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SEROLOGIC TESTING OF BADGERS TO MONITOR PLAGUE IN SOUTHWESTERN IDAHO[1]

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ABSTRACT: Serologic testing of badgers (*Taxidea taxus*) was used to monitor plague (*Yersinia pestis*) in a Townsend ground squirrel (*Spermophilus townsendi*) population in 10,000 ha of the Snake River Birds of Prey Study Area, Idaho. Eighty-six percent of the 294 sera tested in 1975 and in 1976 were positive. Significantly fewer (72%) seropositives occurred in 1977. Seasonal changes in the percentage of seropositives and the decline in 1977 were probably due to the phenology of the Townsend ground squirrel and the proportion of that species in the badger's diet. Eight Townsend ground squirrels found dead had positive bacteriologic tests for plague; however, a high mortality in the ground squirrel population was not observed. Food habits and movement patterns of badgers made them ideal for documenting the geographical and temporal characteristics of the plague focus.

INTRODUCTION

The prevalence of plague (*Yersinia pestis*) in the Snake River Birds of Prey Study Area, Idaho was investigated over a 3-yr period as part of ecological studies of the Townsend ground squirrel (*Spermophilus townsendi*) and North American badger (*Taxidea taxus*).

The passive hemagglutination (PHA) test for detecting antibodies of *Y. pestis* in carnivore sera has been used to determine the extent of plague in rodent populations (Meyer et al., 1965; Archibald and Kunitz, 1971). Most carnivores develop only transient clinical signs to plague, and lethal infection is observed infrequently (Marchette et al., 1962b; Rust et al., 1971). Badgers develop transient infection and measurable antibody response after eating infected animals. Hetlet (1968) and Fitzgerald (1970) observed PHA positive sera in badgers during an epizootic of plague in Gunnison prairie dogs (*Cynomys gunnisoni*) in Colorado. Poland et al. (1973) reported that 10 of 10 badgers sampled during the epidemiologic investigation of a human plague case in Arizona had high titers. Serologic studies in carnivores have been used as a tool for surveillance of plague by personnel of the Centers for Disease Control since the early 1970's (Barnes, 1974). We used the same technique to monitor plague in Townsend ground squirrels and other prey species when we tested badger sera. Our study apparently represents the first instance in which serologic investigation of plague has been integrated with population studies of a rodent host and a closely associated predator.

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STUDY AREA

The Snake River Birds of Prey Study Area (SRBPSA) lies between Walter's Ferry and Indian Cove, Idaho, and encompasses approximately 193,300 ha. Most research during the study was centered in a 10,000-ha area near Swan Falls, Idaho, although sampling occurred throughout the study area.

Vegetation of the Snake River Plain can be characterized as shrub-steppe (Daubenmire, 1970). In the Swan Falls area, there is a mosaic of *Artemisia tridentata/Poa sandbergii* and *Eurotia lanata/Poa sandbergii* associations (Franklin and Dyrness, 1973).

The SRBPSA has a high density of Townsend ground squirrels. In the Swan Falls area in 1975–1977 densities were from five to 10 squirrels/ha. Elsewhere in the study area, densities varied from less than one squirrel/ha to five squirrels/ha (Johnson and Smith, 1978). The only rodent species present in moderate densities sympatric with the Townsend ground squirrel in the Swan Falls area were *Dipodomys ordii* and *Peromyscus maniculatus* (Montan, 1977). *Dipodomys* spp. have been shown to be refractory to

TABLE 1. Number and prevalence of serum samples from badgers positive for antibodies to *Yersinia pestis* in the Snake River Birds of Prey Study Area, Idaho, 1975–1977.

