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SUSCEPTIBILITY TO *YERSINIA PESTIS* IN THE NORTHERN GRASSHOPPER MOUSE (*ONYCHOMYS LEUCOGASTER*)

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ABSTRACT: The laboratory-born progeny from two geographically distant populations of northern grasshopper mice (*Onychomys leucogaster*) were challenged with *Yersinia pestis* to determine their relative susceptibilities to plague. One of the *O. leucogaster* populations was associated with a known epizootic focus of the disease and was found to be nearly 2,000 times more resistant to mortality than were members of another population from an area historically free of plague. The ecology and omnivorous behavior of *O. leucogaster* appears to promote strong selection for resistance to plague in areas where they are naturally exposed.

Key words: *Yersinia pestis*, plague, *Onychomys leucogaster*, grasshopper mouse, population resistance, susceptibility, experimental study.

INTRODUCTION

The northern grasshopper mouse (*Onychomys leucogaster*) has several ecological and behavioral characteristics which implicate it in the epizootiology of wild rodent plague (*Yersinia pestis*). Stark (1970) pointed out that, although grasshopper mice are infrequently trapped, they and/or their fleas appear with surprising frequency in records of plague occurrence.

The range of *O. leucogaster* (Fig. 1) includes most of the areas of enzootic plague in the United States described by Barnes (1982). Kartman (1970) includes the genus *Onychomys* in a list of 10 North American rodent genera recognized to be of importance in the ecology of "sylvatic" plague and plague-positive sera and/or flea pools have been collected from *O. leucogaster* in Arizona, Colorado, Kansas, Montana, New Mexico, North Dakota, Oklahoma, Oregon, Texas, and Utah (Wayson, 1947; Ecke & Johnson, 1952; Kartman et al., 1958; Marchette et al., 1962; Kartman, 1970; Stark, 1970).

Onychomys spp. are unique among North America cricetine rodents in that they are true omnivores (Bailey & Sperry, 1929; Landry, 1970). Although much of their animal prey are probably arthropods (Flake, 1973), they do kill and consume other small rodents. Bailey and Sperry (1929) questioned whether these mice dig their own burrows, or use abandoned burrows or those of their mammalian victims. A review of the published records of fleas collected from grasshopper mice (Thomas, 1988) indicates that these mice do investigate or utilize the burrows of many other rodent species. Fifty-six flea species other than the grasshopper mouse flea (*Monopsyllus exilis*) have been collected from *O. leucogaster*. More importantly, nearly one-half (25/56) of the flea species collected from *O. leucogaster* are actual or potential vectors of plague. Most are found normally on ecologically and geographically associated rodent species; *O. leucogaster* appears to be a "host of opportunity." Because of their omnivorous nature, their habit of secondary burrow use, and their propensity for acquiring and disseminating the fleas of other hosts, grasshopper mice are important in shaping the epizootic character of plague in grassland habitats.

In areas where rodents are associated with the plague organism, selection pressures may result in the development of resistance to the disease as a population characteristic such as that documented by

