinodan, 1977; Callard et al., 1977). Segre and Segre (1976a) attributed this age effect in mice to the large number of suppressor T-cells found in older individuals. Makinodan et al. (1976) listed 3 changes that occur with age in mouse immunologic cells: 1) increase in number and/or activity of suppressor cells, 2) decrease in functional efficiency of immunocompetent cells, and 3) decrease in the number of one type of immunocompetent cell that exists in excess in young. In birds, the bursa of Fabricius is an important site for antibody synthesis (Tizard, 1978). Black (1941) reported that 1-year-old crows do not breed and that the bursa of these birds is still apparent.

Wild crows and other scavenger and predatory birds can become exposed to rabies virus by feeding on rabies-susceptible mammals. This study

supports the suggestion by Jorgenson et al. (1976) that oral infection accounted for the antibody found in sera from wild predatory birds (Gough and Jorgenson, 1976).

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SECTION II. THE VIABILITY OF RABIES VIRUS IN STRIPED SKUNK CARCASSES

INTRODUCTION

Although rabies historically has been regarded as being transmitted naturally by the virus-infected saliva of a rabid animal entering a bite wound, recently, other routes of exposure also have been documented (Afshar, 1979). Of these, the oral route has received the most attention. Rabies virus has been found to replicate in epithelial cells, nerve bundles, and submucosal tissue in the oral cavity of mice (Fischman and Ward, 1968; Correa-Giron et al., 1970; Fischman and Schaeffer, 1971); to be resistant to gastric secretions (Correa-Giron et al., 1970); and to be present in various body tissues for at least 6 days after oral inoculation (Correa-Giron et al., 1970). Experimental inoculations of foxes (Kovalev et al., 1971; Black and Lawson, 1980), skunks (Bell and Moore, 1971; Ramsden and Johnston, 1975), predatory birds (Jorgenson et al., 1976), and crows (see Section I, herein) have shown the susceptibility of nonmurine species to oral infection. Transmission of rabies through cannibalism and scavenging also has been reported (Winkler et al., 1972; Lord et al., 1975; Gething, 1976; Shah and Jaswal, 1976).

Irvin (1970) suggested that transmission of rabies virus by means other than bite is of primary importance in maintaining the virus during the endemic sylvatic cycle of the disease. The opportunity for predator and scavenger species to feed on wild animals dying or dead from rabies is probably at least as great as the opportunity for exposure from a bite. Relatively high levels of both wild predatory and scavenger birds (26% of 69 birds, Gough and Jorgenson, 1976) and wild crows (18% of 332; see Section IV, herein) had specific serum antibodies to rabies virus. Jorgenson

et al. (1976) proposed that the route of this exposure was ingestion of rabid prey or carrion.

The viability of rabies virus in carrion is a major factor affecting the chances that a scavenging animal could contract the disease. Temperature is one of the most important environmental factors affecting pathogen stability in tissues not exposed directly to sunlight. Thus, the purpose of this study was to determine the viability of rabies virus in brain and submaxillary salivary glands of rabid striped skunk (*Mephitis mephitis*) carcasses exposed to different controlled temperatures. This species, an abundant food item of several predator and scavenger species (Bent, 1938: 307; Cahalane, 1947:215; Young and Jackson, 1951:148-149; Hall, 1955:211; Young, 1958:74,93), was selected for the experiment because it is the major reservoir of rabies in the midwestern United States (CDC, 1980).

MATERIALS AND METHODS

Animals

Twenty-three apparently healthy and two naturally rabid striped skunks (1 with furious and 1 with paralytic rabies) were caught with live-traps, and two pen-raised striped skunks were obtained from the Iowa Conservation Commission. All animals were descented, and the nonrabid ones were held in captivity at least 2 months before they were inoculated. During captivity, the skunks were offered canned cat food, pelleted dog food, and water.

Inoculation

All 25 nonrabid animals were sero-negative for rabies viral antibodies by the passive hemagglutination test (Gough and Dierks, 1971) prior to inoculation. The inoculant consisted of 10% suspension of mouse brain infected with challenge virus standard (CVS-27, 6th-12th passages, mean titer of $10^{7 \cdot 2}$MICLD$_{50}$/ml). The diluent was Glasgow's modification of Eagle's medium to which 2% fetal bovine serum and 10% tryptose broth were added. Two ml of the inoculant were injected into the masseter muscles of each animal. Incubation time and clinical signs of subsequent rabies were recorded. Five skunks developed furious and 20 developed paralytic rabies.

Environmental chamber

After the skunks developed clinical signs of rabies and died or were killed in extremis, the submaxillary salivary glands and the brain were exposed using methods similar to those described by Tierkel (1973). All carcasses were then placed in plastic bags to reduce desiccation. Fifteen carcasses (5 furious and 10 paralytic) were placed in an unlighted

environmental chamber (Percival) held at a constant 10°C (30-year average spring temperature in central Iowa; Ruffner, 1976). Twelve carcasses (10 induced paralytic, 1 naturally paralytic, 1 naturally furious) were exposed to a constant 24°C (30-year average summer temperature in central Iowa; Ruffner, 1976).

Samples

Brain samples of approximately 0.3 g were extracted with a 16 ga needle and 10 cc syringe. The needle was inserted into the brain several times for each collection in an effort to extract material from the hippocampus, cerebellum, and brain stem. Salivary gland specimens of approximately 0.2 g were obtained by slicing off thin sections. Impression smears were made from brain and salivary gland samples. Then, these specimens were prepared as a 20% suspension in Glasgow's modification of Eagle's medium and stored at -70°C. Samples were taken daily or every other day until no sample tissue remained in the carcass. The fluorescent antibody test (FA; Dean and Abelseth, 1973) was used to detect the presence of rabies virus on the impression smears, and the mouse inoculation test (MI; Koprowski, 1973) was used to determine the viability and titer of the virus in tissue suspensions. One hundred units of penicillin and 0.1 mg of streptomycin were added per ml of solution to control possible bacterial infection. Time and space did not allow MI tests to be conducted for all dilutions of all samples. Therefore, specimens were selectively tested until enough titers were recorded to establish a linear relationship. The log of the 50% endpoint dilution was then calculated (Lorenz and Bogel, 1973). Because of the high probability of bacterial contamination in the

decaying carcasses, FA tests were applied to brain impression smears from mice that died without exhibiting clinical signs characteristic of rabies, and also from mice that died when inoculated with the highest dilutions of tissue suspensions. The level of significance for statistical testing was $P < 0.05$.

