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SUSCEPTIBILITY OF RODENTS TO ORAL PLAGUE INFECTION: A MECHANISM FOR THE PERSISTENCE OF PLAGUE IN INTER-EPIDEMIC PERIODS

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Abstract: Oral infection of rodents with *Pasteurella pestis* has been demonstrated with both fully virulent and avirulent strains. Sustained rodent plague epizootics have been initiated and maintained in the absence of the classical flea vector. Transmission was due to cannibalism of the dying rodents by their healthy cagemates. Oral infection is considered to provide a plausible mechanism for the persistence of plague in an area where conditions are temporarily unsuitable for flea transmission.

The seasonal nature of epidemics of bubonic plague is quite pronounced, particularly in the Republic of Vietnam and India. Seasonal declines in the intensity of epidemics result from adverse climatic conditions reducing the number of flea vectors.[2,9,18,19,20] In addition, a specific effect of temperature in excess of 27C reduces the ability of fleas to block with *Pasteurella pestis*,[4] thereby decreasing their ability to transmit the agent. In many epidemic areas, the climate becomes so unfavorable for the flea transmission of plague that it is difficult to understand how plague can be maintained in the rodent population to serve as seed for the epidemics that always occur in succeeding years. The fact that each zone of a country experiences its annual plague epidemic on a relatively predictable schedule suggests that plague does indeed persist in the indigenous rodent population, despite adverse climatic conditions.

Prior to the incrimination of the flea as the major agent by which plague infection is conveyed to man, it was believed that oral or mechanical infection was responsible for transmission of the plague bacillus. Experiments performed early in the present century, however, appeared to discount the oral route as a significant portal of entry.[6,17,18,19,21] However, in an attempt to explain the persistence of plague in the absence of fleas, the subject of oral infection of rodents with *P. pestis* has been re-examined, with the conclusion that oral infection may play a significant role in the epidemiology of local plague epidemics and provides a plusausible explanation for the carry-over and persistence of plague through inter-epidemic periods.

MATERIALS AND METHODS

Strains of Pasteurella pestis

The strains of *P. pestis* selected for these experiments were the fully virulent Indian strain 195/P and the attenuated Madagascar vaccine strain EV-76 (51f). The strains were cultivated on brain heart infusion agar or broth (Difco) at 25C or 37C depending upon the requirements of individual experiments. The

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences - National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

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strains were tested by the methods outlined by Surgalla *et al.*[22] for the presence or absence of certain antigens or virulence factors. Strain 195/P contained all of the known virulence factors. Strain EV-76 (51f) was found to be deficient in factor P, but was, however, capable of establishing a fatal infection with 10 to 50 bacilli when administered with ferrous sulfate intraperitoneally to mice. The number of viable *P. pestis* organisms contained in the various inocula were determined by standard plate counts.

The experimental animals used in these experiments were the Swiss Bagg strain of white laboratory mice, the Sprague-Dawley strain of albino rats, and colonized *Mystromys albicaudatus,* a small African white-tailed field rodent. Previous unreported studies have shown that the median lethal dose for all three species was less than 100 *P. pestis* 195/P.

Infection of rats with virulent P. pestis 195/P

For the initial experiments, 200 rats were exposed to infection by offering them standard laboratory chow which had been mixed with 10^7 - 10^8 viable *P. pestis* 195/P. Each rat had been previously conditioned by treating its daily ration of food with sterile broth. The infectious food was consumed without hesitation. In later experiments, 30 animals were fed either the entire carcasses or viscera of mice which had succumbed to a virulent plague infection. In another series of experiments, a spleen homogenate from a guinea pig dead of plague was introduced directly into the stomachs of 10 rats by intubation and 10 additional rats were fed the same material.

Immunization of rats with attenuated P. pestis E-76 (51f).

White mice were inoculated with 100 organisms of *P. pestis* EV-76 (51f) mixed with 40 μg $FeSO_4$ by the intraperitoneal route. As an individual succumbed to the infection, it was fed to a rat. A total of 25 rats were fed in this way. A further group of 30 rats received a subcutaneous inoculation of 10^6 EV-76 (51f) as an immunizing procedure. No fatalities were observed in any of the rats. The rats were bled at intervals for serological tests and were challenged on day 28 either by feeding the rats with mouse carcasses infected with *P. pestis* 195/P or inoculating them with 10^1 *P. pestis* 195/P by the subcutaneous route.

