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Authors: Chinwe U. Thomas, and P. Eric Hughes
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Plague Surveillance by Serological Testing of Coyotes (*Canis latrans*) in Los Angeles County, California

Chinwe U. Thomas and P. Eric Hughes, Los Angeles County Department of Health Services, Comparative Medical and Veterinary Services, 12824 Erickson Avenue, Downey, California 90242, USA

ABSTRACT: Sera from 19 (5.6%) of 338 coyotes (*Canis latrans*) collected in Los Angeles County, California between 1986 and 1990 had antibodies against *Yersinia pestis* using the passive hemagglutination test. Coyotes were excellent sentinels of plague activity in Los Angeles County. Enzootic plague declined dramatically in the county during the 5-yr period.

Key words: Plague, *Yersinia pestis*, coyote, *Canis latrans*, serological survey, prevalence, carnivores, Los Angeles County.

Over 90% of human plague infections in the United States occur in the southwest (Centers for Disease Control, 1988). From 1965 to 1989, 9.4% of human plague cases reported in the United States occurred in California. Three cases of human plague have occurred in Los Angeles County, California since 1978. The majority of human cases in California are epidemiologically associated with plague epizootics among California ground squirrels, *Spermophilus beecheyi*, and their fleas, *Oropsylla* (*Diamanus*) *montanus* (Barnes, 1990). *Spermophilus beecheyi* does not appear to be a reservoir of plague, but serves as an amplifying host (Nelson et al., 1986).

Wild carnivores are excellent sentinels for plague (Willeberg et al., 1979; Hopkins and Gresbrink, 1982; Nelson et al., 1986). Coyotes (*Canis latrans*) are a common nonmigratory wildlife species living in close proximity to human populations in many areas of the southwestern United States. Although coyotes are rarely involved in plague transmission (Barnes, 1982; Von Reyn et al., 1976), serological testing of coyotes can help define the extent of plague activity in rodent prey populations such as California ground squirrels more efficiently than testing rodents directly (Willeberg et al., 1979). We describe the findings from a 5-yr serological survey of coyotes in Los Angeles County, California (USA).

Los Angeles County encompasses more than 10,000 km^2 and consists of 163 cities and unincorporated communities (33°40′ to 34°50′N, 117°40′ to 118°55′W). Many parts of the county have a considerable coyote population (Howell, 1982). Coyotes become infected with *Y. pestis* by ingesting infected rodents or by being bitten by their fleas (Von Reyn et al., 1976; Thomas et al., 1989).

Coyote sera submitted to Comparative Medical and Veterinary Services, a division of the Los Angeles County Department of Health Services, between 1 January 1986 and 31 December 1990 were tested for antibodies to *Y. pestis*. Blood samples were collected by personnel of various city and county agencies as an adjunct to their routine pest abatement and animal control activities in Los Angeles County. The capture location, date of submission, sex and approximate age (adult or juvenile) of the coyotes were recorded. An adult was defined as a coyote estimated to be 1-yr-old or older. Blood was collected by cardiac puncture immediately after the coyotes were shot, and submitted in serum separation tubes within 72 hr of collection. The sera were centrifuged, extracted and kept frozen until tested. The plague Fraction 1 antigen and sensitized red blood cells used in serologic tests were supplied

TABLE 1. Prevalence of antibodies to *Yersinia pestis* in coyote sera from select communities in Los Angeles County, California, 1986–90.

Community	Number tested	Number positive[a]	Capture dates for seropositive coyotes	Inverse of titer
Griffith Park	29	6	Aug 1986	32
			Oct 1986	32
			Mar 1987	32
			Apr 1987	32
			Apr 1988	32
			Oct 1988	128
Sunland	25	3	Dec 1986[b]	64, 64
			May 1987	32
Glendale	17	4	Dec 1986[b]	64, 32
			Jan 1987[b]	32, 16
Los Angeles City	16	1	Mar 1986	16
Glendora	5	2	Apr 1987[b]	32, 32
Burbank	4	1	Jul 1989	32
La Canada-Flintridge	4	1	Aug 1989	32
San Marino	3	1	Sep 1988	32

[a] Serum titer ≥1:16 by the passive hemagglutination test (PHA).
[b] Two seropositive coyotes in month.

by the Centers for Disease Control (Fort Collins, Colorado, USA). Passive hemagglutination (PHA) and hemagglutination inhibition (HI) tests were used to detect the presence of plague antibodies in coyote sera using methods of the World Health Organization (1970). A serum antibody titer ≥1:16 was considered positive. Serum determined to be positive by the PHA test was then tested with the HI test. If the HI titer was below the PHA titer by less than two-fold, the positive PHA titer was considered nonspecific. Chi-square tests or Fisher exact tests were used where appropriate in statistical analyses (Dean et al., 1990).

Sera from 338 coyotes were tested for antibodies to *Y. pestis* during the 5-yr period. Adult coyotes comprised 75% of those tested. Nineteen coyotes (5.6%) had serum antibody titers ≥1:16 according to the PHA test. Three juvenile coyotes were seropositive [Griffith Park (1:32), City of Los Angeles (1:16) and La Canada-Flintridge (1:32)]. Nine (5.6%) of 161 male coyotes and nine (5.8%) of 155 female coyotes were positive for plague antibodies. One of 22 coyotes for which the sex was not recorded was seropositive.