RESULTS

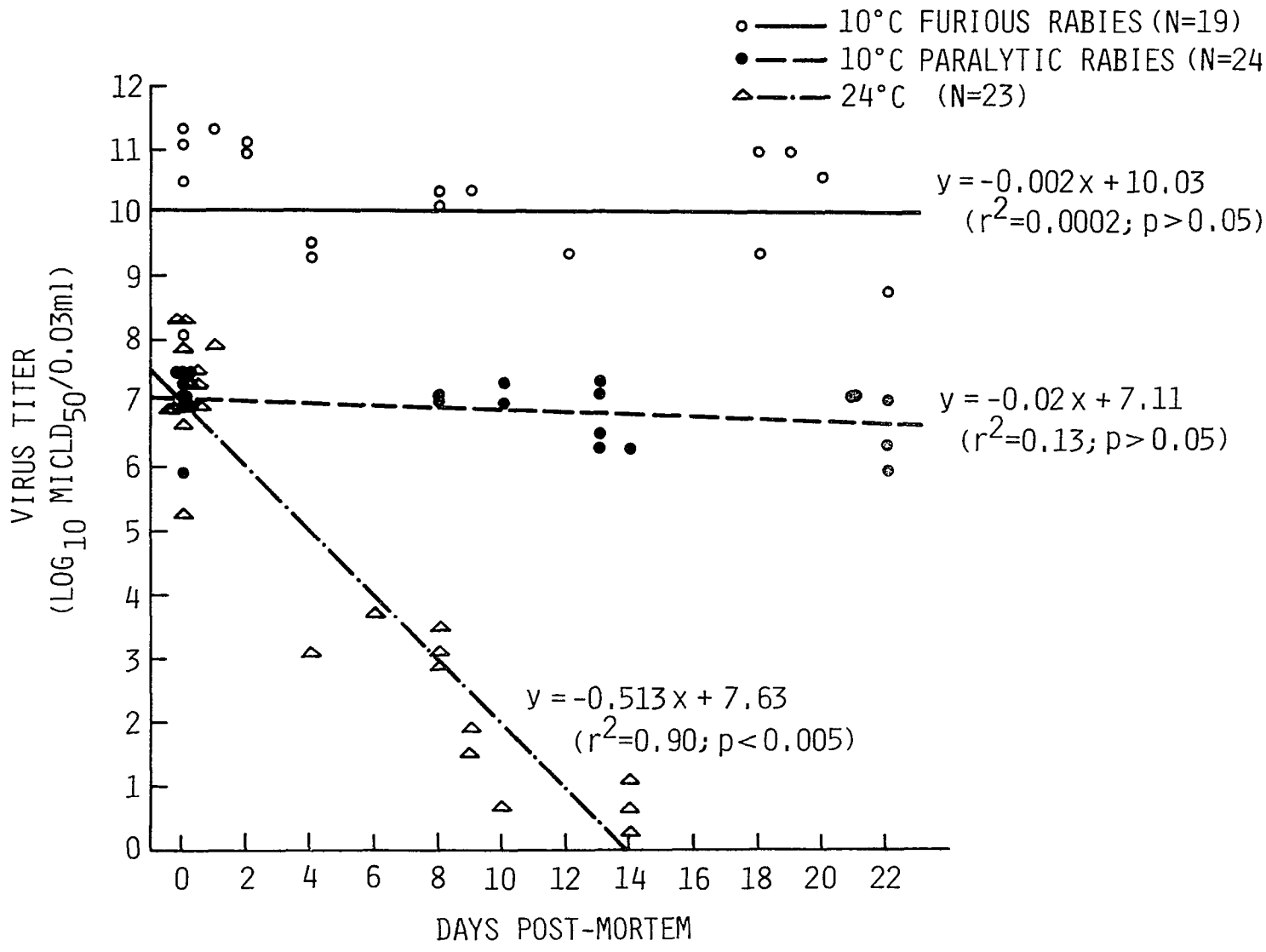
MI test, brain

Differences in titer and viability of virus in brain samples were found between skunks with furious and paralytic rabies (Figure 1). On the day of death, skunks with experimentally induced furious rabies had a higher mean titer (9.58 ± 1.97 log $MICLD_{50}$/0.03 ml, N = 5) than did the other inoculated skunks (7.18 ± 0.66 log $MICLD_{50}$/0.03 ml, N = 20, F = 13.9, df = 1.23, $P < 0.005$). Similarly, brain samples from the two naturally rabid skunks had viral titers of 7.9 log $MICLD_{50}$/0.03 ml (furious rabies) and 5.3 log $MICLD_{50}$/0.03 ml (paralytic rabies) on the day of death.

Rabies virus remained viable at 10°C throughout the 22-day study period in carcasses of skunks that had shown either furious or paralytic rabies. No significant correlations were found between viral titer and time in these two groups (t = 0.053, df = 17, furious; and t = 1.61, df = 22, paralytic; Figure 1). A strong inverse relationship between time and stability of virus was shown with carcasses exposed to 24°C (11 paralytic and 1 naturally furious, t = 13.66, df = 21). The virus was still viable at 2 weeks (24°C), but its strength was greatly diminished.

No significant error in the sampling of brain tissue was detected. Only 2 determinations were greater than 1 dilution more than that for previous samples from the same skunk. At no time was the virus completely inactivated in any brain sample (N = 66).

Figure 1. Viability of rabies virus in brains of rabid skunk carcasses (MI test) held at 2 environmental temperatures. The 24°C sample contained 11 induced paralytic and 1 naturally furious skunk carcasses


10°C FURIOUS RABIES (N=19)
10°C PARALYTIC RABIES (N=24)
24°C (N=23)
y = -0.002x + 10.03
(r2=0.0002; p>0.05)
y = -0.02x + 7.11
(r2=0.13; p>0.05)
y = -0.513x + 7.63
(r2=0.90; p<0.005)
VIRUS TITER
(LOG10 MICLD50/0.03ml)
DAYS POST-MORTEM
0
1
2
3
4
5
6
7
8
9
10
11
12
0
2
4
6
8
10
12
14
16
18
20
22

MI test, salivary gland

Virulent rabies virus was not found by the MI test in some salivary gland samples from skunks in whose brains virus was detected. Out of 28 samples (17 skunks) tested, only 5 (4 skunks) were positive initially. The highest titer (3.3 log $MICLD_{50}$/0.03 ml) was found in a sample taken on the day of death from one of the 5 skunks experimentally infected with furious rabies. However, no rabies virus was detected in samples taken from this animal on days 3 and 5 post-mortem.

The naturally infected skunk with furious rabies had a rabies titer in the salivary gland of 1.5 log $MICLD_{50}$/0.03 ml on the day of death and 0.7 log $MICLD_{50}$/0.03 ml on day 2. This was the longest half-life found for virus in the salivary glands exposed to 24°C. No virus was detected in the salivary glands of the other naturally infected skunk. The last day that a positive salivary gland titer (0.5 log $MICLD_{50}$/0.03 ml) was determined was on day 4 post-mortem from a skunk with paralytic rabies held at 10°C.

FA test

Results of the FA testing were different from the MI results. While all of the brain samples tested by MI (N=66) were positive, 58% of these were negative by the FA test. Sixty-seven percent of 358 FA-tested brain samples were negative (Table 1). The incidence of positive FA reactions in the 10°C group with furious rabies (80%, N=50) was greater than that for the 10°C paralytic group (29%, $\chi^2 = 41.22$, $P < 0.005$) and the 24°C group (22%, $\chi^2 = 56.10$, $P < 0.005$). No difference in rate of identification of rabies virus with the FA test was found between the 2 paralytic rabies