Experimental rodent epizootic

Standard laboratory cages were utilized with the wire screen bottom removed. Such cages were set up with about 2.5 cm of wood chip litter in the bottom. Each of the cages contained 10 rodents, so marked that their identity could be established at the end of the experiment. A single plague infected rodent was introduced into each cage on day 1 of the experiment. Food and water were available at all times and when an animal died, it was left in the cage for its cagemates to devour. As the individual rodents succumbed to oral infection, replacement animals from the normal colony were introduced so that 10 living animals were present at all times. The cages were changed every 5 days and all rodents, including any that may have died from plague infection, were transferred to fresh cages. Five normal animals were then placed in the used cages and held for 20 days so that any infection resulting from fomites or excreta would be noted. The experiments were terminated at the end of 40 days.

Serological methods

Specimens of blood were obtained from rodents that survived infection for a period of 10 days. The sera of such animals were tested for the presence of complement-fixing (CF) and hemagglutinating (HA) antibodies to the fraction I antigen of *P. pestis* by the methods outlined by Cavanaugh *et al.*[5,25]

Pathological studies

Selected rodents succumbing to the infection were subjected to a thorough necropsy; suitable specimens for bacteriological and histological examination were prepared when indicated.

RESULTS

Oral infection of rats with cultures of P. pestis

Over 90% of the animals fed cultures of *P. pestis* incubated at 25C for any period of time through 120 hr died of plague in 2-5 days. By contrast, cultures of *P. pestis* incubated for over 24 hr at 37C failed to kill any of the rats. None of the rats produced antibody to fraction I antigen of *P. pestis*. The 37C cultures incubated for less than 24 hr, however, were as lethal for the rats by the oral route as were the 25C cultures. The viability of cultures of *P. pestis* grown at either temperature was unaffected by exposure to standard laboratory chow for 6 hr.

Examination of the 25C and 37C cultures for the presence of various virulence factors revealed the following: The cultures incubated at 25C for periods up to 120 hr retained all virulence factors; There was no loss of pigmentation, fraction I, pesticin I or II, fibrinolysin, or coalgulase activity in the 37C grown organisms during the 120 hr incubation, however, there was a marked decline in the presence of organisms containing the VW antigen. V+W+ organisms in the 37C culture ranged from 95.6% at 24 hr to less than 5% after 120 hr incubation. The relative innocuousness of the 120 hr 37C culture was further demonstrated by animal experiments. The median time of death of white rats inoculated intradermally with approximately 250 *P. pestis* grown for 120 hr at 25C was 72 hr in contrast to 216 hr for rats inoculated with 410 *P. pestis* grown at 37C.

Oral infection of rats with carcasses of viscera of mice dying of plague

The carcasses or viscera were invariably consumed within several hours of introduction. All rats consuming the infectious meal died of plague within 2-5 days.

Attempts to establish the exact route of infection were made by preparing an infectious mixture of laboratory animal diet and a homogenate of the spleen of a guinea pig freshly dead of plague. None of the rats challenged by intubation died while all of the 10 rats eating the infectious material died in 2-4 days. Further, none of the rats challenged by intubation developed an antibody titer to the fraction I antigen.

An additional 20 rats fed the intact viscera of plague-infected mice died within a period of 2-5 days. Pre-conditioning 10 of the rats on a soft died for 14 days in order to reduce the chance of pre-existing oral lesions did not alter the course of the disease.

Oral immunization of rats with attenuated P. pestis E-76 (51f).

Table I presents the results of a challenge experiment. It can be seen that oral immunization occurs, although not as efficiently as by the parenteral route. It is noteworthy that all rats which survived challenge had demonstrable antibody titers on day 8 following the ingestion of carcasses infected with EV-76 (51f) or parenteral inoculation with EV-76 (51f). On day 8, the geometric mean $\log_2$ titer for CF antibody was 1:1.2

TABLE 1. Immunity of Rats Following Vaccination with **P. pestis** EV-76 (51f) by the Oral or Parenteral Route.

Vaccination EV-76 (51f)		Challenge 195/P		Survival	
Route	Dose	Route	Dose	No./Total	%
Oral	10^8	Oral	10^8	8/15	53
Oral	10^6	Parenteral	10^4	6/10	60
Parenteral	10^8	Oral	10^8	18/20	90
Parenteral	10^8	Parenteral	10^4	10/10	100
None	0	Oral	10^8	0/10	0
None	0	Parenteral	10^4	0/10	0

and 1:8.9 for HA antibody in the sera of the rats. Conversely, those rats which died were uniformly negative for plague antibody by both tests on day 8.

