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EXPERIMENTALLY INDUCED PLAGUE INFECTION IN THE NORTHERN GRASSHOPPER MOUSE (*ONYCHOMYS LEUCOGASTER*) ACQUIRED BY CONSUMPTION OF INFECTED PREY

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ABSTRACT: In this study, 20 laboratory reared *Onychomys leucogaster* from a parental population that is naturally exposed to plague were each fed a white mouse that had been inoculated with *Yersinia pestis*. Three of the 20 *O. leucogaster* died, four survived with antibody titers against *Y. pestis* and 13 survived with no titer against *Y. pestis*. In contrast, when 20 *O. leucogaster* from a plague naive parental population were fed infected prey, seven died and 13 survived with no antibody titer against *Y. pestis*. Our results suggest another means by which *O. leucogaster* from populations that are naturally exposed to plague may acquire the disease.

Key words: *Yersinia pestis*, plague, *Onychomys leucogaster*, grasshopper mouse, infected prey, experimental study, oral transmission.

INTRODUCTION

Plague is a relatively common disease of rodents in the western United States and throughout many arid and semiarid habitats of the world (Kucheruk, 1965). Fleas are the primary vectors of the plague organism (*Yersinia pestis*) among rodents. However, animals that feed on infected rodents may also become infected and develop antibody titers against plague. Mortality due to plague varies from very low among predators such as coyotes (*Canis latrans*), skunks (*Mephitis* spp.), and raccoons (*Procyon lotor*) to relatively high among felids (Barnes, 1982), and ingestion of diseased animals through predation or cannibalism has been suggested as a means for the short-term interepizootic maintenance of the plague organism by Rust et al. (1971).

The northern grasshopper mouse (*Onychomys leucogaster*) is an omnivorous Nearctic cricetine rodent that kills and consumes other rodents (Flake, 1973; Landry, 1970). Many of its prey species are themselves either reservoirs of plague or carry fleas that are known vectors of plague. The range of *O. leucogaster* overlaps most of the distribution of plague in the United States; sera and/or flea pools positive for plague have been collected from these mice in 10 western states. The frequency with which such samples collected from *O. leucogaster* are plague-positive is largely due to their association with a diverse flea fauna (Thomas, 1988). However, their frequency of infection in enzootic areas might be increased by consumption of other infected rodents. Furthermore, this transmission route may expose more plague bacilli to a predator than does inoculation via flea bite due to bacterial amplification in the infected vertebrate. It was the purpose of this study to determine whether *O. leucogaster* could be infected with *Y. pestis* by consuming infected prey and to evaluate whether grasshopper mice from populations naturally exposed to plague have increased resistance to mortality from infections acquired by consuming prey infected with *Y. pestis*.

METHODS

The first-generation (F_1) laboratory-reared progeny of a wild population of *O. leucogaster*

collected from Caddo County, Oklahoma (35°22'N, 98°15'W), an area with no known history of plague activity, and of a population collected from an epizootic focus of plague in Weld County, Colorado (40°41'N, 104°26'W) were used in this study. Forty 6- to 12-wk-old F_1 grasshopper mice (20 from each population) were each fed one live plague-infected 3-wk-old white mouse (*Mus musculus*, NIH—General Purpose Strain, Centers for Disease Control, Center for Infectious Disease, Fort Collins, Colorado 80522, USA). White mice were inoculated subcutaneously with 0.1 ml of a brain-heart infusion (Difco Laboratories, Detroit, Michigan 48232, USA) culture of *Y. pestis* (strain NM77-538) containing an estimated 1×10^9 bacilli/ml. The inoculum was prepared according to the methods of Quan et al. (1985). When one-half of the white mice had died (ca. 24 hr post-inoculation) one of the surviving mice was fed to each of the 40 grasshopper mice. The white mice that survived were not tested for active *Y. pestis* infections and we cannot confirm that all of the live mice fed to *O. leucogaster* were infectious. The *O. leucogaster* were observed twice a day for morbidity and mortality for 21 days postchallenge. We necropsied grasshopper mice that died within 21 days to collect liver, spleen, heart blood, salivary glands and pharynx (using a swab) for bacterial culture, and we noted disease related gross lesions. Surviving animals were bled retro-orbitally on days 10, 21, 30, 60 and 90 postchallenge. Sera were tested for antibodies to *Y. pestis* Fraction 1 (F1) antigen (Centers for Disease Control, Plague Branch Laboratories, Fort Collins, Colorado 80522, USA) by passive hemagglutination/hemagglutination inhibition standard methods (World Health Organization, 1970). Infection of *O. leucogaster* after consuming infected prey was indicated by seroconversion to *Y. pestis* F1 antigen or by mortality and subsequent recovery of *Y. pestis* from tissue samples.