Table 1. Brain sample FA results. (-) and (+) indicate FA- and FA+, respectively

Days post-mortem	Carcass treatment and rabies type											
	10°C furious			10°C paralytic			24°C			Totals		
	-	+	Total	-	+	Total	-	+	Total	-	+	Total
0	1[a]	4[a]	5	8[a]	2[a]	10	9[a]	3[a]	12	18	9	27
1	0	1[a]	1	9	1	10	10	2[a]	12	19	4	23
2	1	3[a]	4	6	4	10	7	5	12	14	12	26
3	0	1	1	6	4	10	10	2	12	16	7	23
4	1[a]	3[a]	4	8	2	10	9[a]	3	12	18	8	26
5	0	1	1	7	3	10	8	4	12	15	8	23
6	0	4	4	5	5	10	11[a]	1	12	16	10	26
7	1	0	1	9	1	10	8	2	10	18	3	21
8	2[a]	2[a]	4	8[a]	2[a]	10	9[a]	1[a]	10	19	5	24
9	0	1[a]	1	6	4	10	7	0	7	13	5	18
10	0	4	4	6	4	10	3	2[a]	5	9	10	19
11	0	1	1	8[a]	2	10	3	1	4	11	4	15
12	0	4[a]	4	10	0	10	4	0	4	14	4	18
13	0	1	1	8[a]	2	10	1	2	3	9	5	14
14	2	2	4	3[a]	4	7	3[a]	0	3	8	6	14
15	0	1	1	4	0	4				4	1	5
16	1	3	4	3	1	4				4	4	8
17	0	1	1	1	3	4				1	4	5
18	1	3[a]	4	3	1	4				4	4	8
19				3	1	4				3	1	4
20				3	1	4				3	1	4
21				3[a]	1	4				3	1	4
22				0	3[a]	3				0	3	3
Totals	10	40	50	127	51	178	102	28	130	239	119	358
(%)	(20)	(80)		(71)	(29)		(78)	(22)		(67)	(33)	

[a]MI+ in at least one sample.

groups (χ^2 = 1.86). Some brain samples were still FA positive on the last sampling day.

Twenty-six percent of the salivary gland samples negative by MI (N = 23) were positive by FA testing. One out of the 5 samples positive by MI from this tissue was negative by FA. Of 358 salivary gland samples tested by the FA procedure, 92% were negative (Table 2). The incidence of positive FA reactions in specimens from skunk carcasses held at 24°C (12%) was greater than that for paralytic carcasses held at 10°C (6%, χ^2 = 4.49, $P < 0.05$), but not different from that for skunks with furious rabies held at 10°C (8%, χ^2 = 0.71). No difference was found between the two 10°C groups (χ^2 = 0.36). The last sample detected as FA+ was on day 20 for the 10°C paralytic group, on day 18 for the furious group, and on day 12 for the 24°C group. Therefore, the FA method did not detect virus as long after death as did the MI testing.

Table 2. Salivary gland FA results. (-) and (+) indicate FA- and FA+, respectively

Days post-mortem	Carcass treatment and rabies type											
	10°C furious			10°C paralytic			24°C			Totals		
	-	+	Total	-	+	Total	-	+	Total	-	+	Total
0	5[a,b]	0	5	10[b]	0	10	10[b]	2[a,b]	12	25	2	27
1	1	0	1	10	0	10	10	2	12	21	2	23
2	4	0	4	10	0	10	10[b]	2[a]	12	24	2	26
3	1[b]	0	1	9	1	10	11	1	12	21	2	23
4	4	0	4	9	1[b]	10	8[b]	4[b]	12	21	5	26
5	1[b]	0	1	9	1	10	10	2	12	20	3	23
6	4	0	4	9	1	10	12[a]	0	12	25	1	26
7	1	0	1	10	0	10	9	1	10	20	1	21
8	4	0	4	10	0	10	10	0	10	24	0	24
9	0	1	1	9	1	10	7	0	7	16	2	18
10	3	1	4	10	0	10	4[b]	1	5	17	2	19
11	1	0	1	9	1	10	4	0	4	14	1	15
12	4	0	4	10	0	10	3	1[b]	4	17	1	18
13	0	1	1	9	1	10	3	0	3	12	2	14
14	4	0	4	6	1	7	3	0	3	13	1	14
15	1	0	1	3	1	4				4	1	5
16	4	0	4	4	0	4				8	0	8
17	1	0	1	4	0	4				5	0	5
18	3	1	4	4	0	4				7	1	8
19				4[a]	0	4				4	0	4
20				3	1	4				3	1	4
21				4	0	4				4	0	4
22				3	0	3				3	0	3
Totals	46	4	50	168	10	178	114	16	130	328	30	358
(%)	(92)	(8)		(94)	(6)		(88)	(12)		(92)	(8)	

[a]MI+ in at least one sample.

[b]MI- in at least one sample.

DISCUSSION

Because of differences in experimental design, it is difficult to critically compare the results of this study with other published rabies viability reports. Burkel et al. (1970) detected rabies viral antigens with FA diagnosis beyond 42 hours in brain samples from striped skunk carcasses exposed to outside mean daily temperatures ranging from 10° to 29°C. Their MI analysis found rabies virus after 69 hours of exposure at 10°C, but not beyond 20 hours at 29°C.

Soave (1966) stated that a CVS strain of rabies virus remained viable in the brains of dead mice for at least 8 days at 25°C and 20 days at 10°C. Winkler and Baer (1976) reported that ERA/BHK-21 rabies virus vaccine remained viable in sausage for more than 30 days at -20° and 4°C, for more than 20 days at 25°C, and for more than 3 days at 35°C. However, Winkler et al. (1975) found that rabies vaccine in bait did not retain a minimum effective dosage for foxes when exposed for 96 hours at 4° or 25°C. Debbie (1974) reported that titers of ERA vaccine remained fairly stable in eggs for 15 days at 6°, 22°, and 37°C.

Rabies virus was detected in only a few salivary gland samples in this study. Sikes (1962) also found that not all (15 of 18) experimentally inoculated skunks secreted virus in their saliva. Several studies have shown that the virus titer in salivary glands is inversely related to the challenge dose (Sikes, 1962; Parker and Wilsnack, 1966; Schneider ahd Hamann, 1969). This would account for the low titers found in this study.

The virus titers of the brain samples from the two naturally infected skunks in this study (5.3 and 7.9) were greater than the range for

naturally infected skunks tested by Howard (1981; 0.7-4.5). The virus titer (3.3) of the salivary gland sample from the naturally infected skunk with furious rabies was within the range reported for salivary glands tested by Howard (1981; 2.8-7.2). No rabies virus was found in the salivary gland from the one naturally infected skunk with paralytic rabies. In earlier reports, Howard (1976) and Samol (1976) detected rabies virus in all salivary glands tested from naturally infected animals.

In this study, the MI test seemed to have a greater sensitivity to rabies virus in the brain samples and a lesser sensitivity to the virus in salivary gland samples than did the FA procedure. However, a high concentration of enzymes in salivary gland samples may have inactivated virus that was antigenically still present and, therefore, stained with the labelled antibody in the FA test. Other reports also have shown differences in results found by these 2 diagnostic tests (Carski et al., 1962; Parker and Wilsnack, 1966; Wilsnack and Parker, 1966). Carski et al. (1962) reported that the FA and MI tests of brain tissue were equally sensitive, but neither completely reliable. They also found that both the FA and MI methods displayed a lower degree of sensitivity to rabies virus in salivary gland than in brain samples.

Another explanation for the FA-brain samples is possible single site localization of the rabies virus as reported by Maserang and Leffingwell (1981). The brain tissue sampling technique used in this study may not be effective for FA testing if the infection sites are localized.

I have shown that, under experimental conditions, rabies virus can remain viable for considerable periods of time in striped skunk carcasses. This evidence suggests that oral transmission of rabies virus among

scavenger species may be a common occurrence, especially during spring when the temperature is relatively cool and the prevalence of skunk rabies is at the yearly peak (Verts, 1967). The viability is shorter-lasting under temperatures duplicating those prevalent in midwestern summers.