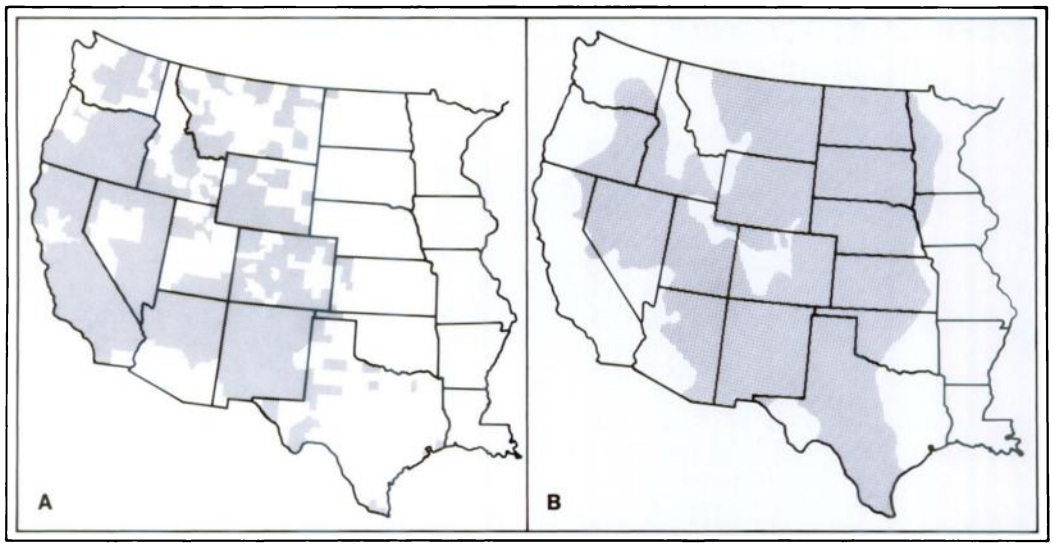

FIGURE 1. The historical occurrence of human and animal plague in the United States (by county) from 1900 to 1986 (A), and the distribution of the northern grasshopper mouse, *Onychomys leucogaster*, in the United States (B).

Shepherd et al. (1986) in South African rodent populations. As a group, omnivores have varying degrees of resistance to plague and variation in susceptibility has been demonstrated for members of a single species from different populations (Quan and Kartman, 1962; Isaacson et al., 1983). When Marchette et al. (1962) compared their data with those of Holdenreid and Quan (1956), they noted a "striking" difference in resistance between subspecies of *O. leucogaster* from New Mexico and Utah. A population from New Mexico was resistant (21 day LD_{50} > 7,000 *Y. pestis* bacilli) while a population from Utah was very susceptible (21 day LD_{50} = 10 *Y. pestis* bacilli). Although these values may represent actual differences between the populations, they may also reflect dissimilarities in experimental design between the two studies. Marchette et al. (1962) used laboratory-reared mice four to eight generations removed from wild parental stock in contrast to the wild caught animals used by Holdenreid and Quan (1956). Holdenreid and Quan (1956) stress that detailed comparisons of the susceptibility of various animal species requires an understanding of the history of plague in the area supplying the experimental animals, as well as the animals' age and sex. Also, this applies to studies on the intraspecific variation in susceptibility to *Y. pestis*.

The purpose of the present study was to compare the relative susceptibilities of two distinct northern grasshopper mouse populations: one population with no history of natural exposure to plague and another from an area of historically active zootic plague. The use of laboratory-bred first generation (F_1) progeny from each of the two geographically distant populations of *O. leucogaster* ensured the use of previously unexposed animals and simultaneously assured adequate representation of each population's reaction to challenge.

MATERIALS AND METHODS

Between June 1983 and December 1984 a total of 34 *O. leucogaster breviauritus* were collected from Caddo County in west-central Oklahoma (35°22'N, 98°15'W), an area without a history of plague or plague-like epizootics. Published records indicate that the nearest collection of plague-positive wild mammals or fleas was made in the Texas Panhandle about 250 km to the west (Barnes, 1982). Between October

1984 and June 1985, we collected 45 *O. leucogaster articeps* from an area of Weld County, Colorado (40°41'N, 104°26'W), where Centers for Disease Control Plague Branch collection data from 1984 indicated plague activity (four positive flea pools; two collected from *O. leucogaster* and one each from *Spermophilus tridecemlineatus* and *Dipodomys ordii*). Ecke and Johnson (1952) described a "plague-like epizootic" among black-tailed prairie dogs (*Cynomys ludovicianus*) which occurred in Weld and Logan counties, Colorado in 1948. Apparently this epizootic originated 40 km east of our collection area and was the first recorded in northern Colorado.

