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Authors: BRUCE W. HUDSON, MARTIN I. GOLDENBERG, and THOMAS J. QUAN

Source: Journal of Wildlife Diseases, 8(3) : 278-286

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-8.3.278

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BRUCE W. HUDSON[1], MARTIN I. GOLDENBERG[2], and THOMAS J. QUAN[1]

Abstract: Wild rodents involved in a plague epizootic were trapped on a bimonthly schedule at 15 trap sites distributed throughout the San Bruno Mountain plague pocket located in northern San Mateo County, California. The percentage of positive sera obtained from *Microtus californicus* varied from zero in two sites in which *Y. pestis* had not been recovered from rodent flea or tissue pools to as high as 90% to 97% positives in *Microtus* trapped in four sites in which *Y. pestis* was recovered.

Analysis of the data available indicates that the rate of seropositive rodents, *Peromyscus maniculatus* and *Microtus californicus*, is correlated with gross numbers of fleas found per trapline.

INTRODUCTION

Detailed descriptions of the San Bruno Mountain wild rodent plague area, including excellent accounts of climate, vegetation, rodent and flea species, and a history of wild rodent plague findings previous to 1957 have been presented by Murray[1] and Kartman et al.[2] San Bruno Mountain is a dominating geological feature of northeastern San Mateo County, California, rising to 400 m at its highest point and extending for about 8 km between South San Francisco and Daly City (SE to NW) and for about 4 km from Colma to the San Francisco City and County line (SW to NE). The landscape varies from brushy slopes to grassy meadows. *Yersinia* (Syn: *Pasteurella) pestis* isolations from the San Bruno Mountain area were first made from collections of rodent fleas in 1942[1]. Since that time evidence of plague infection has been obtained every year in which thorough surveys have been conducted[3,4]. Until 1957, such information was based on isolation of *Y. pestis* from specimens of fleas or tissues collected during surveys of the small mammal populations. More recently investigations have demonstrated the value of serological methods for the study of plague infection in specific sites of the San Bruno Mountain area[3,4]. Materials used in these previous studies were from a limited 2.3-acre area of San Bruno Mountain in which *Microtus californicus* was the primary rodent host. In view of the suitability of serologic methods for investigations of rodent plague in this area, it was of some interest to apply serologic methods and the customary standard bacteriologic techniques in a study of the overall plague-infected area. Accor-

[1] Zoonoses Section, Ecological Investigations Program, Center for Disease Control, Health Services and Mental Health Administration, Public Health Service, U.S. Department of Health, Education, and Welfare, Post Office Box 551, Fort Collins, Colorado 80521, U.S.A.

[2] Formerly, Chief, Microbiology Unit, Zoonoses Section, EIP. Presently, Chief, Central Laboratory, Medical Center for Federal Prisoners, U.S. Department of Justice, Springfield, Missouri 65802, U.S.A.

dingly, we selected several areas of varied habitat immedaitely adjacent to the previous study sites[4] as well as a larger number of sites distributed throughout the area. Bacteriologic and serologic studies were performed, and the results were examined to define major biotic factors influencing serologic response in this area.

MATERIALS AND METHODS

Trapping sites and methods

Figure 1 shows a sketch of the various trap sites used in this study. Trap line 4G consisted of a grid of 98 traps set out at 12 m intervals in the identical site used for the previous study. This particular site was situated in an area consisting of lush, ungrazed annual grasses and forbes with scattered clumps of *Baccharis pilularis*. *M. californicus* was the predominant animal as demonstrated in this and previous studies using the same site. Lines 4N, 4E, and 4S each consisted of 50 trap stations established along the edge of mixed brush thickets consisting predominantly of *Ulex europaeus* with occasional clumps of *B. pilularis*. Line 4SW was established in an area with a moderate cover of annual grass interspersed with low (30-90 cm) growths of *B. pilularis*. The remaining 10 trap lines consisted of 100 traps set at 6-8 m intervals. Cover for the most part consisted of small patches of ungrazed annual grasses interspersed with scattered clumps of *B. pilularis* and other species characteristic of the California coastal chaparral zone. All trap stations were established in November and December and were trapped three times during the ensuing 6-month period. Sherman[3] live traps (8 cm x 8 cm x 25 cm) were set between 13:00 and 15:00