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SECTION III. DAILY MOVEMENT PATTERNS OF THE COMMON CROW IN CENTRAL IOWA

INTRODUCTION

Most of the literature on Common Crow (*Corvus brachyrhynchos*) movement patterns was published more than 30 years ago. These reports (Hasbrouck, 1888; Barrows and Schwarz, 1895; Emlen, 1938, 1940; Kalmbach, 1939; Kalmbach and Aldous, 1940; Black, 1941; Reiman, 1942; Aldous, 1944; Bent, 1946) provide general information on large winter roost distributions and migration routes in North America. Land use and habitat changes during the last 3 decades could have altered these traditional movements (Peterson, 1979). A few researchers have updated regional information on crow distribution (Haase, 1963; Richards and White, 1963; Anderson et al., 1967; Houston, 1969; Richards, 1971). However, data on daily crow movements are lacking. The purpose of this study was to quantify these movement patterns throughout the year in Central Iowa. Such information was needed to be able to determine how large an area a rabies survey of crows would actually monitor.

METHODS

Study areas

Seven study areas located in Webster, Hamilton, Hardin, Boone, Story, Marshall, and Polk counties in central Iowa were chosen. Each area contained a traditional winter roosting site and also supported large numbers of nesting crows. The generally flat topography, broken only by several river valleys is typical of much of Iowa. About 76% of the land is farmed in row crops, especially corn and soybeans (Iowa Development Commission, 1982).

Live-trapping

Nestlings greater than 3 weeks old were tagged and left in their nests. A 9.1 x 18.3 m rocket net (Day et al., 1980:77) and an Australian crow trap (Aldous, 1938; Rowley, 1968) were used to capture most of the older crows during fall and winter. A few birds were caught with monofilament line leg-snares. Nonnestlings were tagged and released at the trap site.

All crows (N = 234) were marked with U.S. Fish and Wildlife Service leg bands. In addition, light blue Herculite wing-tags were folded over the leading edge of the wing, attached with surgical suture wire through the patagium, and fastened with clothing snaps between the 11th and 12th secondaries on 191 crows. A three-unit alphanumeric black identifying code was painted on the outer face (7.6 cm x 17.8 cm) of each tag. The tags, placed on the left wing of nestlings and the right wing of older birds, were visable with the unaided eye at approximately 1 km, and the code was ledgible up to 200 m with a 40x spotting scope.

Radio telemetry

Solar-powered radio transmitters were attached to 14 birds (Dunstan, 1972). A 12-channel receiver, and a car-mounted, as well as a hand-held, Yagi antenna were used to monitor the transmitters. Radio telemetry data were recorded for a total of 66 bird-months. Monitoring time per bird ranged from 1 week to 26 months.

Recording movements

Data on crow movements were obtained from various sources. I was notified by the U.S. Fish and Wildlife Service of all recoveries of Common Crows I banded. I recorded all sightings of patagial-marked birds and visually tracked marked and unmarked birds. Efforts to locate marked birds were concentrated along flight lanes to and from roosts and near nest sites. Radio telemetry techniques primarily were used to locate birds at the beginning of each monitoring period. Once located, birds usually were followed visually. Efforts to locate radioed birds were made at least bi-weekly. Foraging distances from roost sites and nests were determined by radio telemetry and visual observation. Crows traveled to and from each roost site along flight lanes in the 4 major compass directions. Maximum distances traveled by crows on all recorded flights from roost sites and nests were plotted on 1:50,000 scale maps. The outermost plotted points were connected and the enclosed foraging area around each roost site and nest was calculated (Mohr, 1947).

RESULTS

Forty-six percent of all wing-tagged crows were located at least once after tagging (Table 1). Eighty-two percent of all locations (N = 408) were within 2 km of the tagging sites.

April - June

The nesting season in central Iowa started in the beginning of April (mean hatching date, April 29, N = 73; earliest, April 5). Some crows used the same nesting area (farmstead) in successive years. Thirteen sightings of yearlings returning the next year to their original nesting area were recorded. One 2-year-old crow radioed as a nestling was observed at a nest site only 0.5 km from its birth nest, which was again active. All spring sightings of spring wing-tagged birds were recorded within 2 km of their nests (Table 1). The mean foraging area for birds at 7 nests varied little among families ($\overline{X}$ = 151.3 ha, S.D. = 18.1). Nearly all activity within each area was restricted to a few preferred feeding sites and the maximum distance moved from each nest averaged 1.1 km (S.D. = 0.2). The longest recorded flight from a nest was 4.8 km.

July - October

Two-month-old birds joined older crows on foraging flights and stayed within the previously established foraging area until July. At this time, adjacent families started to concentrate at nighttime roosts. Daily, they returned up to 20 km to their respective nest areas to feed (Table 1). In September and October, some families stayed in large flocks throughout the day.

Table 1. Seasonal distribution of located wing-tagged crows in central Iowa during 1979-1981.[a] Locations were plotted using radio telemetry, visual observation, and Fish and Wildlife Service reports

Season tagged	No. tagged	Season recovered: Apr - Jun <2[b]	Apr - Jun 2-20	Apr - Jun 20-200	Apr - Jun >200	Jul - Oct <2	Jul - Oct 2-20	Jul - Oct 20-200	Jul - Oct >200	Nov - Mar <2	Nov - Mar 2-20	Nov - Mar 20-200	Nov - Mar >200	Totals
Apr-Jun	47	104	0	0	0	101	29	0	0	13	1	0	0	248
		(17)[c]	0	0	0	(13)	(7)	0	0	(9)	(1)	0	0	(22)
Jul-Oct	25	0	0	0	0	10	22	0	0	1	0	4	0	37
		0	0	0	0	(3)	(1)	0	0	(1)	0	(1)	0	(3)
Nov-Mar	119	1	0	1	7	0	1	1	4	105	1	1	1	123
		(1)	0	(1)	(7)[d]	0	(1)	(1)	(3)	(55)	(1)	(1)	(1)	(62)
Totals	191	105	0	1	7	111	52	1	4	119	2	5	1	408
		(18)	0	(1)	(7)	(16)	(19)	(1)	(3)	(65)	(2)	(2)	(1)	(87)

[a]Locations of young birds at nest sites before they were capable of sustained flight are not included.

[b]Kilometers from tagging site.

[c](Number of different birds).

[d]All locations were north of tagging sites with the farthest location of 750 km.

November - March

Sightings of crows that were wing-tagged November through March suggest that northern breeders migrated into central Iowa after November 1 (Table 1). No sightings were reported south of any central Iowa tagging sites. All roosts were located at sites that previously had been used in late summer. Four marked crows were found at the same roost site in 2 consecutive years. These roosts contained migrant birds as well as year-round residents. Nine crows marked as nestlings were found in small groups foraging within their nesting area on 13 occasions throughout their first winter (Table 1).

Major roost sizes, after migration was completed, ranged from 60 to 500 crows (Table 2). Most birds remained in large flocks throughout the day at commonly used foraging and staging areas. A strong positive relationship was found between the number of birds in the roost and foraging areas of the roost ($r^2 = 0.83$; $t = 4.159$; $P < 0.005$). The density of birds within the foraging areas of the 7 roosts ranged between 1.6 and 3.0 crows/km^2. Four smaller roosts and 11 families wintering separately also were observed. These were located outside the foraging areas of the larger roosts.

