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ABSTRACT: Carnivores obtain plague infection through ingestion of infected rodents or rabbits or via flea bite. Most are resistant to infection, show little or no symptoms, and produce antibodies to Yersinia pestis which may persist for several months. Consequently, carnivores can be used as serological plague sentinels using the specific passive or indirect hemagglutination test in the laboratory. A carnivore serology program for plague detection began in California in 1974. The program is a cooperative effort incorporating state and federal vector-borne disease units and state and federal predator animal control personnel. The program has proven to be an important tool in the overall statewide plague surveillance program of the California Department of Health Services, and has been effective in detecting plague over broad geographical areas of the state, as well as in areas heretofore unknown to experience plague activity. Carnivore serology has also proven useful in helping to delineate the extent of plague epizootics, and has given insight into the patterns of enzootic plague among wild rodent populations.

INTRODUCTION

Bubonic plague is a disease of rodents caused by the bacterium Yersinia pestis, which exists in widespread discontinuous foci in portions of Africa, Asia, and the Americas. It has been known to exist in the United States since 1899, first introduced into California seaports by infected commensal rats arriving on ships from Asia (Link 1953). Plague was subsequently discovered in native wild rodent populations (ground squirrels) near San Francisco in 1908 and in Los Angeles in 1924. Over the past 76 years it has spread easterly among wild rodent populations as far as eastern Montana, western Texas and Oklahoma.

The disease is characterized by periodic epizootic die-offs among susceptible rodent populations, spread among individual rodents by infected fleas, and by ingestion through cannibalism. During these epizootics, man and other mammals become accidental victims of infection, primarily by flea bite, or by direct contact with infected rodent tissues. Since a 1924 episode in Los Angeles involving commensal rats and human plague, all human cases in the United States have been associated with native wild rodent and flea epizootic sources. Concerns of health authorities continue to be the potential transfer of plague from the firmly entrenched wild rodent sources to commensal rat populations, with subsequent urban epidemics and the potential of pneumonic aerosol spread among humans triggered by an initial case of secondary plague pneumonia. The disease continues to be a very real threat to the public health in this country and has been on the increase in the past 15 years, with 40 cases in 1983, 6 fatal (Centers for Disease Control, personal communication).

Plague surveillance programs are maintained by state and local health agencies in cooperation with the U.S. Department of Health and Human Services Plague Laboratory at Ft. Collins, Colorado. Programs are aimed at determining the animal and vector species involved in each ecological situation, determining the geographic scope of disease activity among rodent populations, and attempting to detect early evidence of potential epizootics in order to implement control measures to prevent human cases. A carnivore serology program to detect plague antibody to Y. pestis through laboratory testing of carnivore blood samples, has become an important component of the surveillance programs in many western states including California.

This paper discusses the importance of the wild carnivore serology program to the surveillance efforts of the California Department of Health Services, and the use of the program in helping to determine patterns of plague activity among wild rodent populations in the state.

BACKGROUND AND METHODS

Carnivores obtain plague infection through ingestion of infected rodents or rabbits or via flea bite. Most carnivore species are resistant to infection, show little clinical evidence of disease, and produce rapid serologic response to infection, developing antibodies to Y. pestis, which may persist for several months. Studies by Rust et al. (1971a) and Rust et al. (1971b) demonstrated that domestic dogs showed this pattern when orally challenged by experimental plague infection. Domestic cats, however, produced more severe clinical symptoms of disease, and experienced 50% mortality in their study. They suggested that dogs might be used in certain areas as serological sentinels of plague activity, especially where they roam freely, and may be associated with wild rodent populations. This methodology has been used with success in both the United States and Africa during plague investigations (Archibald and Kunitz 1971; Taylor et al. 1981).

Based on the studies of domestic carnivores, Barnes (1974) proposed a program of serological testing of wild carnivores for plague detection. It was felt that serological testing of wild

carnivores might give indirect evidence of plague among rodent prey species, even during inter-epizootic periods of low-level plague circulation. Laboratory challenge studies showed responses among coyotes, skunks, and raccoons to be similar to that of domestic dogs as described by earlier workers (Barnes 1982). The development of special filter-paper blood-collection strips made the program quite cost-beneficial as compared to the more costly and labor-intensive collection and testing of wild rodents and fleas over widespread areas. The program was established as a cooperative effort between state and federal wildlife and health authorities.