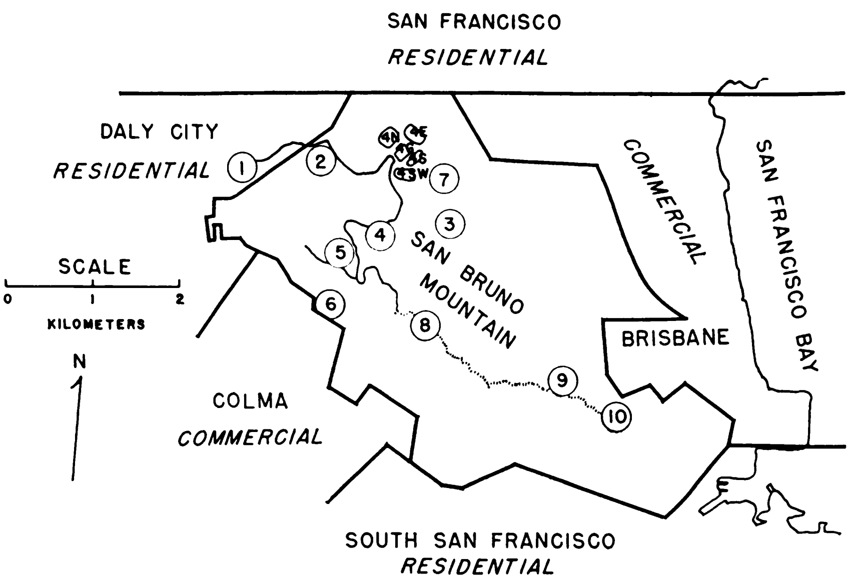

FIGURE 1. A sketch of the San Bruno Mountain plague area showing the location of the trap lines used in this stduy.

[3] Trade names are used as a means of identifying the product, and their use does not constitute endorsement by the Public Health Service or the U.S. Department of Health, Education, and Welfare.

hours, and animals caught were removed the following morning. Traps were then reset and remained until the next morning.

Blood sampling techniques and processing of animals and fleas

All animals captured were placed in 1 litre jars marked with trap site and station, and were taken to the laboratory for further processing. Blood sampling techniques, the passive hemagglutination test, and bacteriologic methods were identical to those used in previous studies[4]. In brief, the animals were lightly anesthetized with ether, brushed vigorously to remove fleas, and bled by cardiac puncture. Of the small mammals trapped in lines 1 through 10, 25% to 65% of the apparently healthy *M. californicus* and 24% to 71% of the apparently healthy *Peromyscus maniculatus* were sacrificed for bacteriologic examination. Continuing studies necessitated capture and release methods for animals trapped in lines 4G, 4N, 4E, 4S, and 4SW. Only dead or sick animals collected from these sites or those animals which succumbed during handling were examined by tissue pool techniques.

All animals found dead or sick in traps were necropsied and portions of spleen and liver excised for mouse inoculation as separate tissue pools. Spleen and liver tissues taken from healthy animals were inoculated as pools according to trap site and host species. Fleas were removed, pooled according to trap site and host, and inoculated into white mice or guinea pigs. Isolates obtained from these inoculations were then identified by standard techniques used in plague bacteriology.

Precise procedural information for the passive hemagglutination test as used in our laboratory has been detailed elsewhere[4]. Essentially, the procedure involves serial dilution of the test and control sera using the "Microtiter" equipment of Cooke Engineering Company, Alexandria, Virginia[3], and admixing a standard volume of tanned sheep erythrocytes sensitized with a purified soluble antigen (*Y. pestis* fraction 1). Minimum titers tested were 1:8, and serums yielding titers of 1:32 or higher were considered to be positive.

RESULTS

Table 1 lists the total number of *Microtus californicus* and *Peromyscus maniculatus* captured and the results of tissue and flea pool inoculations during the 6-month trapping period. Isolations of *Y. pestis* were obtained from two of 55 (3.6%) pools of *M. californicus* tissues and from two of 50 (4%) pools of *P. maniculatus* tissues. Two of the isolations were made from tissues of healthy, live-trapped rodents, and the other two were made from animals found dead in the trap lines as indicated in table 1. Bacterial isolations were made from 37 (13.1%) of 206 pools of *M. californicus* fleas and from 26 (14.5%) of 169 pools of *P. maniculatus* fleas. Completely negative bacteriologic results were obtained only in lines 1 and 10.