Year		All neg.	PHA serum titer									All pos.
			16	32	64	128	256	512	1,024	2,048	4,906	8,192
1975	Number	2	0	1	3	5	2	5	3	1	0	0	20
	Percentage	9.1	0	4.6	13.6	22.7	9.1	22.7	13.6	4.6	0	0	90.9
1976	Number	17	5	7	17	18	12	11	11	7	8	5	101
	Percentage	14.4	4.2	5.9	14.4	15.3	10.2	9.3	9.3	5.9	6.8	4.2	85.6
1977	Number	42	2	12	14	18	20	22	14	7	1	2	112
	Percentage	27.3	1.3	7.8	9.1	11.7	13.0	14.3	9.1	4.6	0.7	1.3	72.3
1975–1977	Number	61	7	20	34	41	34	38	28	15	9	7	233
	Percentage	20.8	2.4	6.8	11.6	14.0	11.6	12.9	9.5	5.1	3.1	2.4	79.2

plague infection (Holdenreid and Quan, 1956). *Peromyscus maniculatus* susceptibility, on the other hand, has been shown to vary substantially from one population to another and to be heterogeneous within populations (Quan and Kartman, 1956). The susceptibility of populations of *P. maniculatus* in Idaho is unknown.

Adult and yearling badgers were resident and restricted their movements to home ranges <400 ha. During the study the Swan Falls area supported a density of up to five residents (adults and yearlings) per 100 ha (Messick and Hornocker, 1981).

MATERIALS AND METHODS

Between spring 1975 and late summer 1977, 294 blood samples were collected from live-captured badgers. Two to 4 ml of blood was taken from one of the superficial veins of the front leg. After the sample clotted, the serum was separated and frozen.

Serum samples were kept frozen and shipped on dry ice to the Plague Branch, Centers for Disease Control, Fort Collins, Colorado, for serologic testing. The passive hemagglutination (PHA) test for specific antibody to Fraction 1 of *Y. pestis* and passive hemagglutination inhibition (PHI) control test on positives to eliminate possible cross-reactions with *Yersinia pseudotuberculosis* antibody were performed on all samples (W.H.O. Committee on Plague, 1970). Results are reported as a dilution ratio of 1:4 to 1:8,192, with titers ≥1:16 considered positive. The numerator has been omitted from tables and graphs for convenience.

Townsend ground squirrels were live-trapped on five grids, each 1 ha in size, during the squirrels' active months, in 1975, 1976, and 1977. Nine ground squirrels found dead or sick were examined at necropsy, and spleen and liver samples were frozen and shipped to the plague laboratory for testing with the fluorescent antibody technique (Moody and Winter, 1959) and for isolation of *Y. pestis*, using standard plating methods and blood agar media. After incubation at 28 C for 24 hr, colonies typical of *Y. pestis* were tested for sensitivity to specific anti-plague bacteriophage.

Ecology of the badger was studied simultaneously and in the same area through capture-recapture techniques, radiotracking and analysis of digestive tracts (Messick and Hornocker, 1981).

RESULTS AND DISCUSSION

Limited serum collections were made in 1975; however, a high proportion of PHA antibody positives (91%) prompted increased sampling during the two ensuing years (Table 1).

Forty-six badgers were recaptured and each was sampled two to seven times during the study. The duration of their elevated antibody titers varied widely, presumably because of reexposure to plague between samples, or relapses of chronic infection. However, the rate of increase or decrease of titers can be estimated with serial samples from those badgers recaptured within a relatively short period of time. For example, one badger showed an increase from a titer of 1:512 to 1:2,048 in 19 days, followed by a decline to 1:32 in 51 days. Four other badgers showed similar changes.

These data suggest that badgers may have antibody responses to plague similar to those of other carnivores. Rust et al. (1971) reported positive PHA test results for dogs and cats by the eighth day after exposure. Peak titers were reached in 8 to 21 days and persisted for at least 300 days. Meyer et al. (1965) reported that mongooses (*Herpestes auropunctatus*) developed antibodies within 14 days of exposure and peak titers within 28 days. Antibody production may be dependent upon the number of organisms initially consumed and the additive effects of repeated infection. For example, the complement-fixation antibody titer measured in adult coyotes was directly proportional to the number of *Y. pestis* to which they were exposed (Marchette et al., 1962b). Mongooses inoculated

