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SEROLOGIC OBSERVATIONS DURING AN OUTBREAK OF RAT BORNE PLAGUE IN THE SAN FRANCISCO BAY AREA OF CALIFORNIA

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Abstract: Results of a serologic study of a plague outbreak among rats (*Rattus norvegicus*) and associated wild rodents are presented. Bacteriologic and serologic evidence points toward mutual exchange of fleas and plague infection between the intermingled rat and wild rodent populations. Results emphasize the value of serologic methods for epizootiologic studies of plague in North American rat populations.

INTRODUCTION

One of the most intensively studied enzootic foci of plague in North America is the San Bruno Mountain region of the San Francisco Bay area of California. A number of papers dealing with specialized aspects of these studies have already been published.[1,3,7] Serologic surveillance was maintained on one of the primary hosts, *Microtus californicus*, from 1962 to 1967. During this period four seasonal plague epizootics among *M. californicus* (California voles) and associated *Peromyscus maniculatus* (white-footed deer mice) were detected bacteriologically and serologically. At the most intense periods of wild rodent plague activity it was not unusual to detect seropositive rates of 80-100% in animals resident at various sites within this plague pocket.[2]

Numerous investigators have pointed out the frequent association of peridomestic populations of rats with wild rodents in the Western United States and the dangers of transmission of plague infection from the wild rodent reservoir to rats.[8,9] Such transmission occurred in the San Bruno Mountain plague pocket and was the subject of a series of investigations which have been summarized by Kartman and co-workers.[6] The continuous danger of the transfer of plague from such wild rodent reservoirs into urban rat populations through rat harborages in suburban areas makes it essential to apply the most complete surveillance methods available in such areas. Since serologic techniques had not been applied to North American rat populations, we reinvestigated a previous rat plague site[5] immediately after the extensive plague epizootic of the fall and winter of 1963-1964. Although these data represent the results of studies performed some 10 years ago, to our knowledge they represent the sole serologic study of rat-borne plague in North America. Owing to continuous urban development in this area, it is unlikely that additional data will be forthcoming from this particular site.

MATERIALS AND METHODS

The study site bordered the San Bruno Mountain Hog Farm study area of 1954. The general area and history of plague infection were described by Kartman *et al.*[5] A sketch of the area, including the previous hog farm site, is presented in Figure 1. Noticeable evidence of rat infestation was present in five areas centering around buildings and sheds of the hog

farming area. Visible evidence of commingling of wild rodents and rats were present at site A immediately northeast of pig sheds and food cookers located at site B. Intensive trapping was performed at sites A through E from April 22 through May 19, 1964. Trapping procedures, processing of animals, ectoparasite and tissue inoculations, passive hemagglutination tests, and fluorescent antibody techniques have been described previously.[3,4]

RESULTS

The trapping effort and animal catch from April 22 through May 19 are summarized in Table 1. The total catch for the area consisted of 57 Norway rats (*Rattus norvegicus*), 22 California voles (*M. californicus*), 25 deer mice (*P. maniculatus*), eight house mice (*Mus musculus*), two harvest mice (*Reithrodontomys megalotis*), one brush rabbit (*Sylvilagus bachmani*), and two weasels (*Mustela frenata*). Four feral dogs inhabiting the area were also shot or captured by hand. An additional seven mummified *R. norvegicus* carcasses were found during the trapping period in buildings scattered throughout the area. Tissues of these seven carcasses were examined and found to be negative by fluorescent antibody techniques. *Yersinia pestis* was isolated from tissues and a pool of 13 *Nosopsyllus fasciatus* from a moribund rat trapped at site B (Figure 1). Inoculation of pooled tissues of the remaining animals yielded negative results. The results of bacteriologic examination of flea pools are presented in Table 2. Fleas collected from animals other than rats, voles, and deer mice were also inoculated but yielded consistently negative results. The 572 fleas collected from rats, California voles, and deer mice were inoculated in a total of 69

TABLE 1. Trapping Effort and Animal Catch Trap Lines A through E, Hog Farm Study Area, San Bruno, Mountain, April 22 through May 19, 1964.

Trap Line	No. and Type of Trap	No. of Trap days	Catch	
			Species	No.
A	10 Young	1280	*Rattus norvegicus*	7
	70 Sherman		*Microtus californicus*	18
			Peromyscus maniculatus	20
			Mus musculus	1
			Reithrodontomys megalotis	1
			Sylvilagus bachmani	1
			Mustela frenata	1
B	7 Young	112	*R. norvegicus*	12
			P. maniculatus	3
C	11 Young	176	*R. norvegicus*	10
			P. maniculatus	1
			M. *musculus*	3
			M. frenata	1
D	14 Young	624	*R. norvegicus*	28
	25 Sherman		*M. californicus*	4
			P. maniculatus	1
			M. musculus	4
E	7 Young	112	None	—

Additional animals consisted of 4 feral dogs (*Canis familiaris*) captured or shot.