Six- to 12-wk-old laboratory-born F_1 progeny of population-specific pairs of grasshopper mice were tested for susceptibility to plague using the technique described by Quan et al. (1985). Laboratory breeding and all challenge techniques involving the use of *Y. pestis* were conducted at the Centers for Disease Control (Plague Branch Laboratory, Fort Collins, Colorado 80522, USA). *Yersinia pestis* strain NM77-538, a fully virulent strain which was isolated from a bubo aspirate obtained from a 1977 human plague case in New Mexico, was used throughout this study. A subculture from reference stock was made on blood agar and incubated at 28 C for 48 hr. Five to 10 typical colonies were transferred to 10 ml of sterile brain-heart infusion (BHI) media (Difco Laboratories, Detroit, Michigan 48232, USA). This culture was incubated at 28 C for an additional 24 hr. Subcutaneous inoculation of 0.1 ml of this suspension into 5-wk-old pathogen-free white mice (*Mus musculus*, CID—General Purpose Strain [Center for Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado 80522, USA]) established the virulence of the bacteria. This strain of white mice was used exclusively throughout the study because of its uniform susceptibility to *Y. pestis*. When these mice died, 3–4 days postinoculation, streaks of spleen and liver tissue were made on blood agar for recovery of *Y. pestis*. Five to 10 typical colonies from these isolates were transferred into 10 ml BHI and incubated at 28 C for 24 hr after which 0.5 ml of this suspension was transferred to 10 ml BHI and incubated an additional 24 hr at 28 C. The estimated bacterial concentration for this culture after 24 hr was 1×10^8 *Y. pestis* bacilli/ml. In order to establish the bacterial concentrations used for inoculation, seven 10-fold serial dilutions were made of this culture and 0.1 ml of the three highest dilutions were spread on each of two blood agar plates. The number of colonies observed after 48–72 hr was used to calculate actual doses inoculated.

Sixty-four F_1 *O. leucogaster breviauritus* from Oklahoma stock were tested in the first trial. For each of eight log dilutions (1×10^0 to 1×10^7 *Y. pestis*/ml^{-1}), eight grasshopper mice were injected subcutaneously in the lower abdomen with 0.1 ml of the inoculum (8×8 LD_{50} trial). Sixty-four 5-wk-old white mice were challenged in the same manner as the control.

In the second trial, 64 F_1 *O. leucogaster articeps* from Colorado stock were inoculated as in the first trial. White mice were used again as a control. Thirty-two *O. leucogaster breviauritus* were inoculated in groups of eight at the four highest dilutions (1×10^0 to 1×10^3 *Y. pestis*/ml^{-1}) to serve as an additional control for this trial and to support the findings of the first LD_{50} determined for these mice. Since an LD_{50} was established for this population in the first trial, it was not necessary to inoculate mice at dilutions lower than those which would produce a 50% mortality rate in 21 days. A third trial included 40 *O. leucogaster articeps*, and white mouse controls, inoculated at the five lowest dilutions (1×10^3 to 1×10^7 *Y. pestis*/ml^{-1}) to support the LD_{50} value determined for these mice in the second trial. Time and space limitations prohibited the simultaneous challenge of all the *O. leucogaster* from both populations and appropriate controls.

All mice were observed for morbidity and mortality twice a day for 21 days postinoculation. Animals that died were necropsied and their liver and spleen tissues were excised for bacteriologic culturing to verify the cause of death as plague. Disease-related gross lesions were then described. Surviving animals were bled retro-orbitally on days 10, 21, 30, 60, and 90 postinoculation. Sera were tested for antibodies to *Y. pestis* Fraction 1 (F1) antigen (Centers for Disease Control, Plague Branch Laboratory, Fort Collins, Colorado 80522, USA) by PHA/PHI standard methods (World Health Organization, 1970). Significant differences in geometric mean positive titer between the two populations were tested for by the Student's *t* paired difference test. The mean lethal dose (LD_{50}) and mean infectious dose (ID_{50}) were calculated for test populations and controls by the method of Reed and Muench (1938).