Experimental plague epizootics

At day 40, when the experiment was terminated, 38 of 50 (76%) mice housed with a mouse dying of plague infection had also died of plague. Twelve of the 38 animals replacing the dying members of the original cage populations also died of plague. None of the mice housed in the cages soiled by the mice dying of plague died of the disease. Two of the 12 surviving members of the original mouse populations and 1 of the 26 surviving replacement mice had antibodies at day 40 to the fraction I antigen. The titers of these mice were 1:32, 1:8192, 1:2048 in the HA and 1:32, 1:256, 1:128 in the CF tests, respectively.

Similar results were obtained with *M. albicaudatus*. Two of 20 *Mystromys* were initially infected by injecting them with *P. pestis*. These animals had died by day 2 and were entirely consumed by the other rodents present. Animals were replaced as they died and all cadavers in excess of 2 were removed at the time of each replacement. Some 144 *Mystromys* died within 31 days, all apparently after oral infection. During the first third of the experiment, the mean survival time was 2 days, while during the final two-thirds, the mean survival time was 4 days. None of the *Mystromys* survived long enough to produce plague antibodies.

Pathological observations on animals dying of experimental oral plague infection

The pathological picture of orally infected rats differed considerably from those infected by other routes (subcutaneous and peritoneal). All animals succumbed with terminal bacteremia. The predominant changes observed were extensive pulmonary edema with transudate in the thoracic cavity and focal necrotic, frequently suppurating, cervical adenitis. In rare instances, the mesenteric lymph nodes were enlarged and contained areas of focal necrosis. An occasional focal, acute hepatitis was noted along with the above pathological changes. The bronchial and mediastinal lymph nodes were not remarkable. The lesions suggested that the organisms gained entrance through the pharyngeal mucosa. *P. pestis* was isolated from the blood and the cut surfaces of the spleen, lungs, and lymph nodes of all animals examined.

DISCUSSION

In the closing years of the 19th century, a pandemic of plague originated in China and gradually spread throughout much of the world. The close association of the human epidemic and the rat epizootic was noted, and upon the isolation of the etiological agent, *P. pestis*,[11,23] it was proven that the same agent was the cause of plague in both human beings and rats. Prior to the incrimination of the flea as the vector of bubonic plague by Liston,[13] it was believed that both rats and human beings were infected by the oral route or by mechanical means.

Many experiments were carried out with rats during this period to prove or disprove the theory that oral infection was the major route of transmission for rats. The majority of the experiments were conducted by infecting various foods with cultures of *P. pestis* and then feeding these infectious materials to rats. Neither the immune status of the rats used nor the cultural conditions which the *P. pestis* utilized in these experiments were specified. The highly variable results of these early experiments indicated that in certain studies, few, if any, rats were susceptible to infection with cultures administered by the oral route while in other studies the majority of animals fed either cultures or infected carcasses succumbed.

In the experiments reported here, rats were entirely susceptible to infection by the oral route when the cultures utilized for infection were incubated at 25 C. By contrast, when the cultures used to infect rats were incubated at 37 C for over 24 hr, none of the rats succumbed to the infection and none of the rats had antibody to the plague bacillus.

The effect of prolonged incubation on the loss of virulence factors has been investigated. It has been reported that 37C cultures have a tendency to shift

from a population of fully virulent V+W+ bacilli to a population of non-virulent V—W— bacilli.[3,5] These reports have been confirmed during this study. There was no detectable shift in the population during incubation at either 25C or 37C in respect to the following virulence factors: pigmentation, fraction I, pesticin I or II, fibrinolysin, or coagulase production. The shift in the V-W factor while not detectable in the 25C culture, was pronounced in the 37C culture, ranging from 4.4%, V—W— organisms at 24 hr to 95.2%, V—W— organisms at 120 hr. The magnitude of this shift appears to be sufficient to account for the difference in lethality by the oral route of the respective culture. It is probable that the variable results experienced by earlier workers was due to similar population shifts in the cultures used.

Despite the fact that failure was experienced with some cultures of *P. pestis* in initiating oral plague infections in rats, little difficulty was experienced in achieving fatal plague infections when the subject rodents were offered the intact carcasses or viscera of mice which had recently died of plague. The rats, housed in individual cages, exhibited no reluctance to consume such materials which were generally eaten as soon as they were placed inside the cages. By contrast, mice housed in groups of 10 may have developed some aversion to eating dead cagemates inasmuch as the last fatality in each of the 5 cages of mice occurred 14-32 days after the experiments were initiated. Nevertheless, 76% of the original mice died of plague during an experimental period of 40 days. When *M. albicaudatus* were tested, 100% of these rodents succumbed, indicating that no such aversion developed among the *Mystromys*. Korobkova *et al.*[12] reported similar results with mice, but not with guinea pigs. They did not mention the existence of cannibalism among cagemates, which may explain the difference in the results between the two species.