RESULTS

All of the potentially infected white mice were killed and at least partially consumed by *O. leucogaster* within 30 min and totally consumed within 24 hr.

Seven of 20 (35%) *O. leucogaster* from the Oklahoma population died after consuming prey mice inoculated with *Y. pestis* (Table 1). One of the seven survived to day 15 postchallenge with a 1:124 anti-F1 titer on day 10. The remaining six survived only 3.33 ± 0.82 days. Slight axial and abdominal skin hyperemia was noted in several of the mice that died, and one of these mice hemorrhaged around the superficial gastric blood vessels. A large hematoma was noted on the left side of the upper lip of one mouse. *Yersinia pestis* was recovered from the salivary glands, heart blood and spleens of five of the seven mice that died and from the livers of six of the dead mice. The plague bacillus was recovered from a pharyngeal swab of only one of the dead mice. None of the mice that survived to 21 days postchallenge had an anti-F1 titer.

Seven of 20 (35%) *O. leucogaster* from the Colorado population also became infected, however only three of the infected mice died. Pathologic lesions observed at necropsy were similar to the Oklahoma group. The time to death for these mice was 3.67 ± 0.58 days. *Yersinia pestis* was cultured from the spleens and salivary glands of all three dead mice and from the livers and heart blood of two of the three. A *Y. pestis* positive pharyngeal swab was also taken from one of these mice. Low anti-F1 titers were measured in four mice that survived infection. The geometric mean positive titer (reciprocal) for these mice was 96, 64, 112, 96 and 16 on days 10, 21, 30, 60 and 90, respectively.

DISCUSSION

Thomas et al. (1988) found the Oklahoma grasshopper mouse population to be moderately susceptible to mortality from subcutaneous inoculation with *Y. pestis* (21 day LD_{50} = 70 bacilli), and the Colorado population very resistant to mortality (21 day LD_{50} = 875,772 bacilli), but no significant difference in infection rate between the two groups ($ID_{50} = \geq 10$ bacilli) was shown. In this study, we had similar results after the grasshopper mice killed and consumed white mice inoculated with *Y. pestis*. While the infection rate was the same for both groups (35%), the mortality rate was lower in the Colorado group. However, Fisher's exact test indicates no statistical difference in mortality rate between groups ($P > 0.25$). A 2 × 2 *G*-test

shows that this may be a function of sample size and 33 mice from each population would need to be used to test for true difference between the mortality rates derived in this study.

Yersinia pestis is a nonmotile bacterium that must passively gain entrance to the body of a mammalian host. Butler et al. (1982) have shown that the pathology of *Y. pestis* infections initiated orally in mice consistently indicates a systemic infection and that the bacterium is not an enteric pathogen.

One of the infected grasshopper mice in this study had a large hematoma in the lip, suggesting infection via a puncture wound. Isolation of *Y. pestis* from the salivary glands in eight of ten of the *O. leucogaster* that died in this study may indicate a portal of entry for *Y. pestis* through the salivary ducts; however, tissue isolates indicated pervasive bacteremia in mice that died and *Y. pestis* may have been isolated from blood in the tissue rather than the salivary gland itself. Since 65% of both groups apparently were not infected and *Y. pestis* positive pharyngeal swabs were collected from only two of the grasshopper mice that died, the intact buccal and gastrointestinal mucosa appear to effectively resist *Y. pestis* introduced orally. The time to death for grasshopper mice in this study, along with a lack of overt disease related gross pathology, further support rapid onset of a systemic infection. Recovery of *Y. pestis* from tissues at necropsy and a mean time to death of greater than 48 hr indicate that death of the *O. leucogaster* was not due to a lethal dose of murine toxin in the mice that were fed to them.

Onychomys leucogaster populations have been shown to develop resistance to plague mortality when naturally exposed (Thomas et al., 1988) and they have a natural association with a large flea fauna including many flea species of known importance in the ecology of plague (Thomas, 1988). The omnivorous feeding habits of *O. leucogaster* provide another means of infecting these mice in areas of active zo-

TABLE 1. Mortality and serological results from *Onychomys leucogaster* following consumption of *Yersinia pestis* infected mice.

Origin of parental stock	Mortality	Seroconversion	Infections
Oklahoma	7/20 (35%)	1/20 (5%)*	7/20 (35%)
Colorado	3/20 (15%)	4/20 (25%)	7/20 (35%)

* Mouse died on day 15 and is counted among the seven deaths.

otic plague. While the amount of mammalian prey consumed by grasshopper mice may be small in comparison to the amount of arthropod and plant material consumed (Hansen, 1975), 35% of those who ate potentially infected mice in this study became infected. Our results further implicate these mice in the ecology of plague in western North America.

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