RESULTS

The two populations of *O. leucogaster* had very different mortality rates. Although 47 of 64 (73%) of *O. leucogaster breviauritus* from the plague naive population in Oklahoma died, only 16 of 64 (25%) of the *O. leucogaster articeps* died. Sex-related difference in mortality rate did not occur. The mortality rate in female *O.*

TABLE 1. Response of a plague naive grasshopper mouse population to experimental challenge with *Yersinia pestis*.

	Calculated LD_{50}[a] Calculated ID_{50}[b]	Number *Y. pestis*[c] inoculated	Mortality ratio	Number sero-positive/ number survivors	Infection ratio	Average days to death	Mortality[d] index
Onychomys leucogaster	70.41[a]	1.4	3/8	0/5	3/8	8.7	4.33
Oklahoma (n = 64)	10.00[b]	14	3/8	5/5	8/8	6.7	5.62
		140	6/8	2/2	8/8	4.7	10.46
		1,400	3/8	5/5	8/8	5.7	6.61
		14,000	8/8	—	8/8	4.4	22.83
		140,000	8/8	—	8/8	3.4	29.59
		1,400,000	8/8	—	8/8	5.4	18.59
		14,000,000	8/8	—	8/8	2.8	36.37
			47/64	12/17	59/64	6.8	
Mus musculus control LD_{50} = 5.04							

[a] Mean lethal dose—number of *Y. pestis* bacilli.
[b] Mean infectious dose—number of *Y. pestis* bacilli.
[c] Number of *Y. pestis*/ml^{-1} inoculated subcutaneously.
[d] Percentage mortality × average days to death (Holdenreid and Quan, 1956).

leucogaster breviauritus was 76% (25/33) and 71% (22/31) for males; the mortality rate for female *O. leucogaster articeps* was 26% (10/39) and 24% (6/25) for males.

The gross lesions most frequently observed in the grasshopper mice that died were axillary and abdominal skin infusions. If an axillary skin infusion was present, in most cases it was accompanied by coagulated free blood in the axilla. Development of a subdermal nonsuppurative nodular mass at the site of infection also was observed frequently. In all cases, *Y. pestis* was isolated from the spleen and/or liver of mice dying within 21 days of inoculation.

None of the 96 *O. leucogaster breviauritus* challenged in the two trials developed buboes, and only three of the 104 *O. leucogaster articeps* (3%) inoculated in two trials developed buboes. One was an axillary bubo which developed 21 days after inoculation. This mouse died of plague on day 29 postchallenge. The other two were inguinal buboes discovered 30 days postinoculation in mice which subsequently survived 90 days postchallenge. Inoculation of white mice with aspirates from these buboes and subsequent culture of the pathogen from the tissues of the dead mice revealed that these buboes contained viable *Y. pestis*. These findings indicate that at least a small percentage of *O. leucogaster* from resistant populations can maintain infections of *Y. pestis* bacilli for 30 days or more after introduction.

The F_1 progeny of *O. leucogaster* trapped from a population in northern Colorado having a known natural association with plague had an LD_{50} nearly 2,000 times higher than that of a plague naive population of grasshopper mice from Oklahoma. The calculated inocula used in the two 8 × 8 susceptibility trials were very close (1.4 and 1.8 *Y. pestis* bacilli × log dilution/ml^{-1}, respectively) and all of the inocula had classic virulence characteristics in white mouse controls (21 day LD_{50} < 10 *Y. pestis* bacilli). The *O. leucogaster breviauritus* population had 21 day LD_{50} values of 70.4 bacilli in the 8 × 8 trial (Table 1) and 444.4 bacilli in the second (4 × 8) trial. These values indicated a moderate susceptibility. The LD_{50} value for the *O. leucogaster articeps* population was 8.75×10^5 bacilli in the 8 × 8 trial (Table 2). In the second *O. leucogaster articeps* LD_{50} trial, a staphylococcal contaminant

TABLE 2. Response of a grasshopper mouse population from an area of enzootic plague to experimental challenge with *Yersinia pestis*.

