Susceptibility of New Mexico Rodents to Experimental Plague

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DURING the last three decades, extensive studies have been made to elucidate the plague vector capacity of many flea species infesting wild rodents (*1*). On the other hand, there has been a lack of adequate investigation concerning the susceptibility to *Pasteurella pestis* infection of the wild rodent hosts of these fleas. Until both factors, vector potential and host susceptibility, are determined, the ecology of sylvatic plague will be difficult to understand. A flea whose natural host is refractory to the development of *P. pestis* bacteremia is unlikely to be a plague vector.

In the early laboratory studies of the susceptibility of wild rodents to *P. pestis*, McCoy (*2*, *3*) and McCoy and Smith (*4*) established that several species of animals succumbed to experimental infection. Subsequently, other rodents of these species were found in the field infected from natural sources. Although these investigators produced infection in seven species of rodents, the relative susceptibility of these animals to plague infection could not be evaluated from their data because the number of bacteria either injected or introduced by skin scarification was not recorded. But a rough comparison of species susceptibility was presented. For example, all 19 inoculated California ground squirrels (*Citellus beecheyi*) died of plague while only 8 of 15 inoculated valley pocket gophers (*Thomomys bottae*) died, an indication that of these 2 species the ground squirrel was more susceptible.

Later, from an extensive series of investigations on California ground squirrels, Meyer (*5*) concluded that young squirrels were more susceptible than adults; adult males were more susceptible than adult females; squirrels from a known plague focus were more resistant to infection than were squirrels from a plague-free area.

For detailed comparisons of the susceptibility of various animal species to plague, Meyer's work indicated the need for considering the plague history of the area supplying the experimental animals, as well as their age and sex.

With this background, the ecology of wild rodents and their ectoparasites was studied at the Santa Fe Field Station of the Communicable Disease Center, Public Health Service.

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We present here the results of testing locally trapped adult rodents for susceptibility to experimental plague.

Procedure

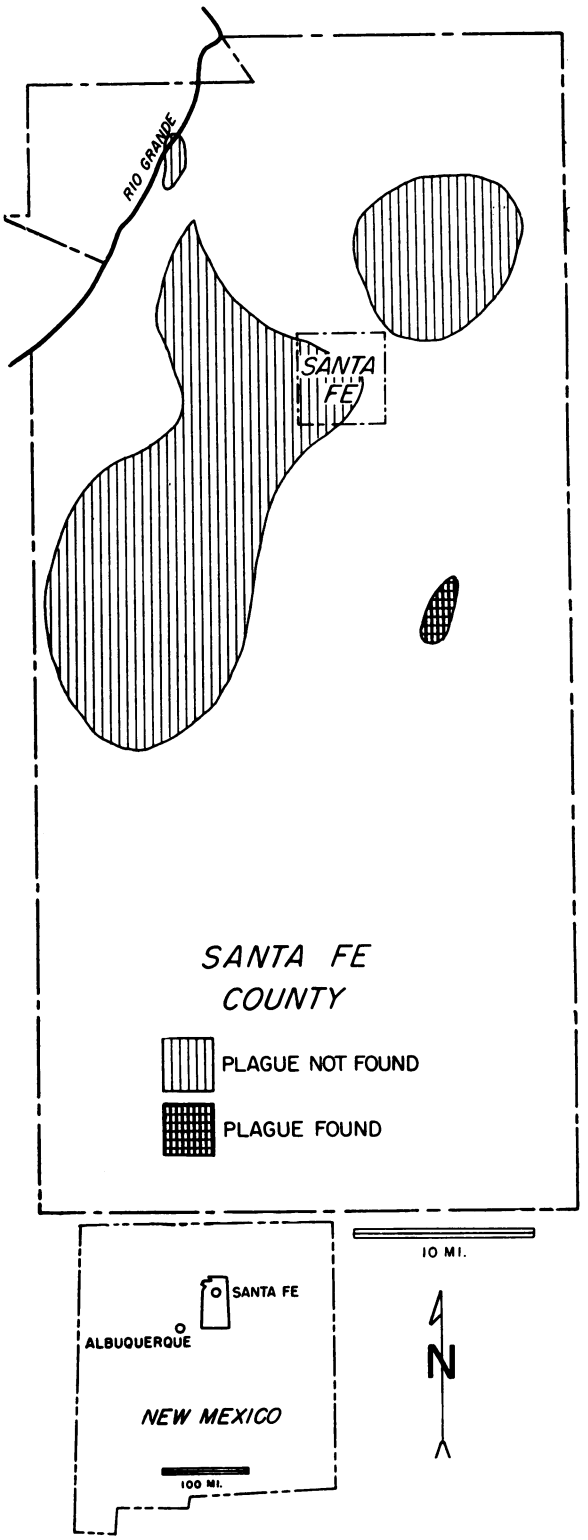
Test animals were trapped between September 21 and December 17, 1953. After a liberal dusting with pyrethrum powder to kill their fleas, the rodents were held in the Santa Fe laboratory for testing between October 21 and December 18, 1953.