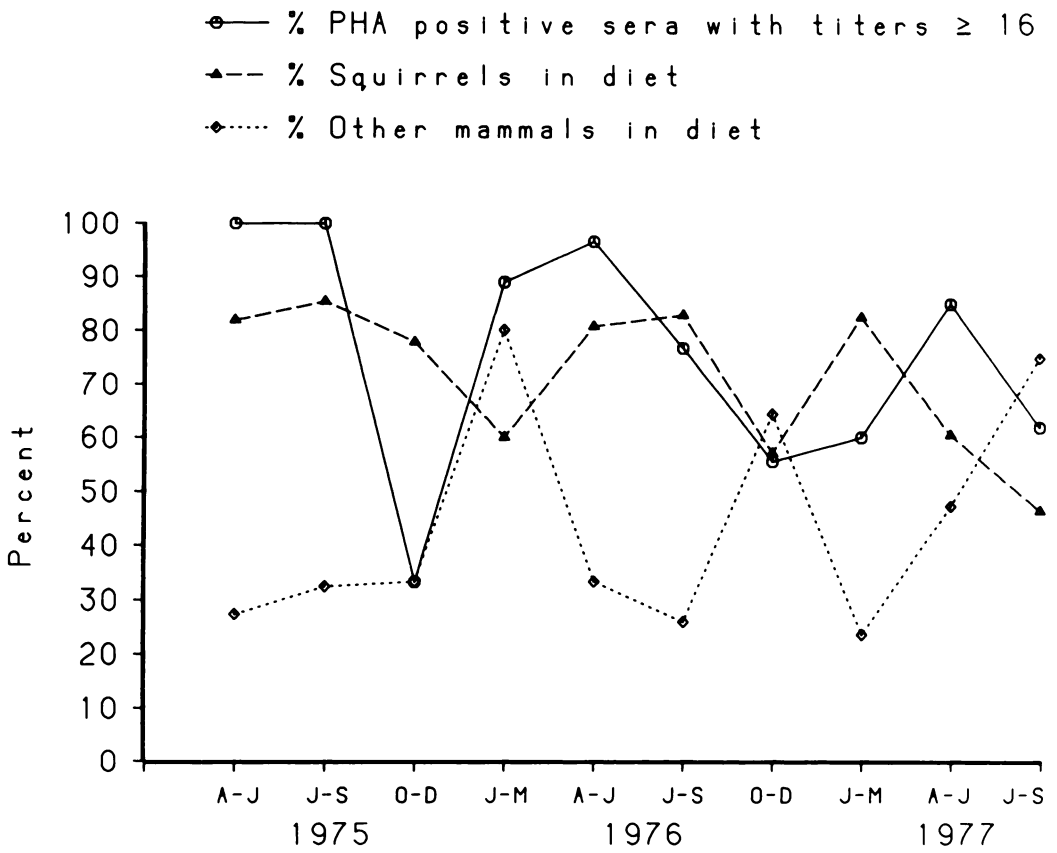

FIGURE 1. Seasonal changes in % PHA positive sera with titers ≥16 to *Yersinia pestis* in 294 badger sera, and percentage frequency of occurrence of Townsend ground squirrels and all other mammals combined, in 427 badger food samples. There were fewer positive sera in 1977 ($\chi^2 = 8.37$, df = 1, $P < 0.01$). Badger food samples contained fewer Townsend ground squirrels and more of other mammals in 1977 than in 1975 and 1976 ($\chi^2 = 27.33$, df = 1, $P < 0.01$). A–J = April, May and June samples, J–S = July, August and September samples, O–D = October, November and December samples and J–M = January, February and March samples.

with a small dose of plague showed a decline of antibodies within 28–96 days, but the antibodies persisted in those animals treated with larger doses (Meyer et al., 1965).