The oral route appears to be particularly advantageous to *P. pestis,* even attenuated bacilli being capable of entry into the body of the experimental animal by this route and persisting for some time. The attenuated *P. pestis* strain EV-76 as described by Girard[7] is an effective vaccine strain, capable of limited multiplication in the body of the host and evoking an antibody response to the fraction I antigens of *P. pestis* if such multiplication occurs. Over 50% of the rats ingesting the carcasses of mice which had died from an artificially contrived infection with EV-76 (51f) responded by developing CF and HA antibodies to the fraction I antigen of *P. pestis* and immunity to a challenge infection with fully virulent *P. pestis.* It is also of interest that only those animals which produced demonstrable antibody were resistant to fatal infection by either oral or parenteral routes.

The exact route by which *P. pestis* ingested by rats gains entry into the body is somewhat uncertain. It was, however, definitely possible to rule out infection by way of the gastrointestinal tract. None of the rats receiving infectious material containing 10^6 median lethal doses via stomach tube succumbed to the disease. Unlike the reports of the Plague Research Commission,[17] we did not observe enlarged mesenteric buboes in a significant number of orally infected rodents. Two other possibilities exist: infection via the tonsillar-pharyngeal route or via the pulmonary system. The changes observed at autopsy, extensive pulmonary edema and focal necrotic, frequently suppurating, cervical adenitis with little or no involvement of the bronchial or mediastinal lymph nodes, are more compatible with the tonsillar route than the pulmonary route of infection. Meyer[15] reported that in one aerosol infection experiment, cervical buboes with septicemia and secondary pulmonary involvement occurred in 60% of the test animals as opposed to primary pneumonia in 20%. He concluded that the larger particles which had impinged on the mucosa of the upper respiratory tract probably initiated the infections. Feeding the rats a soft diet prior to challenge with infected viscera negated the question as to whether or not the bone fragments, etc. contained in intact carcasses were primarily responsible for entry of the plague bacillus by mechanical injury of mucosal surfaces. The important features of the

post mortem changes were that all animals examined had cervical adenitis, with or without pulmonary edema, and terminal bacteremia.

The significance of the terminal bacteremia is self-evident. Such rats could serve to infect vector fleas, should any fleas be infesting the animal during the final phase of the disease.

As a rule, the lymph nodes tributary to the site of inoculation are the most extensively involved in plague infection. In India, during the early portion of this century, some 4,000 rats naturally infected with plague were examined and 75% of these rats had pronounced cervical adenitis.[17,18] Pollotzer[18] also states that a large cervical bubo was the most evident feature in the plague rats examined by him in China. In India, it was believed that the large cervical bubo observed in plague rats was due to the fact that fleas congregated on the neck of the hosts and that the neck region was the site most often bitten by infected fleas. In the light of these experiments, however, an equally plausible explanation might be that many of the rats examined in the field had ingested either rats infected with *P. pestis* or plague-infected fleas and that not all of the rats had been infected by the bites of infective fleas. Meyer[14] noted that many field rodents found dead of plague had enlarged submaxillary glands that suggested infection through cannibalism.

Fleas are undoubtedly the major conveyors of plague to man in epidemics of bubonic plague. Flea control is the most rapid means available of preventing human disease. It is not the purpose of this report to cast doubt on the importance of flea vectors in the epidemiology of bubonic plague. It is also true, however, that reliance on non-residual insecticides which do not pollute the environment for vector control is fast becoming a general policy.[21] Mechanisms permitting plague infection to smoulder in a rodent population, until adequate numbers of vector fleas permit the recrudescence of human disease, justify study aimed at the eventual complete interruption of all infectious cycles in plague foci. The role of soil contaminated with the remains of animals dying of plageu serving as a source of plague infections of burrowing rodents has been demonstrated, i.e., rodents reoccupying burrows of rodents dying of plague being frequently infected.[1,10,16] The cannibalism of the tissues of plague-resistant rodents infected, but not fatally so, with fully virulent *P. pestis* by rodent species sensitive to fatal plague infection is another mechanism by which plague may be maintained in a natural plague focus.[25] In the experiments presented in this report, a fatal plague infection was the invariable result when susceptible commensal rats or mice or one species of field rodents ingested the carcass or viscera of a mouse which had died of plague. Considering the general habits of commensal rats, the ingestion of dead rats by other rats is not an unlikely event in nature. Indeed, in many areas, where epizootics continue to some extent through adverse climatic conditions, oral transmission, with the other mechanisms cited above, may be the reason for the short-term persistence of plague in a given locale. In such areas, rat abatement in conjunction with a coordinated program of flea control would be the only efficacious means of eradicating plague.

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