The inoculum was obtained from 24-hour cultures of *P. pestis*, Alexander strain, New Mexico (*6*), grown at approximately 28° C. in brain-heart infusion broth and in dilutions of the broth varying from 10^{-1} to 10^{-8} in 1 percent peptone water. The number of viable organisms in an inoculum was calculated from the counts of the bacterial colonies, obtained by spreading on each of several blood-agar plates 0.2 ml. portions of 10^{-6} and 10^{-7} dilutions of the culture and allowing the bacteria on these agar plates to grow for 2 days in a 28° C. incubator. White laboratory mice, Princeton strain, inoculated with dilutions of the cultures, served as susceptible controls.

Each rodent in the experiments received initially 0.05 ml., inoculated intracutaneously into a shaven area over the right thigh. Intracutaneous inoculations were used on the assumption that they simulate infection by flea bites more closely than either subcutaneous or intraperitoneal inoculations. Some survivors of the initial injection were reinoculated intracutaneously 17 days after the first inoculation. A few survivors of the second injection were inoculated a third time. This was done intraperitoneally with 0.5 ml. of undiluted culture 41 days later.

On death, animals were autopsied. Evidence that plague was the cause of death was obtained from microscopic examination of stained smears of lymph nodes, spleen, liver, or heart blood exhibiting the typical bipolar bacillus; from appropriate macroscopic pathology; and from plague organisms identified on blood-agar culture of tissue smears. Tissues from test animals failing to show plague by one or more of these three processes were subinoculated by scar-

Areas in Santa Fe County, N. Mex., from which rodents were collected for susceptibility tests to experimental plague infection.



ification into mice. On death, the subinoculated mice were subjected to the same procedure followed for the test animals.

To investigate the possible development of latent infections, some of the animals that had survived at least 21 days after inoculation were sacrificed. Their spleen, liver, and, if present, enlarged lymph nodes, were pulped and inoculated into guinea pigs or mice.

Eleven rodents that survived previous inoculations were inoculated intraperitoneally with 0.5 ml. of plague toxins prepared according to the method used by Quan and associates (*7*) and Goodner and associates (*8*).

Results and Discussion

A total of 398 wild rodents of 21 species and 105 white control mice were inoculated with varying numbers of plague organisms. The wild rodent species ranged in susceptibility to infection from being as uniformly sensitive as the control mice to complete refractoriness. The LD_{50} for the laboratory mouse was about 7 bacilli.

The species found as homogeneously susceptible as the control strain of laboratory mouse were:

Reithrodontomys megalotis aztecus Allen, western harvest mouse
Perognathus flavus flavus Baird, silky pocket mouse
Peromyscus leucopus tornillo Mearns, white-footed mouse
Peromyscus truei truei (Shufeldt), piñon mouse
Neotoma albigula albigula Hartley, whitethroat woodrat
Neotoma mexicana fallax Merriam, Mexican woodrat
Eutamias minimus operarius Merriam, least chipmunk (probably as susceptible)

Although some individuals of the following species were resistant, the majority died of plague after infection with 1 to 1,000 mouse LD_{50}:

Peromyscus maniculatus rufinus (Merriam), deer mouse
Peromyscus boylii rowleyi (Allen), brush mouse
Peromyscus nasutus nasutus (Allen), rock mouse
Neotoma micropus canescens Allen, southern plains woodrat
Eutamias quadrivittatus quadrivittatus (Say), Colorado chipmunk

Those moderately resistant to infection (more than 1,000 mouse LD_{50}) were:

Citellus variegatus grammurus (Say), rock squirrel
Citellus spilosoma marginatus Bailey, spotted ground squirrel
Microtus longicaudus mordax (Merriam), longtail vole
Onychomys leucogaster pallescens Merriam, northern grasshopper mouse
Thomomys bottae nervagus Merriam, valley pocket gopher
Thomomys talpoides fossor Allen, northern pocket gopher

On the other hand, the following were refractory to intracutaneous inoculations of about a million mouse LD_{50}:

Dipodomys spectabilis baileyi Goldman, bannertail kangaroo rat
Dipodomys ordii medius Setzer, Ord kangaroo rat

The number of rodents in each species dying of plague after the experimental infection and the total number of rodents inoculated at each dilution are tabulated as fractions in tables 1 and 2.