Table 2. Characteristics of crows using 7 major roosts in central Iowa during winters 1979-1980 and 1980-1981

No. of crows in roost	
Mean ± SD	244.3 ± 159.9
Range	60-500
Flight lane distance (km)	
Mean ± SD	7.5 ± 4.6
Range	1.6-24.0
Foraging area (km^2)	
Mean ± SD	110.8 ± 63.2
Range	25.9-165.9

DISCUSSION

The onset of the nesting season in Iowa was within the range found in Illinois (Black, 1941) and Ohio (Good, 1952). Although the tendency for birds to return to the same nesting area each year had not been documented previously for Common Crows, this fidelity has been shown for other migratory birds (e.g., Hickey, 1943; Bergstrom, 1951; Sladen and Tickell, 1958).

I could find no references in the literature of helper crows at nest sites, although it has been found in some jays (Woolfenden, 1975). Skutch (1961) suggested that this behavior may function as an important learning phase for social species.

Both Black (1941) and Good (1952) stated that flocks of several hundred birds also were found throughout spring. Good (1952) suggested that these consisted of yearling, unpaired birds. Although I did not see any sizeable spring flocks, flock-forming during the late summer in Iowa was similar to that reported by Black (1941), Good (1952), and Haase (1963).

The arrival time of northern migrants into central Iowa was consistent with that found in other midwestern states at the same latitude (Barrows and Schwarz, 1895; Black, 1941; Good, 1952; Haase, 1963). The strict north-south migration route of winter-tagged crows in this study differs slightly from the more northwest-southeast route used by crows banded in Kansas and Oklahoma (Kalmbach and Aldous, 1940). Recoveries of birds banded between the Mississippi and Ohio rivers indicate shorter and more random migration routes that are probably limited by the Great Lakes (Good, 1952). The fact that no birds were recovered south of the banding sites

and the sightings of permanent residents in central Iowa suggest a "leap frog" migration pattern (Peterson, 1979).

Use of the same traditional roosting areas each year is not easily explained. Black (1941) speculated that the winter crow distribution in Illinois was controlled by the former distribution of forests and prairies, the present distribution of grain crops, the location of large rivers that are open all year, and snowfall. Emlen (1938, 1940) suggested that the winter ranges of the crow in New York and California were delimited by an innate homing factor. Kalmbach and Aldous (1940) reported that crows were quite sedentary for the duration of a single winter in Oklahoma, but did not show yearly roost site tenacity. Their banding information of 33 winter-to-winter recoveries showed that these birds wintered an average distance of 189 miles (304 km) from the previous winter banding site.

Most previous reports have focused on the large winter roosts without devoting much attention to the scattered solitary families. However, Emlen's theory for traditional roost sites (1938) would also explain why year-round residents outside the influence of large roosts remained in small family groups.

The roosts I found were smaller and their flight lanes shorter than those reported in earlier studies. Researchers in the past frequently referred to roosts containing between 1,000 and 200,000 birds compared to 60-500 in this study. Good (1952) followed flight lanes of 25 to 30 miles (40 to 48 km) and Black (1941) reported a feeding range of 15-20 miles (24 to 32 km). Repeated use of the same flight lanes and feeding areas by individuals within a roost was also found by Aldous (1944). A decrease in roost sizes during the past 40 years suggests either that the number of

wintering crows decreased or, as I believe, that the large roosts dispersed into smaller ones as midwestern farmland became more open and intensely cultivated and roost sites became more fragmented.

This paper is the first known quantitative report of daily crow movements in the United States. The information presented complements that previously published on crow foraging and migration patterns. Because nesting birds have a relatively small foraging area and tend to stay in that area well after the breeding season, a rabies survey of those crows would monitor the prevalence of the disease within a local population. However, wintering concentrations contain both local and migrant birds and, thus, a winter survey of crows would not be indicative of the rabies in local populations.

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SECTION IV. THE COMMON CROW AS A SENTINEL SPECIES OF RABIES IN WILDLIFE POPULATIONS

INTRODUCTION

Since the development of effective rabies vaccines for dogs and cats (Habel, 1973), the source of most rabies in the United States has shifted from pet animals to wildlife species (CDC, 1980). However, it is not known whether the increase in laboratory diagnosis of sylvatic rabies should be attributed to an actual increase in the occurrence of the disease or to a greater awareness of, and more testing for it. There is, at present, no unbiased, convenient, and economically feasible means available to determine the true prevalence of rabies in wildlife.

Wild animals generally are submitted to diagnostic laboratories for confirmation of the presence of the viral agent only if they are suspected of being rabid and if there has been potential human or domestic animal exposure. Because of this, the percentage of submitted animals that are rabies-positive probably is greater than the true prevalence in wildlife populations (Tabel et al., 1974).

A survey of trapped mammals tested for rabies may give a conservative estimate of incidence because rabies-infected individuals may become less susceptible to trapping, as suggested by Verts (1967) and Niemeyer (1973). Also, any rabid animals that die are not sampled.

Much effort has been exerted in recent years to develop rabies vaccines (Debbie, 1974; Winkler et al., 1975; Winkler and Baer, 1976; Dubreuil et al., 1979; Black and Lawson, 1980) and techniques for administering vaccines (Glosser et al., 1970; Wandeler et al., 1974; MacInnes and Johnston, 1975; Frost and Kiefert, 1978; Bogel et al., 1981) to wild mammal populations with the ultimate goal of eradicating rabies. Without a reliable

technique for measuring the effectiveness of vaccination programs, efforts to control and eradicate rabies have been severely hampered.

Although birds generally are considered resistant to clinical rabies infections (Kraus and Clairmont, 1900; Marie, 1904; von Lote, 1904; Schneider and Burtscher, 1967), Gough and Jorgenson (1976) found that some wild predatory and scavenger birds possessed rabies antibody titers. Jorgenson et al. (1976) reported that a Great Horned Owl (*Bubo virginianus*) developed an immune response after ingesting a rabid Spotted Skunk (*Spilogale putorius*). Because of this pathogenic relationship between rabies virus and avian hosts, it seems logical that a reliable index of the prevalence of this disease in wildlife populations might be established by periodically surveying bird species that feed on rabies-susceptible mammals. Furthermore, surveys involving avian scavengers seem reasonable because Schaefer (see Section II, herein) found that the virus remained active in Striped Skunk (*Mephitis mephitis*) carcasses for at least 3 weeks at 10°C and for at least 2 weeks at 24°C.

The feasibility of using the Common Crow (*Corvus brachyrhynchos*) as a sentinel species of rabies in wildlife was investigated in this study. This species is an abundant year-round resident in many areas of the United States (Goodwin, 1976) and commonly eats carrion (Barrows and Schwarz, 1895; Crook, 1936; Platt, 1956; Plott and Sherrod, 1974). Also, crows orally inoculated with rabid mouse brains and carcass suspensions developed significant antibody titers within 1 week, and the antibody persisted only about 2 weeks (see Section I, herein). Because of these findings, the Common Crow seems to be a possible sentinel species.

The objectives of this study were to determine: 1) the importance of rabies-susceptible mammals in the crow's diet and 2) if a significant correlation exists between the prevalence of rabies antibodies in wild crows and rabies antigens in Striped Skunks, the major reservoir of rabies in the midwestern United States.