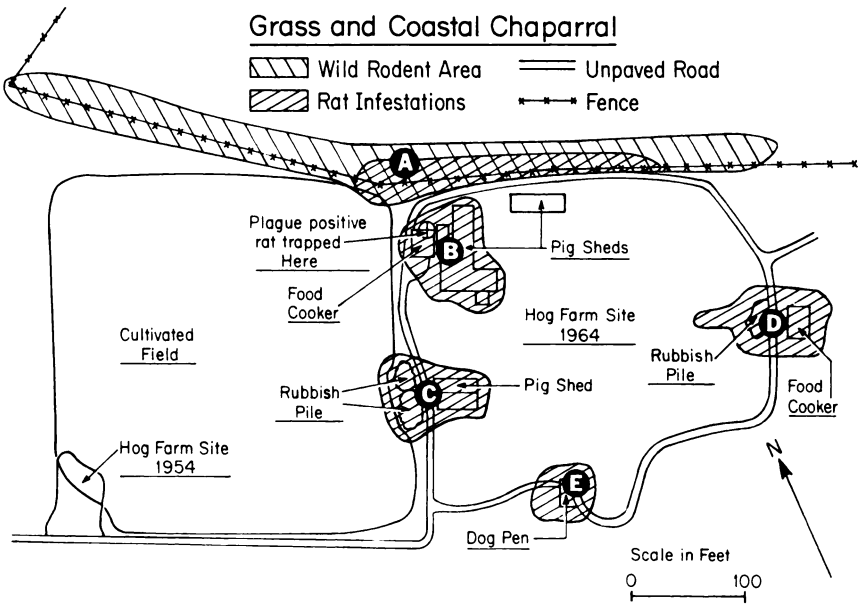

FIGURE 1. A sketch of the study site and areas trapped from April through June 1964. The present hog farm is situated on the border of the hog farm site previously studied in 1954.[5]

pools; 12 of the 69, including the pool of 13 *Nosopsyllus* mentioned above, yielded positive results. All positive pools were obtained from sites A, B, and C, which were also those sites most closely associated with wild rodents. Eight of the positive pools were made up of *Nosopsyllus* removed from Norway rats. The remaining four positive pools consisted of the wild rodent fleas, *Malaraeus telchinum* and *Catallagia wymani*, which were removed from voles and rats in areas A, B, and C.

The results of passive hemagglutination tests of rodent sera (Table 3) were consistent with bacteriologic findings. All positive rat sera and all but one of the positive wild rodent sera were obtained from sites A, B, and C. Of a total of 57 rat sera, seven gave positive results. Eight of the 22 vole sera and eight of the 25 deer mouse sera also gave positive results. Serum samples were collected from three dogs and two weasels (*Mustela frenata*) captured in the study area. Two of the dogs and one weasel yielded positive passive hemagglutination titers.

After May 19, rat and ectoparasite control procedures were instituted by local and state public health workers. These were completed by June 1964. Intensive trapping efforts in the same areas from June 2 through June 5 yielded a total catch of five from sites A, C, and D; six *Microtus* and five *Peromyscus* from site A; and 12 house mice from sites A, B, C, and D. These animals were not infested by fleas and were negative bacteriologically. Positive sera were obtained, however, from one rat and one vole from site A. Serum titers were 1:512 and 1:128, respectively.

DISCUSSION

Examination of ectoparasites indicated a flea exchange between *R. norvegicus* and the wild rodents involved (*M.*

californicus and *P. maniculatus*). A total of 400 fleas were collected from *R. norvegicus* caught in the four trap lines listed in Table 2; of these, 21 were *M. telchinum* and *C. wymani*, species normally associated with voles and deer mice in this area. Two of the 9 pools composed of these 21 fleas yielded *Y. pestis* after animal inoculation. The remaining 379 rat fleas (*N. fasciatus* and *Leptopsylla segnis*) yielded eight positive *N. fasciatus* pools. Similar results were obtained with the fleas from *Microtus* and *Peromyscus*. Of the 28 flea pools tested, one positive *M. telchinum* pool and one positive *N. fasciatus* pool were

TABLE 2. Results of Bacteriologic Examination Study of 69 Flea Pools Obtained April 22-May 19, 1964.