Positive serologic results were observed only in animals taken from trap lines where bacteriologic isolations of *Y. pestis* were obtained from fleas or rodent tissues. Tables 2 and 3 contain the serological data for all lines. Results are listed for *Microtus* and *Peromyscus* serum samples only. As in the previous study[3], all other rodent and insectivore species examined showed no evidence of serum antibody to *Y. pestis* by the passive hemagglutination test. Data listed in table 3 are from results of tests of sera taken at the time of first capture only. Of the total number of *Microtus* and *Peromyscus* captured and bled, 50.2% and 15.3%, respectively, showed evidence of serum antibody to *Y. pestis*. Trap lines 1 and 10, as mentioned above, yielded negative results. The remaining trap lines contained from 8% to 97% seropositive *Microtus* and from 5% to 33% seropositive *Peromyscus*. Trap line 4G yielded 50% positive sera in *Peromyscus* in a total sample of only eight animals.

As discussed in previous publications, these serum antibody rates reflect infec-

TABLE 1. Result of Animal Inoculations for Bacteriologic Recovery of **Yersinia pestis** from Tissues and Fleas of **Microtus californicus** and **Peromyscus maniculatus**, San Bruno Mountain, San Mateo County, California.

Trap Line	Animal		Tissue Pool Inoculation			Flea Pool Inoculations		
	Species	No.	No. Animals Examined	No. Pools	No. Pools ① Positive	Total No. Fleas	No. Pools	No. Pools Positive
1	M.c.	37	16	4	0	71	8	0
	P.m.	44	16	4	0	144	8	0
2	M.c.	38	16	4	0	346	17	5
	P.m.	21	11	3	0	178	10	2
3	M.c.	28	17	4	0	284	17	4
	P.m.	69	20	4	0	340	17	4
4	M.c.	46	20	6	0	398	21	0
	P.m.	7	5	2	1(LT)	83	6	2
5	M.c.	47	20	5	0	217	13	2
	P.m.	40	23	6	0	197	11	0
6	M.c.	46	16	4	0	151	11	2
	P.m.	64	16	3	0	202	13	0
7	M.c.	59	19	6	0	315	20	0
	P.m.	107	26	6	0	552	29	4
8	M.c.	20	13	5	1(DT)	52	7	1
	P.m.	56	17	4	0	154	9	0
9	M.c.	19	12	4	1(LT)	201	11	4
	P.m.	36	21	6	0	150	9	4
10	M.c.	21	8	3	0	122	8	0
	P.m.	25	14	3	0	244	10	0
4G	M.c.	134	10	5	0	831	35	15
	P.m.	9	3	3	0	41	2	1
4N	M.c.	27	1	1	0	131	9	0
	P.m.	68	2	2	0	337	17	1
4E	M.c.	7	0	—	—	43	4	1
	P.m.	82	4	3	0	350	17	7
4S	M.c.	16	3	2	0	78	6	0
	P.m.	37	1	1	1(FD)	128	8	0
4SW	M.c.	28	4	2	0	405	19	3
	P.m.	6	0	—	—	16	3	1
Totals	M.c.	573	175	55	2	3645	206	37
	P.m.	671	179	50	2	3116	169	26

① Notations: LT — live trapped, DT — dead in trap, FD — found dead.

TABLE 2. Summary of Results of **Yersinia pestis** Fraction 1-Specific Passive Hemagglutination Tests Performed on **Microtus californicus** and **Peromyscus maniculatus** Serum Samples Collected from San Bruno Mountain.