STUDY AREA

Seven study areas located in Webster, Hamilton, Hardin, Boone, Story, Marshall, and Polk counties in central Iowa were chosen. Each area contained a traditional crow winter roosting site and also supported large numbers of nesting crows. The generally flat topography, broken only by several river valleys, is typical of central Iowa. About 76% of the land is farmed in row crops, especially corn and soybeans (Iowa Development Commission, 1982). Central Iowa also has a high prevalence of rabies based on the geographical distribution of confirmed rabies cases from the Iowa State Department of Health records over the past 40 years.

MATERIALS AND METHODS

Vertebrate foods of crows

A total of 184 free-flying crows were collected and the contents of the esophagus and gizzard were removed. Fifty-two samples of food being feed to nestling crows were obtained using the ligature technique (Johnson et al., 1980). All food samples were preserved in 10% formalin. The percentage occurrence and percentage volume of vertebrate food items were determined using methods similar to Swanson and Bartonek (1970). Because of the large volume of vertebrate foods consumed by a few birds, volume measurements are expressed as the average percent volume in the sampling period (aggregate percent; Swanson et al., 1974). All observations of crows feeding on carrion also were recorded.

Prevalence of antibodies in crows

Blood samples were obtained from 182 dead and 150 live-trapped, free-flying crows. Samples also were taken from 105 one-week-old nestlings to determine whether rabies virus can be passed embryonically and from 196 four-week-old nestlings to determine whether young crows are infected from the food they eat. One bird per nest was held in captivity from 4 weeks of age and serologically monitored biweekly for at least 8 weeks.

Blood samples from all dead crows and one-week-old nestlings were taken by cardiac puncture and from all other birds by jugular venipuncture. Sera were examined for rabies antibodies by the passive hemagglutination procedure (Gough and Dierks, 1971). Corneal impression smears and oral swabs of the pharyngeal cavity also were taken from birds whenever possible

and examined for the presence of rabies viral antigens by the fluorescent antibody (Dean and Abelseth, 1973) and the mouse inoculation (Koprowski, 1973) tests.

Prevalence of antigens in mammals

Three methods were used to sample mammalian wildlife populations: records of wild Striped Skunks submitted to the Iowa State Department of Health Diagnostic Laboratory from the 7 counties were examined; carcasses of 236 Striped Skunks that were trapped in the 7 counties were obtained from fur buyers; and 45 road-killed Striped Skunks were collected from the study areas. The brain and salivary glands from the fur buyer and road-killed skunks were examined for presence of rabies viral antigens (Dean and Abelseth, 1973; Koprowski, 1973).

Correlations between antibody and antigen prevalence in study areas

Data from all samples were arranged into usable variables. Because nestling crow samples within nests were not believed to be independent, a nest variable was created. Nests were classified as positive if at least 1 nestling sample from the nest was positive. All adult (free-flying) crow samples were considered to be independent of one another and formed another variable. Skunk samples were grouped by sampling methods. Due to missing data and low numbers of positive samples during some months, crow data (nests and adult crows) collected in all months were combined for each study area. Similarly, skunk data also were combined. A Pearson Correlation Coefficient then was calculated to determine if a relationship existed between the prevalence of antibodies in crows and antigens in skunks for the 7 study areas.

Log-likelihood ratio tests (Bishop et al., 1975) were used to determine whether the two species had different rates of rabies, and also whether the area from which an animal was taken affected its chance of testing positive for rabies. To perform these tests, animals were classified according to 3 factors: species, whether they tested positive or negative for rabies, and the area in which they were captured. The level of significance for all statistical testing in this study was $P < 0.05$.

RESULTS

Vertebrate foods of crows

The importance of vertebrate food to adult crows varied seasonally (Figure 1). This food occurred less frequently July through October than both November through March ($\chi^2 = 15.70$, $P < 0.005$) and April through June ($\chi^2 = 7.41$, $P < 0.01$). The only difference detected in percent volume was between spring (April-June) and summer (July-October) ($\chi^2 = 3.9$, $P < 0.05$). The occurrence of vertebrate foods in nestlings' diet was not different from that in adults' diet during the nesting season (60.0% vs. 45.8%, $\chi^2 = 0.61$). However, the percent volume for nestlings was greater than that for adults (48.2% vs. 26.9%, $\chi^2 = 4.21$, $P < 0.05$).

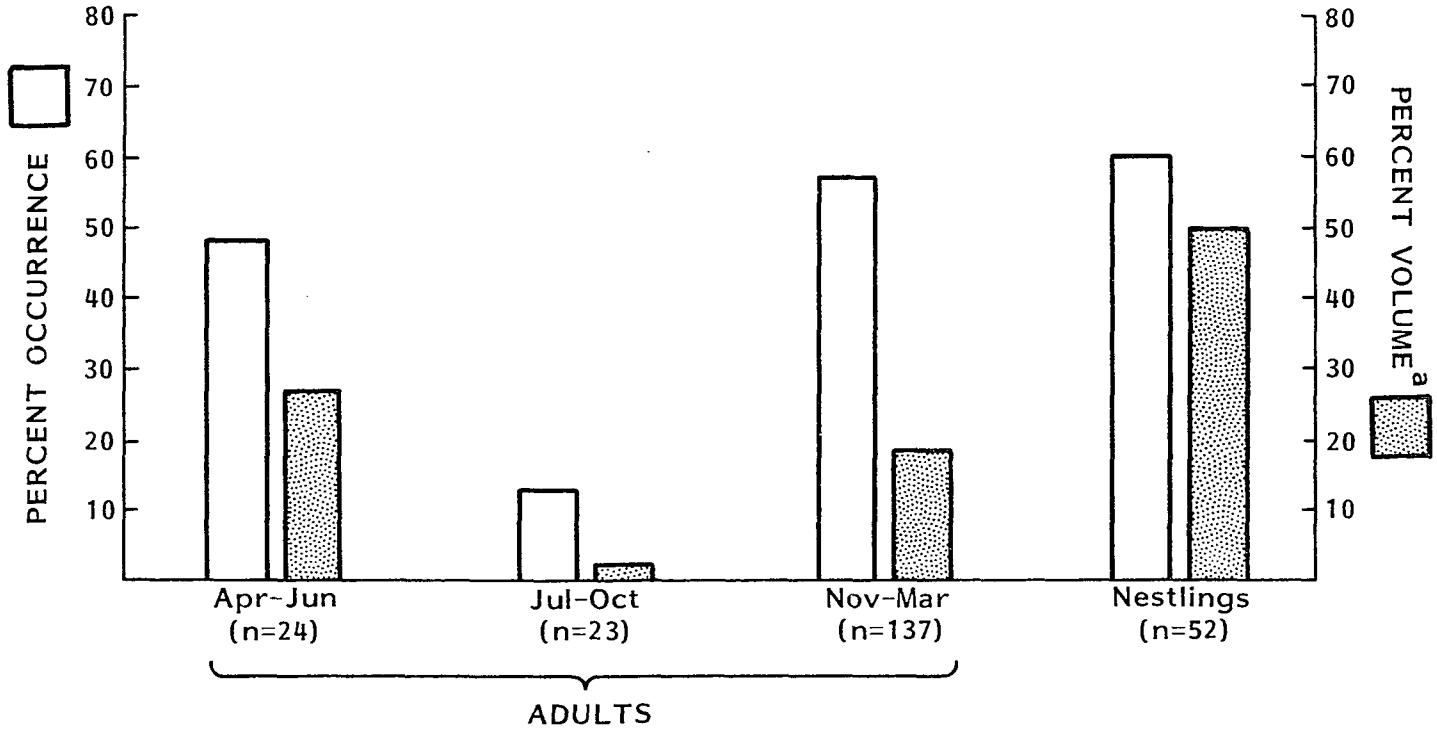
It was difficult to determine if the vertebrate food, which included hair, feathers, bones, and whole nestling birds and mice, was eaten live or as carrion. Maggots were found only in three samples when vertebrate material also was present. Fifty observations of crows scavenging on road-killed carcasses were recorded. On 8 of these occasions, crows were feeding on Striped Skunks.