The juvenile members of badger family groups (adult female and young) always had lower PHA titers than the parent (Table 2). Samples for Table 2 were collected from late March to early June (the period between birth and dispersal of young). It is possible that the litter acquired antibodies via placental transfer or from the adult's milk. Maternal origin of antibodies to plague was documented in Norway rats (*Rattus norvegicus*) (Williams et al., 1974, 1977). It is also possible that young badgers acquired their initial antibodies after feeding on infected prey brought to the maternal den by the adult. Either possibility would account for the similar PHA titers in litter mates (Table 2). Higher titers in the parent may result from differential transfer to the offspring, larger doses of infected prey and the additive effects of repeated exposure.

There were significantly ($\chi^2 = 8.37$, df = 1, $P < 0.01$) fewer seropositive samples in 1977 than in 1975 and 1976 (Table 1). PHA antibody positives in badgers also varied seasonally (Fig. 1). Both the decline in seropositive results in 1977 and the seasonal variation in seropositives was probably related to the food habits of badgers; namely, the percentage of Townsend ground squirrels in the badgers' diet (Fig. 1).

TABLE 2. Hemagglutination titers to *Yersinia pestis* in serum samples from badger family groups sampled in 1975–1977 on the Snake River Birds of Prey Study Area, Idaho.

Adult female			Juvenile no.							
			1		2		3		4	
Animal number	Titer	Date[a]	Titer	Date[a]	Titer	Date[a]	Titer	Date[a]	Titer	Date[a]
92	1,024	07/5/76	256	01/5/76	128	04/5/76	64	05/5/76		
98	512	11/5/76	64	29/5/76	64	29/5/76	64	30/5/76		
	2,048	30/5/76								
101	512	14/5/76	128	09/5/76	64	10/5/76				
104	1,024	17/5/76	128	17/5/76						
37	512	24/5/76	64	23/5/76						
			64	03/6/76						
37	256	29/5/77	Neg.	29/5/77	Neg.	29/5/77	32	13/6/77	32	13/6/76
96	512	04/3/77	128	10/5/77	128	16/5/77	128	17/5/77		
	1,024	31/5/77								
155	2,048	12/5/77	512	11/5/77	256	12/5/77				
43	256	26/5/77	32	13/5/77	32	25/5/77	Neg.	13/6/77		
			16	25/5/77	Neg.	15/6/77				

[a] Day/month/year.

Some time lag in duration and variation of the immunologic response would be expected.

Townsend ground squirrel remains were present in 70% of 427 badger stomachs, colons, and scats examined during the study. Other mammals in badger food habit samples and their percentage frequency of occurrence included: *Lepus californicus* (4%), *Sylvilagus nuttali* (1%), *P. maniculatus* (11%), *Microtus montanus* (4%), *Dipodomys* spp. (6%), *Reithrodontomys megalotis* (2%), *Perognathus parvus* (1%), and *Onychomys leucogaster* (1%). The combined frequency of occurrence of all mammals (see Messick and Hornocker, 1981) other than the Townsend ground squirrel was 44%.

Seasonal and yearly changes in food habits of badgers were related to the availability and phenology of the Townsend ground squirrel (Messick and Hornocker, 1981). In 1975 and 1976 Townsend ground squirrels emerged from hibernation in February. Young were born in March and emerged from natal burrows in early April. Adult males and females began estivating in late May and early to mid-June, respectively. Juveniles estivated in late June and early July. By the second week of July, few squirrels were still active (Johnson and Smith, 1978).

Predation by badgers was greatest during the period of squirrel activity and after reproduction had increased their density. Badgers consumed fewer squirrels and more of other animals during the fall and winter (Fig. 1).

The ground squirrel cycle in 1977 differed from the pattern described for 1975 and 1976. A drought in the region in 1977 resulted in failure of the ground squirrel population to reproduce. The ground squirrel population estivated in late April and early May. By mid-May, no squirrels were active (Johnson and Smith, 1978).

The proportion of Townsend ground squirrels in the diet of badgers decreased correspondingly between April and September 1977 (Fig. 1). The proportion of mammals other than Townsend ground squirrels (Fig. 1) and of nonmammalian foods (see Messick and Hornocker, 1981) eaten by the badgers was predictably higher in 1977 than in the two previous years.