Trap Line	Species	Nov - Dec No. Pos./No. Tested	Nov - Dec Range of ① Pos. Titers	Jan - Feb No. Pos./No. Tested	Jan - Feb Range of ① Pos. Titers	Mar - Apr No. Pos./No. Tested	Mar - Apr Range of ① Pos. Titers	Nov - Apr No. Pos./No. Tested	Nov - Apr GMPT ②
1	M. californicus	0/20	—	0/6	—	0/10	—	0/36	—
	P maniculatus	0/25	—	0/9	—	0/9	—	0/43	—
2	M. c.	17/17	256-4096	10/10	128-2048	9/10	32-256	36/37	440
	P. m.	4/15	32-256	3/3	32-128	0/3	—	7/21	100
3	M. c.	11/11	64-2048	9/9	128-4096	4/5	128-256	24/25	440
	P. m.	4/36	32-2048	5/17	32-256	5/16	32-256	14/69	110
4	M. c.	8/25	64-512	15/15	64-4096	5/6	128-256	28/46	480
	P. m.	1/6	1024	—	—	0/1	—	1/7	1024
5	M. c.	3/17	32-128	6/22	128-1024	5/8	32-512	14/47	160
	P. m.	0/27	—	3/9	32-128	2/4	32-64	5/40	50
6	M. c.	0/7	—	1/13	32	7/24	64-1024	8/44	200
	P. m.	0/23	—	3/17	32-128	0/23	—	3/63	60
7	M. c.	21/24	32-2048	18/18	32-4096	12/15	32-512	51/57	350
	P. m.	2/41	64-128	11/39	32-2048	11/27	32-2048	24/107	160
8	M. c.	2/4	512	2/2	512-4096	7/13	64-128	11/19	450
	P. m.	0/20	—	3/18	64-256	4/16	32-256	7/54	90
9	M. c.	1/2	256	3/3	1024-2048	5/13	32-4096	9/18	700
	P. m.	3/15	64-2048	4/13	32-512	3/8	32-128	10/36	150
10	M. c.	0/1	—	—	—	0/20	—	0/21	—
	P. m.	0/11	—	0/10	—	0/4	—	0/25	—
TOTALS									
	M. c.	63/136	400②	64/98	670②	54/124	180②	181/350	380
	P. m.	14/219	180②	32/135	130②	25/111	90②	71/465	120

① Reciprocal of titer.
② GMPT — Geometric mean positive titer.

TABLE 3. Summary of Results of **Yersinia pestis** Fraction 1-Specific Passive Hemagglutination Tests Performed on **Microtus californicus** and **Peromyscus maniculatus** Serum Samples Collected from Area 4, San Bruno Mountain.

Trap Line	Species	Nov - Dec		Jan - Feb		Mar - Apr		Nov - Apr	
		No. Pos. No. Tested	Range of ① Pos. Titers	No. Pos. No. Tested	Range of ① Pos. Titers	No. Pos. No. Tested	Range of ① Pos. Titers	No. Pos. No. Tested	GMPT ②
4G	M. californicus	16/53	32-2048	22/29	64-4096	9/9	128-512	47/91	400
	P. maniculatus	—	—	3/7	64-512	1/1	256	4/8	180
4N	M. c.	0/7	—	2/15	64-512	0/3	—	2/25	180
	P. m.	0/28	—	2/15	1024-4096	2/6	32-512	4/49	510
4E	M. c.	0/2	—	4/5	128-2048	—	—	4/7	430
	P. m.	2/31	32-64	3/16	1024-4096	5/17	32-512	10/64	190
4S	M. c.	0/5	—	3/9	128-512	—	—	3/14	260
	P. m.	0/14	—	3/8	64-2048	2/7	32-128	5/29	170
4SW	M. c.	5/5	1024-4096	10/11	128-2048	2/2	64	17/18	710
	P. m.	0/4	—	—	—	1/1	64	1/5	64
TOTALS									
	M. c.	21/72	420②	41/69	560②	11/14	200②	73/155	440
	P. m.	2/77	50②	11/46	620②	7/14	90②	24/155	210

① Reciprocal of titer.

② GMPT — Geometric mean positive titer.

tion rates in a population of wild rodents that suffers low mortality during seasonal plague outbreaks. It was expected that the density of vector fleas should have a demonstrable effect on antibody levels. For analysis of these data, the basic unit used was the trapline. Each trapline was considered to be a single locus partially or fully separated physically from the others. Data for each trapline was further segregated into those relevant for each of the two major species populating the area (i.e. *Peromyscus* and *Microtus*) .The correlation method used was Student's test[12]. Geometric mean positive titers per trapline were found to have a positive, statistically significant ($P > .95$) correlation to the mean number of fleas per animal for both *Microtus* ($r = 0.795$) and for *Peromyscus* ($r = 0.762$). The correlation coefficients (r) for the two species were not statistically different. Percent positive serums found in bacteriologically positive traplines were significantly correlated to the mean number of fleas per animal per trapline for both *Microtus* and *Peromyscus*. The correlation coefficients from data from positive lines only (Mc, $r = 0.635$; and Pm, $r = 0.291$) were significantly different at the 95% level. [Mc positive serum more dependent on number of fleas/animal than Pm.] Only data from *Peromyscus* in all lines produced a positive significant correlation ($r = 0.715$; $P > 95\%$) between percent positive serums and percent positive flea pools. Correlation coefficients for *Peromyscus* and *Microtus* ($r = 0.437$) were not found to be statistically different at the 95% level.