Prevalence of rabies antibodies in wild crows

Blood samples from 332 adult crows were tested for the presence of rabies antibodies (Table 1). Of these, 18.3% were positive. The prevalence of antibodies during July through October was considerably greater than that during other periods ($P < 0.005$).

The prevalence of serum rabies antibodies varied among age classes of nestling crows (Table 1). Antibodies were not detected in one-week-old

Figure 1. The importance of vertebrate foods (eaten live and as carrion) in the crow's diet



[a]Percent volume = sum of %vol./n (see Swanson et al. 1974)

Table 1. Prevalence of serum rabies antibodies in wild crows sampled in central Iowa during 1979-1981

Adult crows			Nestling crows				
				Birds		Nests	
Sampling season	n	% positive	Age of nestlings	n	% positive	n	% positive
Apr - Jun	40	20.0	1 week	105	0	30	0
Jul - Oct	31	67.7	4 week	196	12.8	70	22.9
Nov - Mar	261	12.3	>4 week[a]	27	22.2	27	22.2
Totals	332	18.3					

[a]When 4 weeks old, 1 nestling per nest from 27 of 70 nests was taken into captivity and sampled biweekly for 8 weeks. If a positive sample was obtained during this time, the bird was classified as positive.

nestlings. The prevalence found in nestlings older than 4 weeks was not different from that in younger birds ($\chi^2 = 1.78$).

Prevalence of rabies antigens in wild skunks

Large discrepancies in the prevalence of rabies antigens in wild Striped Skunk populations were found among the 3 data sources (Table 2). Rabies was detected more frequently in skunks submitted to diagnostic labs than those sold to fur buyers ($\chi^2 = 86.99$, $P < 0.005$) and road-killed skunks ($\chi^2 = 37.29$, $P < 0.005$). A dramatic increase in the prevalence of positive skunks submitted to diagnostic laboratories occurred from 1979 to 1981 ($\chi^2 = 44.42$, $P < 0.005$). Data from the other 2 sources did not reflect this trend.

The chronologic pattern of rabies antigen prevalence in Striped Skunks (Figure 2) was different from the rabies antibody prevalence in crows (Table 1). Although more skunks were submitted to diagnostic labs during the summer, rabies seemed to be less prevalent then than during other seasons (Figure 2).

Correlations between antibody and antigen levels in 7 study areas

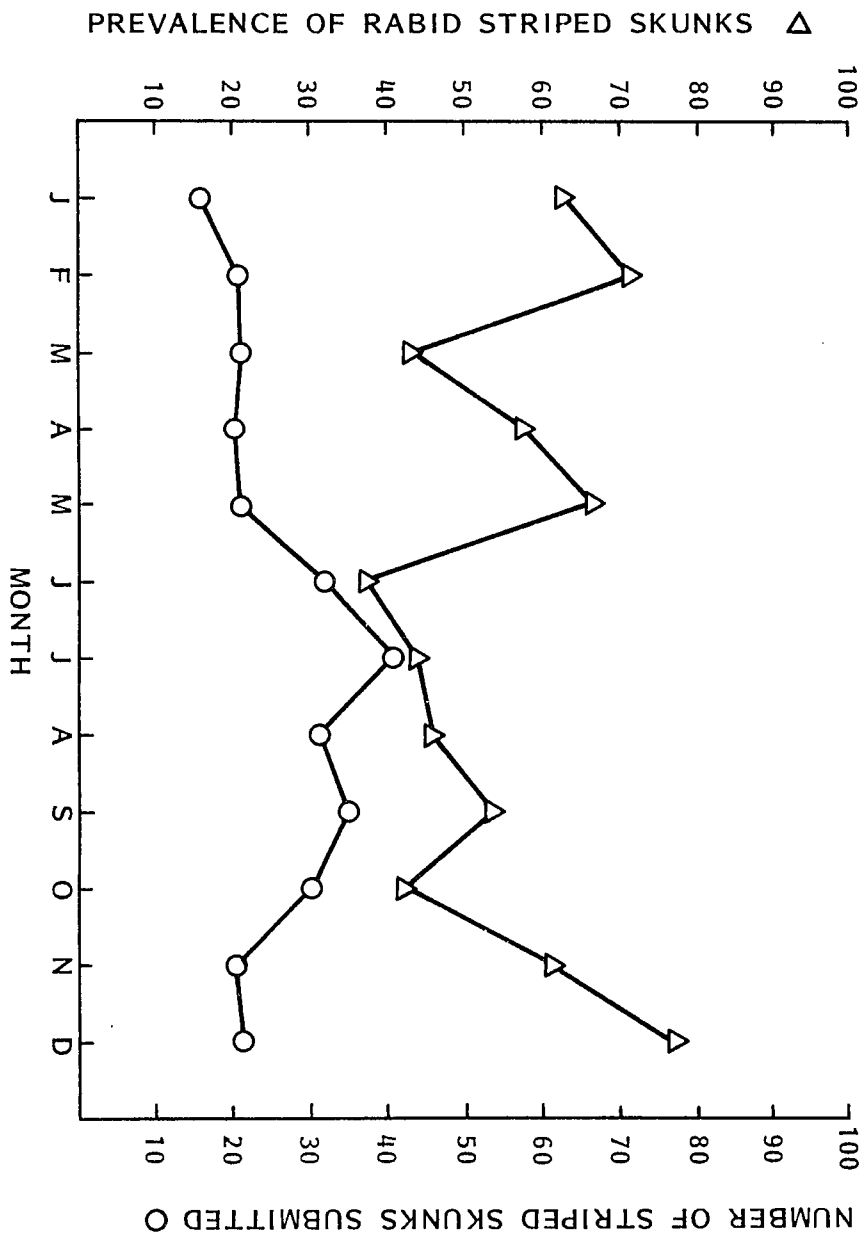
No correlations between the prevalence of rabies antibodies in crows and the prevalence of rabies antigens in skunks were found when using either standard data ($r^2 = 0.115$, $F = 0.65$) or arcsin transformed and weighted by sample size data ($r^2 = 0.138$, $F = 0.80$).