Trap Line	Host Species	Flea Species[a]	Total No. Fleas	No. Pools Inoculated	No. Pools Positive
A	*R. norvegicus*	*M.t.*	13	3	1
		N.f.	55	4	2
	M. californicus	*M.t.*	61	6	1
		H.l.	2	2	
		C.w.	2	2	
	P. maniculatus	*M.t.*	1	1	
		H.l.	1	1	
		O.k.	6	2	
		N.f.	2	1	
		Unidentified	45	3	
B	*R. norvegicus*	*C.w.*	1	1	
		N.f.	79	7	5[b]
	P. maniculatus	*M.t.*	1	1	
		O.k.	1	1	
		N.f.	4	1	1
		Unidentified	4	1	
C	*R. norvegicus*	*M.t.*	1	1	
		C.w.	1	1	1
		L.s.	5	2	
		N.f.	43	3	1
	P. maniculatus	Unidentified	4	1	
D	*R. norvegicus*	*M.t.*	5	3	
		N.f.	197	11	
	M. californicus	*M.t.*	26	4	
		H.l.	2	1	
		C.w.	2	2	
		N.f.	7	2	
	P. maniculatus	*M.t.*	1	1	

[a] *M.t.* = *Malaraeus telchinum*; *N.f.* = *Nosopsyllus fasciatus*; *H.l.* = *Hystrichopsylla linsdalei*; *O.k.* = *Opisodasys keeni*; *C.w.* = *Catallagia wymani*; *L.s.* = *Leptopsylla segnis*.

[b] One pool of 13 *N. fasciatus* was removed from *Y. pestis* positive rat.

TABLE 3. Results of Passive Hemagglutination Tests for **Y. pestis** Antibody.

Trap Line	Species[a]	No. Tested	No. Positive	Range of Titers[b]	Geometric Mean Titers
A	*R. norvegicus*	7	3	1:128-1:512	1:400
	M. californicus	18	7	1:32 -1:512	1:170
	P. maniculatus	20	7	1:16 -1:256	1:80
	M. frenata	1	1	1:32	——
B	*R. norvegicus*	12	2	1:512-1:2048	1:1020
	P. maniculatus	3	2	1:512-1:1024	1:720
C	*R. norvegicus*	10	2	1:32 -1:640	1:140
	P. maniculatus	1	0	——	——
	M. frenata	1	0	——	——
D	*R. norvegicus*	28	0	——	——
	M. californicus	4	1	1:2048	——
	P. maniculatus	1	0	——	——

[a] Other species tested (all negative) were:
M — 8; *R. megalotis* — 1; *S. bachmani* — 1
Feral dogs (*C. familiaris*) trapped or shot.
3 sera tested, 2 positive titers of 1:128 and 1:256.

[b] Minimum titers tested — 1:8, Minimum titers considered positive — 1:16.

obtained from *M. californicus* and *P. maniculatus*, respectively. The latter finding may be of some importance since it indicates that infected rat fleas may possibly be transferred from rats to wild rodent hosts although it is possible that infection of the rat fleas may have been the result of feeding on wild rodent hosts. In all, 13 of the 109 identified fleas from voles and deer mice were northern rat fleas, *N. fasciatus*.

The epizootic of 1964 was closely followed during the 6-8 months before April-May and has been reported elsewhere.[2] Very briefly, the first serologic evidence of an increase in infection rate in the San Bruno Mountain area was detected in *M. californicus* and *P. maniculatus* during November and December 1963, at study sites located 3-5 km east of the hog farm area reported here. Progressively increasing infection, directly associated with flea indices, was detected at 10 other trap sites during the winter months of 1963-1964. There is little question in the opinion of the authors that the source of rat infection was the preexisting epizootic in *Microtus* and *Peromyscus*.

The presence of plague-infected wild rodent fleas on rats and of infected rat fleas on wild rodents was previously reported in this area,[6,8] and our report does not constitute new data on rat-borne plague at this site. The analysis of serologic results during this outbreak is of more direct interest. Site A, which yielded the best evidence for commingling of wild rodents, rats, and their respective fleas also yielded the highest prevalence of seropositive rats. In this site, 17 of the 45 rodent sera tested yielded positive results. The prevalence of positive rodent sera was only 1 in 33 at site D, the area with the least number of wild rodent captures. Although not essential, Fisher's exact test applied to the results from site A and combined results from sites B, C, and D yielded significant values ($P = .033$) for the association of seropositive rats with site A.

These data point out not only the value of serologic methods for investigations of plague in rats but also the coincidence of wild rodent plague and rat plague in suburban areas. The demonstration that diagnosis of plague by serologic methods is possible in North American rat populations and that such methods can be applied with relative ease and economy should be of interest to workers concerned with maintaining plague surveillance in urban rat populations. Although the present investigation was conducted during and immediately after a plague epizootic, under conditions conducive to the recovery of *Y. pestis* from both animal tissues and fleas, serologic methods also have potential value for use during inter-epizootic periods when bacteriologic recoveries are difficult to obtain. Indeed, during the latter periods, serologic methods may be the only ones which will yield positive results. Previous work has already demonstrated the prolonged existence of *Y. pestis* antibody in *Microtus* and *Peromyscus* after plague epizootics in San Bruno Mountain area.[3] A similar effect in rats should allow retrospective diagnosis of plague in rats for several months after patent infection occurs.

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