Wild carnivore blood sampling for plague antibody detection began in California in the northeastern portion of the state in 1974. Samples are collected in the field by predator trappers and other cooperators during the normal course of their operations. Special filter paper sample strips are utilized for collection of blood. Specimens are air-dried, placed in individual envelopes with collection data and sent to state coordinators who catalog the data and forward specimens to the Plague Laboratory, Center for Disease Control, Department of Health and Human Services, Ft. Collins, Colorado, for testing. Specimens on filter paper strips are tested by specific passive or indirect hemagglutination and inhibition tests as described by Wolff and Hudson (1974). Titers of 1:32 or greater are reported as positive.

Samples from coyote, bobcat, badger, skunk, raccoon, fox, bear, and a number of wild carnivores less frequently taken, are submitted for testing. The majority of samples are from trappers of the U.S. Fish and Wildlife Predator Animal Control Program, with trappers and researchers also contributing.

RESULTS AND DISCUSSION

Wild carnivore blood samples have been collected for serological testing for plague antibody in 45 of California's 58 counties, 1974-1982. Data are not completed for 1983 as of this presentation, and not reflected in the totals; however, they are cited as they relate to patterns of plague activity. A total of 402 samples was found positive for antibody to Yersinia pestis by PHA and PHI tests among 3466 specimens tested as presented in Table I. Positives represent 25 of the 45 counties from which samples were taken. The percent positives by year from California reflected in Table I compare closely to that reported by Hopkins and Gresbrink (1982) for the state of Oregon.

Table I. Wild carnivore sera tested for antibody to Yersinia pestis in California, 1974-1982.

YEAR	NO. TESTED	NO. POSITIVE*	% POSITIVE
1974	117	9	7.8
1975	133	13	9.8
1976	255	29	11.3
1977	372	11	2.9
1978	357	55	15.4
1979	584	107	18.3
1980	591	61	10.3
1981	591	73	12.3
1982	466	44	9.4
TOTALS	3466	402	11.6

* Positive antibody to Yersinia pestis by passive hemagglutination test.

Wild carnivore data are shown by species, percentage of the species among the total, and percentage of the total with plague antibody in Table II. Species positive include coyotes, representing 75.5% of the total sample, and bobcats, bears, badgers, raccoons, skunks, grey foxes, and pine martens representing smaller percentages, 6.6 to 0.8%, respectively. The high percentages of antibody shown among spotted skunks, pine martens, and bobcats may indicate their preference for rodent prey as compared to the more omnivorous carnivore species which also feed on other matter. The high incidence of plague antibody in black bears in California remains as yet unexplained. Mountain lion, fisher, ringtail cat, weasel, and kit fox were sampled infrequently, mostly during special studies by researchers, and were negative for plague antibody. Three positives among other specimens tested include two feral cats and a feral dog on a military installation.

Comparison of wild carnivore serological data by political boundaries (e.g., county), or by monthly or annual intervals, as collected in the described program is limited in accuracy by built-in sampling biases. Samples are collected somewhat randomly during normal predator control operations and are dictated primarily by animal damage requests. Nelson (1980), at a previous conference, discussed the ecology of plague among wild rodent species in California and the existence of the disease within known and suspected plague foci. Both reservoir and recipient rodent species are necessary for a

Table II. Wild carivore sera tested for plague antibody, by species in California, 1974-1982.

Species	No. Tested	Percent of Total Tested	No. Positive *	Percent Positive
Coyote	2617	75.5	279	10.6
Bobcat	230	6.6	42	18.2
Badger	85	2.45	6	7.0
Raccoon	36	1.0	1	2.7
Skunk	115	3.3	8	6.9
Spotted Skunk	2	< 0.1	2	100.0
Gray Fox	62	1.8	4	6.4
Black Bear	203	5.85	47	23.1
Pine Marten	29	0.8	10	34.4
Mountain Lion	5	0.1	0	--
Ringtail Cat	2	< 0.1	0	--
Fisher	1	< 0.1	0	--
Weasel	2	< 0.1	0	--
Kit Fox	1	< 0.1	0	--
Other (includes feral cat, dog and misc. non-carnivores)	76	2.2	3	3.9
Totals	3466	100.0	402	11.5

* Positive for antibody to Yersinia pestis by passive hemagglutination test.

natural focus of the disease to exist. The disease is cyclic, periodically spreading outward during epizootics among susceptible (recipient) species, then retreating once again within the focus where it is maintained among resistant (reservoir) species. Since wild carnivores obtain transient infection and resulting antibodyfrom ingestion of a variety of rodent prey over potentially widespread areas, carnivore serological data is best interpreted as it relates to the ecology of the disease, the occurrence of epizootics, and the knowledge of persistance of the disease within known and apparent plague foci.

