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 cage mates. 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. In addition, a specific effect of temperature in excess of 27°C reduces the ability of fleas to block with Pasteurella pestis, 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. 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 plausible 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 25°C or 37°C 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. 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 EV-76 (51f).

White mice were inoculated with 100 organisms of P. pestis EV-76 (51f) mixed with 40 mg FeSO₄ 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 10^4 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 10^4 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 cage mates 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.

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 25°C 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 37°C failed to kill any of the rats. None of the rats produced antibody to fraction I antigen of P. pestis. The 37°C cultures incubated for less than 24 hr, however, were as lethal for the rats by the oral route as were the 25°C 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 25°C and 37°C cultures for the presence of various virulence factors revealed the following: The cultures incubated at 25°C for periods up to 120 hr retained all virulence factors. There was no loss of pigmentation, fraction I, pesticin I or II, fibrinolysin, or coagulase activity in the 37°C 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 37°C culture ranged from 95.6% at 24 hr to less than 5% after 120 hr incubation. The relative innocuousness of the 120 hr 37°C 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 25°C was 72 hr in contrast to 216 hr for rats inoculated with 410 P. pestis grown at 37°C.

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 diet and a homogenate of the spleen of a guinea pig freshly dead of plague did not alter the course of the disease.

Oral immunization of rats with attenuated P. pestis EV-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 viscera infected with EV-76 (51f) or parenteral inoculation with EV-76 (51f). On day 8, the geometric mean log₂ titer for CF antibody was 1:1.2

<table>
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<tr>
<th>Vaccination Route</th>
<th>Challenge Route</th>
<th>Survival No./Total</th>
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with the above pathological changes. The areas of focal lymph nodes were enlarged and adnexitis. In rare instances, the mesentery was necrotic, frequently suppurating, cervical Infection and none of the rats had anidioexous and poritoeat those Infected rats differed considerably from those Infected rats. The mean survival time was 4 days. The mean survival time was 2 days, while during the final two-thirds of the experiment, the mean survival time was 4 days.

**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 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*. It was proven that the same agent was the cause of plague in both human beings and rats. Prior to the inoculation of the fleas as the vector of bubonic plague by Liston, 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 those 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 corpses 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 27°C cultures have the tendency to shift
from a population of fully virulent V+W+ bacilli to a population of non-virulent V—W— bacilli. 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 egestivates 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. albicans was tested, 100% of these rodents succumbed, indicating that no such aversion developed among the Mystromys. Korobkova et al. reported similar results with mice, but not with guinea pigs. They did not mention the existence of cannibalism among cage-mates, 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 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 10^7 median lethal doses via stomach tube succumbed to the disease. Unlike the reports of the Plague Research Commission, 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 and mediastinal lymph nodes, are more compatible with the tonsillar-pharyngeal route than the pulmonary route of infection. Meyer 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 ani-
mals examined had cervical adenitis, with
or without pulmonary edema, and ter-

minal bacteremia.

The significance of the terminal bac-

teremia 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 infect-
ed with plague were examined and 75%
of these rats had pronounced cervical
adenitis.\(^{17}\) Pollotzer\(^{2}\) 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\(^{16}\) noted that
many field rodents found dead of plague
had enlarged submaxillary glands that
suggested infection through cannibalism.

Fleas are undoubtedly the major con-

veyors 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 impor-
tance of flea vectors in the epidemiology
of bubonic plague. It is also true, how-
ever, that reliance on non-residual insecti-
cides which do not pollute the environ-
ment for vector control is fast becoming
a general policy.\(^{17}\) Mechanisms permit-
ing plague infection to smoulder in a
rodent population, until adequate num-
bbers of vector fleas permit the recrudes-
cence of human disease, justify study
aimed at the eventual complete interrup-
tion of all infectious cycles in plague foci.

As a rule, the lymph nodes tributary
to the site of inoculation (i.e., the most

bacteremiac regions involved in plague infection, remains of animals dying of plague serve as a source of plague infections of burrowing rodents has been demonstrat-
ed, i.e., rodents reoccupying burrows of
rodents dying of plague being frequently
infected.\(^{1,16}\) The cannibalism of the
tissues of plague-resistant rodents infect-
ed, but not fatally so, with fully virulent
\(P.\) \(pestis\) by rodent species sensitive to
fatal plague infection is another mechan-
ism by which plague may be maintained
in a natural plague focus.\(^{1,16}\) In the experi-
ments 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
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 a re-
rat abatement in conjunction with a co-
ordinated program of flea control would
be the only efficacious means of eradicat-
ing plague.

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