	Calculated LD_{50}[a] Calculated ID_{50}[b]	Number Y. *pestis*[c] inoculated	Mortality ratio	Number sero-positive / number survivors	Infection ratio	Average days to death	Mortality[d] index
Onychomys leucogaster	875,772.00[a]	1.8	0/8	6/8	6/8	—	0.00
Colorado (n = 64)	1.80[b]	18	0/8	7/8	7/8	—	0.00
		180	1/8	7/7	8/8	9.0	1.39
		1,800	1/8	7/7	8/8	9.0	1.39
		18,000	1/8	7/7	8/8	7.0	1.79
		180,000	3/8	5/5	8/8	4.3	8.66
		1,800,000	4/8	4/4	8/8	5.0	10.00
		18,000,000	6/8	2/2	8/8	4.3	17.32
			16/64	45/48	61/64	5.3	
Mus musculus control LD_{50} = 7.08							

[a] Mean lethal dose—number of Y. *pestis* bacilli.
[b] Mean infectious dose—number of Y. *pestis* bacilli.
[c] Number of Y. *pestis* ml^{-1} inoculated subcutaneously.
[d] Percentage mortality · average days to death (Holdenreid and Quan, 1956).

on the blood agar plates prevented quantification of a dilution factor for the inocula. However, the dilution at which 50% mortality occurred was exactly 1×10^6 which indicates a theoretical LD_{50} value of $\geq 1 \times 10^6$ bacilli. This supports the results of the first trial and indicates very high resistance in this population.

None of the *O. leucogaster breviauritus* survived inoculation of greater than 1,400 plague bacilli indicating an LD_{100} between this number and 14,000 bacilli (Table 1). There were no deaths in mice from the Colorado population at the highest two dilutions (1.8–18 Y. *pestis* bacilli) and only three of the 40 mice challenged at the highest five dilutions (1.8–18,000 Y. *pestis* bacilli) died. Two of eight *O. leucogaster articeps* challenged with an inoculum containing 1.8×10^7 Y. *pestis*/ml^{-1} in the second trial survived (Table 2).

The *O. leucogaster breviauritus* which survived had a significantly higher geometric mean positive titer (t = 2.576, P = 0.005) against Y. *pestis* F1 antigen at 10, 21, 30, 60, and 90 days postinoculation than did the more resistant *O. leucogaster articeps* inoculated at the same dilutions (Fig. 2). It is apparent that a high serum titer to this virulence determinant is not a primary factor in grasshopper mouse resistance to plague mortality since the more susceptible population developed the higher mean anti-F1 titers. The mice challenged in the smaller replicate trials were inoculated with different dilution ranges and the trials were of different sizes. Therefore, the only realistic comparison of titer values to be made is of those from the two 8×8 LD_{50} trials. However, even in this comparison there is a disparity since no *O. leucogaster breviauritus* survived inoculation with more than 1,400 Y. *pestis* bacilli. For this reason, a titer curve for *O. leucogaster articeps* survivors challenged with 1.8–1,800 Y. *pestis* is added to Figure 2 for a more direct comparison.