In California over the past decade serological testing of wild carnivores has provided additional insight into the occurrence and persistence of plague in apparent foci, has been useful in defining the extent of epizootics among wild rodent populations in specific localities, and has demonstrated disease activity over broad geographical areas of the state.

PERSISTENCE OF PLAGUE IN APPARENT FOCI

Persistence of plague activity among wild rodent populations within plague zones, or apparent plague foci, has been demonstrated through the use of wild carnivore serology in several different areas of California. In Great Basin desert habitat of the Honey Lake Valley in northeastern California, serological positives have been taken among coyotes and bobcats in seven of the past nine winter/spring seasons, 1974 to 1982. Positives have occurred among coyotes and badgers in nine of the past ten winters since 1974 in mountain valleys of Siskiyou and Modoc counties. Periodic ground squirrel epizootics have occurred in many of these intermountain valleys over this past decade. A human plague case associated with one of these epizootics occurred in Scott Valley, Siskiyou County, in summer 1978. Following this case, monthly serum samples of coyotes taken by predator trappers showed continuing plague activity among wild rodent populations in Scott Valley and in neighboring Shasta Valley over an 18-month period.

Persistence of plague activity has also been demonstrated through wild carnivore serology in an apparent plague focus in Alpine County south of Lake Tahoe. Percentages of positive plague antibody among coyotes, bobcats, and grey foxes in this area have ranged from 20.5 to 68.4% among those sampled during each of six winters, 1977 to 1983, as shown in Table III. Epizootics among ground squirrels and chipmunks occurred in this area in the summer of 1977, and again in summer 1980, lending validity to the wild carnivore serological data, and existence of an apparent plague focus in this region.

Table III. Prevalence of antibody to Yersinia pestis among wild carnivores* sampled in consecutive fall/winter seasons, Alpine County, California, 1977-1983.

	Number Positive**/Number Tested					
Month	1977/78	1978/79	1979/80	1980/81	1981/82	1982/83
November	-	3/4	0/2	2/4	5/7	2/3
December	2/15	6/7	3/9	4/15	8/10	12/13
January	2/12	8/14	1/3	4/4	2/2	4/15
February	3/7	3/9	0/3	-	-	-
Totals	7/34	20/34	4/17	10/23	13/19	18/31
Percentage Positive	20.5%	58.8	23.5	43.4	68.4	58.0

*Samp1es from coyote, bobcat, and grey fox; vicinity of Fredericksberg and Markleeville, CA.
**Positive antibody to Yersinia pestis by passive hemagglutination test.

DELINEATION OF PLAGUE EPIZOOTICS

Messick et al. (1983) reported on the use of serologic testing of badgers to monitor plague activity in Townsend ground squirrels in the Snake River Birds of Prey Study Area in Idaho. We report here on the use of serologic testing of wild carnivores to help define the extent of plague epizootics among woodrats, deermice, and Beechey ground squirrels in sagebrush-juniper habitat, and among golden-mantled ground squirrels and chipmunks in coniferous forest habitat in California.

Plague was confirmed in bushy-tailed woodrats, deermice, Beechey ground squirrels and their fleas at Lava Beds National Monument, California in 1976-1977. Plague antibody was demonstrated in two badgers and one spotted skunk in the National Monument during this epizootic using serological methods. Antibody to Y. pestis was also shown in coyotes sampled by predator trappers within a 20 mile (32 km) radius of the National Monument in the Tulelake-Lower Klamath Region of California and Oregon, preceding, and during this confirmed epizootic, further defining the extent of plague activity (Table IV).

In Nevada County north of Lake Tahoe, in the Sierra Nevada Mountains in coniferous forest habitat, a plague epizootic was confirmed among wild rodent populations centered in the vicinity of the City of Truckee in summer 1976. Plague antibody was demonstrated with carnivore serology in six of ten coyotes taken from nearby Hobart Mills to Webber Lake, some 20 mile (32 km) distance this same summer, giving further definition to the extent of the epizootic in this habitat (Table V). In 1979-1980, serological samples of pine martens taken at Sagehen Field Station by a California Department of Fish & Game researcher showed a high percentage of positive plague antibody, demonstrating continuing plague activity in this Truckee area, at least among prey rodent species upon which pine martens were feeding.