Reithrodontomys megalotis aztecus and *Perognathus f. flavus* were more susceptible to experimental plague infection than were the white mice controls. The five species of *Peromyscus* were quite susceptible and succumbed to about the same number of organisms required to kill the controls, but certain individuals of both *P. maniculatus rufinus* and *P. n. nasutus* showed a fairly high degree of resistance. None of the three species of *Neotoma* survived inoculation of 10,000 organisms, but a few survived doses of less than 10 organisms. Reinoculated *Neotoma* survivors succumbed to 1,000 organisms. It was found that the diluted bacterial suspension initially inoculated into these animals was noninfectious. *Microtus*, *Citellus*, and *Eutamias* appeared to be more resistant than the *Neotoma*.

Thomomys and *Onychomys* showed a high degree of resistance, especially upon reinoculation. This may have been the result of an immunizing effect of the first inoculation. Two *Thomomys* that survived two previous intracutaneous inoculations succumbed to 100 million organisms inoculated intraperitoneally. Both species of *Dipodomys* were amazingly resistant

Table 1. Number of rodents dying of plague per number inoculated with varying numbers of Pasteurella pestis organisms

Rodent species	Original inoculation									First reinoculation [1]			
	10^7	10^6	10^5	10^4	10^3	10^2	10	1–5	<1	10^7	10^5	10^4	10^3
White mice controls [2]		6/6	6/6	16/17	17/17	15/16	14/16	4/16	0/11				
Reithrodontomys megalotis aztecus				4/4				4/4					
Perognathus flavus flavus				1/1				3/3					
Peromyscus boylii rowleyi				5/6				5/5				1/1	
Peromyscus leucopus tornillo				5/5	9/9	10/10	9/11	6/14				0/1	
Peromyscus maniculatus rufinus		5/6		9/10	6/7	12/15	8/8	6/17			0/5		
Peromyscus nasutus nasutus				6/7				3/5				2/2	
Peromyscus truei truei			4/4	19/19	16/16	16/16	13/16	1/3				2/2	
Neotoma albigula albigula				7/7	4/4		5/8	6/11	0/2				
Neotoma mexicana fallax				4/4			1/1	3/6	1/2				2/2
Neotoma micropus canescens				6/6			2/3	4/8	0/3				2/2
Eutamias minimus operarius				6/6	1/1			4/6					2/2
Eutamias quadrivittatus quadrivittatus				3/4				3/5			3/3		
Citellus lateralis lateralis			2/2										
Citellus spilosoma marginatus				3/4				3/4			1/2		
Citellus variegatus grammurus				3/5				0/4			4/6		
Microtus longicaudus mordax				4/5	0/6			1/4			2/4		
Onychomys leucogaster pallescens	1/1			1/4				0/5		2/4	1/4		
Thomomys talpoides fossor	1/1		0/1	0/3				0/4		2/7			
Thomomys bottae nervagus			0/2										
Dipodomys ordii medius	0/2	0/2	0/4	0/9				0/5		0/10			
Dipodomys spectabilis baileyi	0/2		0/3	0/4				0/4		0/7			

[1] First reinoculation given 17 days after the original.
[2] Albino mice controls for all experiments.

to infection. None died upon intracutaneous inoculations of as many as 10 million organisms, but some succumbed to 100 million organisms inoculated intraperitoneally.

The number tested in each rodent species was insufficient to indicate possible variation in susceptibility between sexes and different ages. The lack of an opportunity to experiment at another time than the October–December period leaves unanswered the possibility that there may be a seasonal change in host receptiveness to infection.

Following the completion of the plague susceptibility experiments some of the surviving rodents were injected with plague toxins. Each of these animals received a toxic dose equivalent to 600 times the LD_{50} for white laboratory mice. Each of two *Onychomys* receiving toxins died. The two *Thomomys*, one each of species *talpoides* and *bottae*, also succumbed. Three of the four *Dipodomys ordii medius* and one of three *Dipodomys spectabilis baileyi* were killed. All deaths occurred within 24 hours after the injection of poisons. The controls for each species survived for more than 3 weeks.

For four species of the rodents, the susceptibility to *P. pestis* of rodents from a plague-free area was compared with that of animals trapped in an area in which plague-positive rodents and fleas had been found (*9*). The map shows the proximity of the two areas. No difference was observed in the degree of susceptibility of the two sets of rodent species, *Neotoma a. albigula*, *Peromyscus t. truei*, *Peromyscus leucopus tornillo*, and *Peromyscus maniculatus rufinus* (table 2). These results contrast with the observations made by Meyer (*5*) on California ground squirrels. The reasons for these contrasting observations are not readily apparent. It is possible that the animals trapped in the plague-focus area were from populations without previous plague experience. Actual geographic limits of the plague focus could not be delineated, and no evidence could be found to substantiate an assumption that the infection spread throughout the trapping area. The difference between Meyer's observations and those in the present study may possibly be explained by the different species of hosts used.