DISCUSSION

The data indicate that, although mortality is positively correlated to inocula, considerable variability in individual susceptibility to Y. *pestis* exists within and between populations. While the two populations tested represent distinct subspecies, and subspecific epithets are used in this manuscript, we do not believe that the results can be extrapolated to represent any

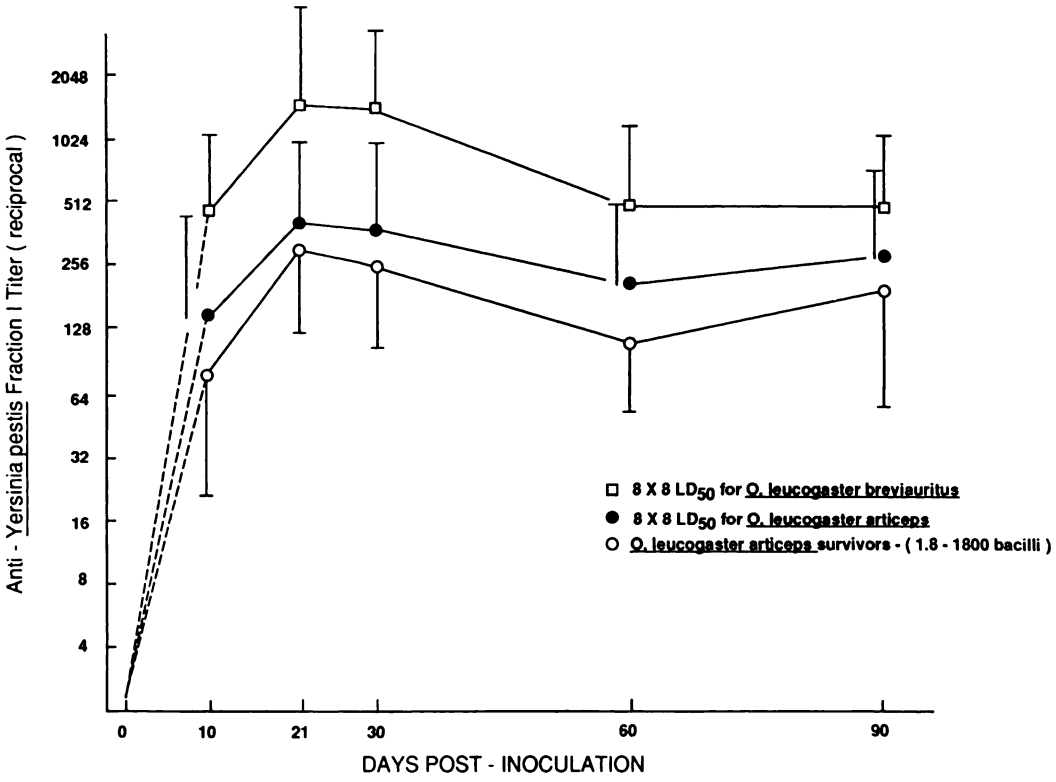

FIGURE 2. Ninety day anti-F_1 titer curves (mean ± standard error) for survivors from two populations of *Onychomys leucogaster* challenged with plague (*Yersinia pestis*).

other population of either subspecies. It is our conclusion that the observed differences in susceptibility are due to the natural association of the more resistant population with plague. This is supported by the observation of increased resistance in South African rodents by Shepherd et al. (1986). They demonstrated acquired resistance in rodent populations for which LD_{50} values had been established several decades earlier. The detailed history of plague in Colorado given by Ecke and Johnson (1952) points to the possibility that the present resistance to plague found in *O. leucogaster arriceps* from Weld County has developed during the past 40 yr. This illustrates how rapidly disease, acting as an agent of selection, can alter a host population's susceptibility to a pathogen. The selection pressures that resulted in the population's resistance probably are associated with the behavior of these mice. The omnivorous *O. leucogaster* can be exposed to plague by ingesting infected rodents. Grasshopper mice also frequent the burrows of other animals. Both of these behavioral traits expose grasshopper mice to a diverse flea fauna including many species known to be of importance in the ecology of plague. The distribution and behavior of northern grasshopper mice make them likely candidates as epizootic hosts for *Y. pestis*. The results of this study show that populations of grasshopper mice can acquire increased resistance to plague from natural exposure to the disease. Therefore, these rodents may be very important in maintaining the epizootic cycle of plague in at least one focus of the disease in North America.

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