If the assumption is made that the maximum geometric mean positive titer and the percent of positive sera in *Microtus* are a reflection of the period of maximum intensity of *Y. pestis* infection for each trapping area, and values for these periods only are used in correlating flea indices with serum antibody responses, the data can be fitted to a dose-effect curve significant at the 95% level. Table 4 presents these data by traplines in the order of increasing flea densities and figure 2 presents the regression line obtained for the data listed in table 4 according to the method of Litchfield

TABLE 4. Relationships Between Mean Flea Densities and Rates of Serum Positives in **Microtus californicus.**

Trap Line	Trapping Period in Which Highest Geometric Mean Titers were Obtained	Average Number of Fleas Per Animal	Percent Serum Positives
4N	Jan - Feb	2.9	13
6	Mar - Apr	3.3	29
5	Jan - Feb	3.9	27
4S	Jan - Feb	5.3	33
4G	Jan - Feb	5.8	76
4E	Jan - Feb	6.6	80
8 & 9	Jan - Feb	6.6	100 (95.6)*
7	Jan - Feb	6.7	100 (95.6)*
4	Jan - Feb	9.6	100 (99.5)*
4SW	Nov - Dec	11.2	100 (99.9)*
2	Nov - Dec	11.5	100
3	Jan - Feb	14.1	100

* Corrected values according to Litchfield and Wilcoxon (1949).

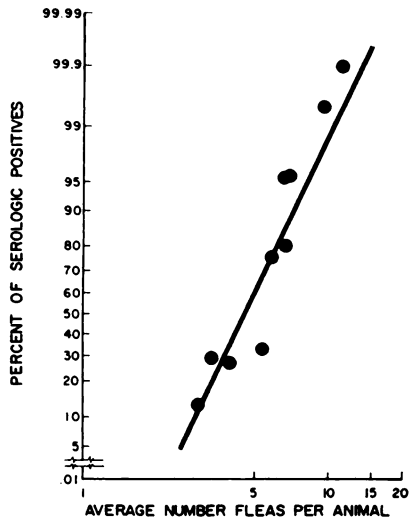

FIGURE 2. Relation between percent serologic positives (probits) and average numbers of fleas per **Microtus californicus** during an epizootic period in 10 trapsites.

and Wilcoxon[5]. Because of the small number of animals captured in lines 8 and 9 during the period in which highest geometric mean titers were demonstrated, the data of January and February for these lines have been combined. Bacteriologically negative samples from traplines 1 and 10 have been excluded. Data for *Peromyscus* from the same periods showed no evidence of linear relationship by this procedure.

DISCUSSION

Extensive studies of wild rodent plague in the San Francisco Bay area have emphasized the sharp limitation in space of wild rodent plague infection. Each known site of infection has been called a "nidus" or "pocket" of wild rodent plague, and it has been concluded that epizootics in this area are localized with little evidence of spread at a distance[6,7]. It has also been stated that plague - resistant populations of the California vole, *Microtus californicus*, together with its fleas, constitutes the *modus vivendi* of wild rodent plague in this area[6]. In our studies, roughly 50% of all *Microtus* captured show the presence of *Y. pestis*-specific antibody as contrasted to only 15% of all *Peromyscus maniculatus* although the bacteriologic data indicated no major differences in plague activity between the two rodent species. Isolations of *Y pestis* were made from 3.6% and 4.0% of the tissue pools of *Microtus* and *Peromyscus*, respectively, and also from 13.6% and 15.4% of the flea pools from the same two hosts, respectively. We conclude that although the serologic evidence indicates a possible higher infection rate on *Microtus*, bacterologic evidence does not allow us to imply a major or minor role to either *Microtus* or *Peromyscus* during the period covered in this report.