It is probable that the epizootiology of plague, including the diet of badgers, contributed to the seasonal and yearly fluctuations in seropositive results. Seasonal fluctuation in numbers of *M. californicus* and *P. maniculatus* with serum antibodies to *Y. pestis* have been observed in Utah and California (Hudson et al., 1964; Cavanaugh et al., 1965; Hudson and Kartman, 1967). Plague is often most prevalent among ground squirrels during the period of highest density, a time when they are most likely to exchange flea vectors (Evans et al., 1943).

Such seasonal fluctuations in the prevalence of plague could account for the observed correlations between PHA antibody positives and squirrel phenology. However, the percentage

of Townsend ground squirrels in badger food samples appears to reflect seasonal changes in the percentage of positive serums (Fig. 1). That would be expected if badgers acquired antibodies primarily from feeding on infected Townsend ground squirrels. Relatively small numbers of other mammals in the badger's diet and the fact that they are not associated with a seasonal change in seropositives (Fig. 1) suggest, indirectly at least, that badgers were exposed to plague primarily through Townsend ground squirrels. Furthermore, eight of nine ground squirrels found dead in 1975–1977 within 6 km of Swan Falls were positive for *Y. pestis* by the fluorescent antibody test; two of eight cultures from these rodents were positive. No other dead ground squirrels were found during live-trapping operations. Because we did not evaluate the rate of infection among living squirrels serologically, their rate of exposure is not known. However, the number of seropositives among badgers and the information obtained on their prey relationships and their movements suggest that there was a widespread and general epizootic with little mortality among Townsend ground squirrels during our study. Thus, Townsend ground squirrels may be relatively tolerant of plague infection, at least as they are exposed to *Y. pestis* in the southwestern Idaho ecological milieu. Vector efficiency of the flea species, flea abundance, and the infectivity and pathogenicity of the strains of *Y. pestis* were not evaluated, nor did we challenge Townsend ground squirrels with virulent plague strains in the laboratory. Studies by Williams et al. (1978) on *Y. pestis* suggest that antibody production in natural populations may be elicited by avirulent strains, but there is no indication that such strains play a significant role in the ecology of plague. For example, avirulent strains would not be expected to produce a bacteremia in ground squirrels and thus, in turn, produce antibodies in badgers following ingestion of squirrels.

Yersinia pestis may persist from year to year among the ground squirrels, in infected ground squirrel fleas, or in resistant rodent species. *Peromyscus maniculatus* and *D. ordii* are the only other rodents present in moderate densities sympatric with the ground squirrel population and that exist in the habitats used by badgers. *Peromyscus maniculatus* has been repeatedly associated with plague in California (Nelson, 1980). *Peromyscus* spp. and *D. ordii* were suspected to be reservoir species in Utah (Marchette et al., 1962a; Thorpe et al., 1963). In our study, *Peromyscus* spp., *Dipodomys* spp. and *Microtus* spp. were found in 11, 6 and 4% of badger food samples, respectively. The relatively low frequency and small biomass of these potentially bacteremic hosts make it impossible for us to determine their rates of infection using the serology from badgers.

The persistent occurrence of antibody to *Y. pestis* among badgers over the 3 yr suggests that plague may be enzootic in the shrub-steppe community of southwestern Idaho. That the phenomenon is general rather than localized is illustrated by badgers which we sampled in 1977 near Bruneau, Idaho, 57 km from Swan Falls. Five of six of these samples were positive; titers ranged from 128 to 2,048. However, 1975–1977 were peak years for plague throughout the western United States (Centers for Disease Control, Vector-Borne Diseases Division, 1975, 1976, 1977). The cyclic or sporadic amplification patterns and geographic expansion of *Y. pestis* are well known, as is its ultimate withdrawal to enzootic foci (Pollizer, 1954). The prevalence of animals with titers to plague in our study area with evidence of past infection could therefore represent a portion of this cycle rather than indicate that plague is enzootic among the study population in southern Idaho. Continued and long-term monitoring of these animal populations for evidence of *Y. pestis* during the period of recession would be necessary to evaluate this possibility.

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