DETECTION OF PLAGUE OVER BROAD GEOGRAPHICAL AREAS

Prior to the inception of the carnivore serology program, plague was known to occur in California in certain zones of activity as documented by known rodent epizootics and human plague cases. K. F. Murray prepared a map depicting these zones of epizootic potential in 1964 (unpublished). The presence of plague antibody in wild carnivores demonstrated plague activity among rodent populations, for the first time, well to the west of these known plague areas, in the north coast area of Humboldt and Mendocino counties, and in the coast ranges of Trinity County. Plague activity was also shown for the first time since the 1940s on the Modoc Plateau, in Alpine County south of Lake Tahoe, in Alameda County just east of San Francisco Bay, and in the area of Vandenburg Air Base south of Santa Barbara.

Wild carnivore serological sampling over the past decade has supplied the means to detect plague activity over a much broader area of the state than was ever before possible. The resulting data and continuing investigations of epizootic events have enabled us to update our knowledge of plague zones in California considerably, as shown by Nelson (1981).

The wild carnivore serology program has proven to be a valuable element in plague surveillance. If we are to reduce human exposure to sylvatic plague in the future, a knowledge of early detection of epizootic events is essential. This will only be gained through a continuing understanding of the ecology of plague among animal and flea populations.

Table IV. Correlation of wild carnivore sera testing, with standard laboratory testing of rodent sera and flea pools to delineate a plague epizootic in juniper/lava rim habitat, Tulelake-Lower Klamath Region, California-Oregon, 1976-1977.

Date	Antibody Titer in Coyotes, Tulelake-Lower Klamath Region* CALIFORNIA-OREGON	Antibody Titer in Rodents and Carnivores, Bacterial Isolation From Rodent Fleas (+), Lava Beds National Monument - CALIFORNIA
2-4-76	5 mi. SE Lorella, ORE** (1:128)***	---
7-16-76	15 mi. S Newell, CAL (1:64)	---
7-26-76	5 mi. W Timber Mtn., CAL (1:64)	---
8-21-76	6 mi. W Timber Mtn., CAL (1:64)	---
12-9-76	---	Deermouse, Labyrinth Cave (1:1024)
12-10-76	21 mi. S Tulelake, CAL (2)(1:64; 1:128)	---
2-9-77	---	Flea pool, wood rat, Skull Cave (+)***
		Flea pool, woodrat nest, Labyrinth Cave (+)
2-15-77	---	Badger, west boundary (1:128)
3-29-77	---	Spotted skunk, Sentinel Cave (1:256)
3-31-77	---	Flea pool, woodrat nest, Post Office Cave (+)
4-2-77	---	Woodrat, Sunshine Cave (1:2048)
5-19-77	---	Woodrat, Blue Grotto Cave (1:1024)
5-20-77	---	Badger, Capt. Jack's (1:4096)
6-14-77	---	Flea pool, woodrat, Gillem's Camp (+)
6-17-77	---	Flea pool, Beechey ground squirrel, Park Headquarters (+)
8-29-77	10 mi. SE Lorella, ORE** (1:64)	---
11-16-77	---	Woodrats (2), Blue Grotto Cave (1:4096) (2)
11-30-77	9 mi. SE Lorella, ORE** (1:128)	---

*All locations are within 32 km of Lava Beds National Monument.

**Records of Oregon Dept. of Human Resources, Health Division, Vector Control Section, 1978.

***Positive antibody to Yersinia pestis by passive hemagglutination test;

(+)Bacterial isolation of Y. pestis from rodent fleas.

Table V. Hemagglutination titers to Yersinia pestis in serum samples of coyotes, preceding an epizootic among wild rodents in coniferous forest, Sierra Nevada Mountains, California, summer, 1976.

Date	Animal	Location	Plague Findings*
7-1	Coyote	Hobart Mills	1:64
7-23	Coyote	Hobart Mills	1:128
7-23	Coyote	Hobart Mills	1:64
7-23	Coyote	Hobart Mills	1:128
7-29	Coyote	Webber Lake	1:64
8-2	Coyote	Webber Lake	1:64
8-31	Golden-mantled ground squirrel	Truckee	+
9-7	Golden-mantled ground squirrel	Donner Lake	+
9-30	Yellow-pine Chipmunk	Nr. Hobart Mills	+
9-30	Chipmunk (Sp.)	Nr. Hobart Mills	+

*Antibody to Yersinia pestis by PHA and PHI tests; bacterial isolation of Y. pestis (+) from rodent tissues.

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