The mortality indexes of four rodent species

(table 2) are shown in table 3. The mortality index (*10*) is the ratio of the percent mortality to the average survival time in days and is identical to the mouse protective index as originally used by Meyer and Foster (*11*) to evaluate human serum with mice. The mortality index for the species of rodents that were homogeneously susceptible to infection varied directly with the dose of the infecting inoculant, whereas the index for the heterogeneously responding species (*P. maniculatus rufinus*) did not. The calculated range of the 95 percent fiduciary probability is well within the observed results.

Three *P. maniculatus rufinus* yielded tissues infected with *P. pestis* 25 to 34 days after they received their last inoculation. These animals showed no obvious signs of illness prior to the time they were sacrificed. The significance of the recovery of virulent *P. pestis* in tissues of apparently healthy mice, 5 weeks after inoculation, is not known. Obviously, the ultimate fate of the infecting organisms, had the mice continued to live, could not be determined. Of all the species tested, only *P. maniculatus rufinus* was found to harbor the organisms while remaining alive in apparent good health.

Olitzki (*12*) recently reported the isolation of plague bacilli from the spleen of *Microtus guentheri* and from the abscess at the inoculation site 6 weeks after the subcutaneous injection of 1 million bacteria. At the George Williams Hooper Foundation, University of California, Quan (unpublished data) recovered virulent *P. pestis* from apparently healthy

Table 2. Comparison of susceptibility to experimental plague in rodents from plague and nonplague foci: number of rodents dying per number inoculated with varying numbers of Pasteurella pestis organisms

Rodent species [1]	Original inoculation								First reinoculation	
	10^6	10^5	10^4	10^3	10^2	10	1–5	<1	10^5	10^4
Neotoma albigula albigula:										
Plague focus			3/3	4/4		3/5	2/5			
Plague free			4/4			2/3	4/6	0/2		
Peromyscus truei truei:										
Plague focus			6/6	6/6	6/6	6/6				
Plague free		4/4	13/13	10/10	10/10	7/10	1/3			2/2
Peromyscus leucopus tornillo:										
Plague focus				4/4	5/5	5/5	1/5			
Plague free			5/5	5/5	5/5	4/6	5/9			0/1
Peromyscus maniculatus rufinus:										
Plague focus	5/6		6/6		5/6		0/5			
Plague free			3/4	6/7	7/9	8/8	6/12		0/5	

[1] Species totals for plague-free and plague-focus areas are shown in table 1.

Table 3. Mortality indexes according to number of Pasteurella pestis inoculated and LD_{50} doses of four rodent species and control mice

Rodent species	Mortality index [1]					LD_{50} [2]	
	10^4	10^3	10^2	10	<10	Dose	95 percent fiducial limits
Neotoma albigula albigula		33. 3	26. 4	12. 9	10. 0	2. 6	1. 1–5. 8
Peromyscus truei truei	36. 4	33. 3	32. 8	29. 1	16. 7	2. 8	1. 3–6. 2
Peromyscus leucopus tornillo		28. 6	31. 3	25. 7	9. 1	3. 8	2. 0–7. 2
Peromyscus maniculatus rufinus	17. 0	25. 7	19. 8	6. 2	0	38. 0	1. 4–2040
White control mice	32. 2	27. 0	20. 6	17. 6	7. 6	7. 0	4. 0–10. 0

[1] Percent mortality per average life of rodents that died (in days); same as mouse protective index used by Meyer and Foster (*11*).

[2] Method of Litchfield and Wilcoxon (*10*).

guinea pigs killed more than 30 days after inoculation. Although recovery of virulent microorganisms from apparently healthy animals, 4 to 6 weeks after inoculation, may suggest how the plague bacillus could be maintained in wild rodents during interepizootic periods, this finding cannot be regarded as latent plague (*13*, *14*) without proof that disease will finally occur (*15*).

Summary

A total of 398 wild rodents of 21 species were inoculated intracutaneously with 0.05 ml. of aqueous suspensions containing various numbers of *Pasteurella pestis* (Alexander strain, New Mexico) to test their susceptibility to plague infection in comparison with white laboratory mice inoculated identically.

The wild rodent species ranged in susceptibility from homogeneous sensitivity equal to that of the control mice to complete resistance.

The majority of the rodents that survived plague inoculation, regardless of species, died of toxemia after receiving about 650 LD_{50} of plague toxins intraperitoneally.

The comparison of four species, *Neotoma albigula albigula*, *Peromyscus truei truei*, *Peromyscus leucopus tornillo*, and *Peromyscus maniculatus rufinus*, trapped in an area where plague was found with those collected from a plague-free area, demonstrated no differences in susceptibility to experimental *P. pestis* infection.

Since the available number of animals of any one species tested was small, such factors as sex and age could not be evaluated.

The possible effect of seasons on the susceptibility of the rodents was not investigated. The persistence of plague in the area where it occurred was not determined.

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