Seropositive coyotes were detected in only one-third of the sample sites during the 5-yr period. Eight cities and unincorporated communities in Los Angeles County had serological evidence of plague (Table 1). Serological prevalence was significantly higher in coyotes from Griffith Park (20.7%) (Fisher Exact test; $P = 0.0029$) and Glendale (23.5%) (Fisher Exact test; $P = 0.0107$) than in coyotes captured elsewhere in the county. Positive sera from Griffith Park were collected between August 1986 and October 1988. All four seropositive coyotes from Glendale were submitted over a 2-mo-period (December 1986 to January 1987).

Serological prevalence steadily declined in the county over the 5-yr period (Chi-square test; $P = 0.013$). Seven of 50 (14%) coyotes tested in 1986 were seropositive; 7 of 89 (7.9%) coyote sera in 1987, 3 of 63 (4.8%) in 1988, 2 of 59 (3.4%) in 1989 and 0 of 77 (0.0%) in 1990 were positive for plague antibodies.

Serological prevalence peaked in April (17.4%) and December (12.5%). Seropositive coyotes submitted in April were captured in Glendora in 1987 ($n = 2$) and in Griffith Park in 1987 ($n = 1$) and 1988 (n

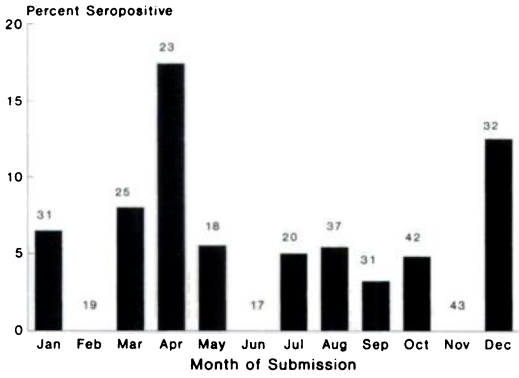

FIGURE 1. Prevalence of plague antibodies in Los Angeles County coyotes (*Canis latrans*) by month, 1986 to 1990 (serum titer ≥1:16 by the passive hemagglutination test (PHA)). Sample size is indicated at top of each bar.

= 1). The four positive sera submitted during December were collected from two areas, Sunland (n = 2) and Glendale (n = 2) in 1986. All coyote sera collected in February (n = 19), June (n = 17) and November (n = 43) during the 5-yr period were negative for plague antibodies (Fig. 1).

Serum antibody titers detected in this study ranged from 1:16 to 1:128. Only four of the 19 seropositive coyotes had titers greater than 1:32. The highest titer (1:128) was detected in a female coyote in Griffith Park in October 1988. Titers of 1:64 were found in each of two male coyotes from Sunland in December 1986, and in one male coyote from Glendale in December 1986.

The overall serological prevalence of 5.6% in coyotes in this 5-yr survey is the same as that reported for wild carnivores in Los Angeles County from 1978 through 1985 (Nelson et al., 1986). However, serological prevalence declined in the county during the past 5 yr (1986 to 1990), based on our findings. Similarly, fewer positive sera were detected in the ground squirrel populations in Los Angeles County; only one seropositive ground squirrel was reported in Los Angeles County in 1990 (Smith and Wilson, 1991). Sylvatic plague activity typically fluctuates over time (Willeberg et al., 1979; Christie, 1982; Barnes, 1982).

Plague-endemic foci have distinct patterns of activity. For example, in a large survey of carnivores in Oregon from 1974 to 1979, the highest serological prevalence occurred in June (21.7%) and the lowest rates were seen in December (3.0%) (Hopkins and Gresbrink, 1982). These prevalences contrast with those determined for coyotes in Los Angeles County. However, the methods used in the Oregon study differed from this study in that coyotes made up 81.5% of the sample, Nubuto paper strips were used for blood collection and a positive titer was defined as 1:32 or greater. Using 1:32 as the minimum positive titer in a separate analysis, we found no association between the month of sera collection and serological prevalence of plague antibodies. This finding is consistent with reports that hemagglutinating antibodies persist for more than 10 mo in domestic dogs (Rust et al., 1971) and at least 2 mo in black bears (*Ursus americanus*) (Clover et al., 1989). In a serological study of coyotes, Barnes (1982) found that hemagglutinating antibodies became detectable in 8 to 14 days, generally peaked in 20 to 30 days and diminished to undetectable levels within 6 to 8 mo. Persistence of serum antibody titers may depend on the number of organisms initially ingested and the cumulative effects of repeated infection (Messick et al., 1983). The presence of *Y. pestis*, as determined in this survey, is affected by plague antibody persistence and such data should not be used for risk assessment.

Plague appears to have disappeared from previously seropositive areas of Los Angeles County. However, the coyotes sampled in this study were dependent on the degree of participation by various trapping agencies in the surveillance program and the location of trapping activities within Los Angeles County. In recent years, more coyotes have been tested from areas in the county that had not been sampled in the past. This shift in testing areas (sam-

pling bias) may partially explain the recent decline in the number of seropositive coyotes; overall prevalence may have been diluted by increased testing in nonendemic areas.

The serological prevalence data reported here correlates well with that obtained from other methods of plague surveillance. Coyotes are efficient sentinels of plague activity in Los Angeles County. A dramatic decline in plague activity occurred during the 5-yr period. Any substantial increase in serological prevalence in coyotes may signal the development of a plague epizootic in the county.

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