Using a log-likelihood ratio test, 3 factors (species, + or - sample results, area) were found not to be independent ($G = 223.50$, $P < 0.01$). Additional testing was conducted to examine this joint dependence among the

Table 2. Prevalence of rabies antigens in wild Striped Skunks sampled in central Iowa during 1979-1981

Iowa State Department of Health Diagnostic Lab Records			Road-kills		Fur buyers		
	n	% positive	n	% positive		n	% positive
1979	56	12.5	25	4.0	1979-80	101	21.8
1980	120	57.5	8	0	1980-81	135	8.9
1981	143	65.0	12	8.3			
Totals	319	53.0	45	4.4		236	14.4

Figure 2. Mean monthly prevalence of rabid Striped Skunks and mean monthly number of submitted Striped Skunks from diagnostic lab data collected for central Iowa during 1979-1981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3 factors. A test of independence between species and sample results was significant ($G = 25.51$, $P < 0.01$), indicating that the probability of an animal being positive for rabies differed depending upon whether it was a skunk ($P = 0.34$) or a crow ($P = 0.19$). A further test showed that this relationship varied by area ($G = 18.47$, $P < 0.01$). The results of all statistical testing suggests that there is no constant relationship between the prevalence of antibodies in wild crows and antigens in wild Striped Skunk populations among areas.

DISCUSSION

There are several important premises to consider before accepting the use of the Common Crow as a sentinel species of rabies in wildlife populations. First, there must be a means for natural exposure of crows to rabies. Secondly, a reliable method for detecting the prevalence of exposure in crow populations must be available. Finally, this rate must be positively correlated with the rabies prevalence in wildlife populations.

Several previous reports (Scott, 1884; Barrows and Schwarz, 1895; Crook, 1936; Kalmbach, 1939; Platt, 1956) and this study have documented crows scavenging on rabies-susceptible mammalian carrion. In another study, rabies titer levels of brain samples from rabid skunk carcasses held at 10°C did not diminish significantly during a 3-week trial; but an inverse relationship between titer level and exposure time was found in samples held for 2 weeks at 24°C (see Section II, herein). Others also have reported lengthy viability of rabies antigens at various temperatures in carcass tissues (Soave, 1966; Burkel et al., 1970), sausage baits (Winkler et al., 1975; Winkler and Baer, 1976), and eggs (Debbie, 1974). Because of this prolonged viability, the potential for natural oral transmission of rabies virus by scavengers is high.

Experimental oral transmission of rabies to crows has been shown (see Section I, herein). After ingesting rabid mouse brains and carcasses, crows developed serum antibodies, but did not contract clinical rabies. Jorgenson et al. (1976) earlier documented humoral immune response to experimental oral infection in a Great Horned Owl.

No correlations were found between rabies prevalence in crow and skunk populations. The log-likelihood ratio tests also did not indicate a constant relationship in the probability of being positive when species were compared among areas. The difficulty of this type of analysis is evidenced by the fact that other studies (Barnes, 1975; Bigler et al., 1975) have proposed to use wildlife as sentinel species of different zoonoses and pollutants without even attempting to prove statistical relationships. It is possible that different results could have been found if a larger and broader data base were obtained. A team approach involving a number of investigators would provide the best way to gather sufficient samples to test this hypothesis adequately.

Another problem in trying to show a positive correlation between the prevalence of rabies antibodies in wild crow populations and the prevalence of rabies antigens in wild mammals is the biases inherent in mammalian sampling. Diagnostic laboratory sampling involves testing primarily those animals that have been involved in actual or potential, human or domestic animal exposure. A survey of trapped mammals is biased because individuals infected with rabies may change in behavior and susceptibility to trapping (Verts, 1967; Niemeyer, 1973). There is further bias in mammalian surveys because any rabid mammals that die are not sampled.

An unknown influence on the use of crows as a sentinel of rabies is the possibility of the existence of an undocumented virus that is antigenically similar to rabies virus. If such an agent exists, a serosurvey for rabies in crows could produce false positive samples and a misinterpretation of the prevalence of rabies in crows.

If it could be shown that there is a relationship between rabies prevalence in crow and skunk populations, I believe sampling 4-week-old nestling crows would provide the best index of rabies prevalence in wildlife populations. Rabies is not transmitted embryonically in crows (Table 1), but rabies-susceptible animals are important components of nestlings' diet (Figure 1). Using data from Kalmbach (1939), the relative volume of carrion and other noninsect animal matter also was greater in nestling samples (25.4%) than in adult samples taken at different times of the year (April-June, 14.4%; July-October, 5.6%; November-March, 8.9%). The prevalence of rabies antibodies in crow nests was less only than that found for adult crows sampled during the summer. Because of the difficulty in trapping adult crows in summer, it is more feasible to sample a sufficient number of nestling crows. Active nests can be located easily in spring before leaf-out. About one man-hour of work is required to obtain samples at each nest site.

Because of the rapid antibody development (<1 week; see Section I, herein), short persistence of antibodies in crows (<2 weeks, see Section I, herein), and the limited home range of crows during the nestling season (<2 km , see Section III, herein), the prevalence of rabies antibodies in nestling crow populations would be an index of the current prevalence of rabies in local wildlife populations.

The use of crows as an epidemiologic tool for monitoring the incidence of sylvatic rabies in the spring would aid public health officials in locating high risk areas. Warnings and control measures then could be implemented prior to the season when most human and domestic animals are

exposed to rabies. Researchers also could more accurately measure the effectiveness of oral vaccination and other rabies control programs.

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SUMMARY AND DISCUSSION

Four projects were undertaken to test the feasibility of using the Common Crow as a sentinel species of rabies in wildlife. In one project, 3-month, 1-year, and 2-year-old captive crows contracted rabies orally when fed rabies infected mice brains. Virus shedding was detected in 62.1% of all experimental birds (N=29). One-year-old crows developed an earlier humoral immune response with a greater mean antibody titer than did 2-year-old birds. An inverse relationship also was found between the inoculant dilution and the time after inoculation when humoral antibodies were detected. Antibody levels persisted for about 2 weeks. No birds died or showed overt clinical signs of rabies disease during the experiment.

In another project, rabies virus remained viable in Striped Skunk carcasses for about 2 weeks at 24°C and throughout the 22-day study period at 10°C. The skunks with experimentally induced furious rabies had a higher mean titer than did the other skunks. The results of this project suggest that oral transmission of rabies virus among wild susceptible scavenger species may be a common occurrence.

In order to determine the geographic area that a survey of rabies in crows would monitor, information was collected on crow foraging areas and movement patterns. The mean foraging area around the nest site was 151 ha with the longest recorded flight from a nest of 4.8 km. During July and August, crow families continued to feed in their respective nest areas but joined other crows at nighttime roosts. Northern breeders migrated into central Iowa in November. Some crows were year-around residents. The

mean foraging area for winter roosting crows was 110.8 km^2 and varied directly with the number of birds in the roost.

In the fourth project, two final aspects were investigated: 1) the importance of rabies susceptible animals in the crow's diet and 2) the relationship between the prevalence of rabies antibodies in wild crows and rabies antigens in wild skunks. Vertebrate foods occurred in more than 40% of the food samples collected from November to June. Fifty observations of crows scavenging on road-killed carcasses were recorded. On 8 of these occasions, crows were feeding on Striped Skunks. The prevalence of rabies antibodies in 332 wild adult crows and 70 crow broods was 18.3% and 31.4%, respectively. Because of mammalian survey biases, large discrepancies in the prevalence of rabies antigens in wild Striped Skunks were found among the 3 data sources. Rabies was detected more frequently in skunks submitted to diagnostic laboratories (53%) than those sold to furbuyers (14.4%) and road-killed skunks (4.4%). No statistical correlations were found between rabies prevalence in crows and skunks. Sampling 4-week-old nestling crows is recommended as the best index of rabies prevalence in wildlife.

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