The widespread nature of plague infection during this period, as contrasted to the limited nature of plague infection demonstrated during the most complete previous investigation, may have been caused by two factors: the occurrence of *Microtus*, which were found in all traplines, and the high absolue density of fleas on both *Microtus* and *Peromyscus*. Previous studies at 25 other sites situated throughout the San Bruno Mountains yielded *Y. pestis* isolations from only two areas closely associated with a sharply delineated epizootic site[1]. The latter report mentioned the relative sparseness of *Microtus* populations and presented figures indicating total flea indices of 0.6 and 2.0 for *Microtus* and *Peromyscus*. Murray[1] also presented data separately for one site, number 25, where *Y. pestis* was isolated from one flea pool. *Microtus* flea indices of 1.1 to 3.1 for this area were above the average for the remaining 24 sites. In view of the correspondence between serologic evidence of infection and the numbers of fleas found per trapline demonstrated in the current study, a logical explanation for the limited distribution of wild rodent plague in the previous study as contrasted with the widespread occurrence found in the present study could lie in the relative difference in the total number of fleas found. The potential importance of these factors was emphasized in previous studies in this area[6]. This study constitutes a verification of these previous statements in that a quantiative relationship between flea densities and serologic evidence of infection is apparent.

LITERATURE CITED

1. MURRAY, K. F. 1957. An ecological appraisal of host ectoparasite relationships in a zone of epizootic plague in central California. Amer. J. Trop. Med. 6: 1068-1086.
2. KARTMAN, L., V. I. MILES, and F. M. PRINCE. 1958a. Ecological studies of wild rodent plague in the San Francisco Bay area of California. I. Introduction. Amer. J. Trop. Med. 7: 112-124.
3. HUDSON, B. W., S. F. QUAN, and M. I. GOLDENBERG. 1964. Serum antibody responses in a population of *Microtus californicus* and associated rodent species during and after *Pasteurella pestis* epizootics in the San Francisco Bay area. Zoonoses Res. 3: 15-29.
4. HUDSON, B. W., and L. KARTMAN. 1967. The use of the passive hemagglutination test in epidemiologic investigations of sylvatic plague in the United States. Bull. Wildl. Dis. Ass. 3: 50-59.
5. LITCHFIELD, J. T., and F. WILCOXON. 1949. A simplified method of evaluation dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99-113.
6. KARTMAN, L., S. F. QUAN, and H. E. STARK. 1962. Ecological studies of wild rodent plague in the San Francisco Bay area of California. V. The distribution of naturally infected fleas during an epizootic in relation to their infection rates. Amer. J. Trop. Med. 9: 153-157.
7. KARTMAN, L., S. F. QUAN, and H. E. STARK. 1962. Ecological studies of wild rodent plague in the San Francisco Bay area of California. VII. Effects of plague in nature on *Microtus californicus* and other wild rodents. Zoonoses Res. 1: 99-119.
8. STARK, H. E., and V. I. MILES. 1962. Ecological studies of wild rodent plague in the San Francisco Bay area of California. VI. The relative abundance of certain flea species and their host relationships on coexisting wild and domestic rodents. Amer. J. Trop. Med. 11: 524-534.
9. QUAN, S. F., L. KARTMAN, F. M. PRINCE, and V. I. MILES. 1960. Ecological studies of wild rodent plague in the San Francisco Bay area of California. IV. The fluctuation and intensity of natural infection with *Pasteurella pestis* in fleas during an epizootic. Amer. J. Trop. Med. 9: 91-95.
10. KARTMAN, L., and F. M. PRINCE. 1956. Studies on *Pasteurella pestis* in fleas. V. The experimental plague-vector efficiency of wild rodent fleas compared with *Xenopsylla cheopis*, together with observations on the influence of temperature. Amer. J. Trop. Med. 5: 1058-1070.
11. KARTMAN, L., F. M. PRINCE, and S. F. QUAN. 1958b. Studies on *Pasteurella pestis* in fleas. VII. The plague-vector efficiency of *Hystrichopsylla linsdalei* compared with *Xenopsylla cheopis* under experimental conditions. Amer. J. Trop. Med. 7: 317-322.
12. OSTLE, B. 1954. *Statistics in Research*. Iowa State University Press, Ames, Iowa.

Received for publication August 12, 1970