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Review article

Adaptive strategies of *Yersinia pestis* to persist during inter-epizootic and epizootic periods

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Abstract – Plague is a flea-borne zoonotic bacterial disease caused by *Yersinia pestis*. It has caused three historical pandemics, including the Black Death which killed nearly a third of Europe's population in the 14th century. In modern times, plague epizootics can extirpate entire susceptible wildlife populations and then disappear for long time periods. Understanding how *Y. pestis* is maintained during inter-epizootic periods and the factors responsible for transitioning to epizootics is important for preventing and controlling pathogen transmission and ultimately reducing the burden of human disease. In this review, we focus primarily on plague in North American foci and discuss the potential adaptive strategies *Y. pestis* might employ to ensure not only its survival during inter-epizootic periods but also the rapid epizootic spread and invasion of new territories that are so characteristic of plague and have resulted in major pandemics and establishment of plague foci throughout much of the world.

plague / *Yersinia pestis* / flea / epizootic

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1. INTRODUCTION

Plague, caused by *Yersinia pestis*, is a severe, primarily flea-borne zoonotic disease characterized by quiescent and epizootic periods. Although most commonly associated with rodents, nearly all mammals can become infected with *Y. pestis* [42, 88]. Similarly, the range in flea species found to be infected is wide, spanning over 80 species. The apparent diversity of potential mammalian hosts and flea vectors is misleading, however, as many species of mammals fail to develop the high bacteremias required to infect feeding fleas or harbor few potential flea vectors. Also, many flea species occur only rarely on important mammalian hosts or fail to transmit plague efficiently [42, 50, 88]. With few exceptions, the mammalian and vector species most likely

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to meet these requirements are rodents and their fleas, respectively. When sufficiently abundant, these highly susceptible rodent species and their fleas often sustain rapidly spreading epizootics that can decimate other wildlife species and elevate human exposure risks.

Despite more than a century of investigation into the epidemiology and ecology of plague we still know remarkably little about many key aspects of the natural cycles of *Y. pestis* and the strategies used by this bacterium to support its survival. For example, the means by which *Y. pestis* persists during inter-epizootic periods remains largely unknown and has been the focus of speculation for over a century. Proposed explanations include long-term persistence in fleas, hibernating hosts, or soil [6, 11, 14, 28, 42, 51, 72, 75, 88, 103]. More commonly, within plague-endemic areas, *Y. pestis* is believed to be transmitted in maintenance cycles involving "enzootic" hosts that, at a population level, display a heterogeneous response to infection [41, 42, 86, 87]. That is, some hosts are highly susceptible to infection, while others are resistant. Occasionally, the infection spreads from enzootic hosts to highly susceptible populations resulting in an amplification or epizootic phase. While this proposed interplay between epizootic and enzootic cycles has dominated the literature for the last few decades [5, 39, 41, 42, 88], very little evidence exists to support the occurrence of truly independent enzootic and epizootic transmission cycles. Instead, the vertebrate-flea enzootic cycles that maintain *Y. pestis* may differ from those that serve to amplify infection during epizootic periods [42, 88] only in the rates of transmission and numbers of hosts infected. In this review, we focus primarily on plague in North American foci and discuss the potential adaptive strategies *Y. pestis* might employ to ensure not only its survival during inter-epizootic periods but also the rapid epizootic spread and invasion of new territories that are so characteristic of plague and have resulted in major pandemics and establishment of plague foci throughout much of the world.

2. HIGH VIRULENCE IN THE HOST MAXIMIZES THE LIKELIHOOD OF FLEA-BORNE TRANSMISSION: A DESCRIPTION OF THE COURSE OF INFECTION IN THE HOST

As recently as 1 500–20 000 years ago, *Y. pestis* evolved from *Y. pseudotuberculosis*, a relatively benign enteric bacterium that is transmitted through contact with contaminated food and water [1]. In striking contrast to its closely related progenitor, *Y. pestis* is extremely virulent, with mortality of untreated infections in humans ranging from 50–100%, depending on the route of exposure [69, 86]. As part of its transition from an enteric to vector-borne pathogen, *Y. pestis* has acquired two unique plasmids (pPla and pMT1), shed genes required for persistence outside the vertebrate or vector and gained apparently new functions for several chromosomal genes [28, 48, 50, 82, 83, 108]. Although much of this material has been reviewed elsewhere, it is worth summarizing some of the adaptations of *Y. pestis* to produce the extremely high bacterial concentrations that typify plague infections.

In laboratory animals, bacterial concentrations usually reach 10^8–10^9 cfu/mL of blood [17, 27, 93]. This markedly high level of bacteremia and associated virulence to the vertebrate host is likely to have evolved as a result of the bacteria's reliance on transmission by infectious fleas [70]. Because of the small volume of blood consumed (0.1–0.3 μL per blood meal [50, 82]) by a feeding flea, bacteremia in the host must be at least 10^6 cfu/mL to reliably infect these insects [37, 70]. While attaining such high bacteremias raises the risk that hosts will die of the septic shock, systemic inflammatory response syndrome, multi-organ failure and hemorrhaging associated with late stage *Y. pestis* infections, this apparent disadvantage appears to be offset by the likelihood that at least some fleas will complete feeding prior to the host's death and thus acquire sufficient bacteria to become infectious to other animals during subsequent blood meal feedings [42]. In addition, pathogen-induced host mortality forces newly infected fleas to seek alternative hosts, thus increasing the likelihood

of transmission to another host of the same or different species [50]. It should be noted that unlike ticks or mosquitoes which seek new hosts for each subsequent blood meal they consume, fleas are likely to feed repeatedly on the same host and will change hosts only under such circumstances as the death of the host, close social contact among hosts of the same species, or the appearance of newly born host young in nests or burrows.

After an infectious flea bites a susceptible vertebrate host, several bacterial genes are upregulated allowing *Y. pestis* to evade the immune system and disseminate to the lymphatic system. Pla, a surface protease, encoded on the pPla plasmid that was acquired after divergence from *Y. pseudotuberculosis*, is typically believed to be required for dissemination from the intradermal bite site [65]. However, recent work has cast doubt upon this conclusion [46, 104]. Upon entry to the host's body *Y. pestis* can infect macrophages which may carry the bacteria to regional lymph nodes where they multiply, express F1 antigen (caf1) and pH6 antigen, and give rise to the characteristic buboes of plague (bubonic plague), which are highly swollen lymph nodes that are heavily infected with *Y. pestis* [101]. *Y. pestis* bacteria leaving the macrophage for an extracellular existence bear F1 capsular antigen and express pH6 antigen (PsaA), both of which help render the bacterium resistant to further phagocytosis [29] and enable it to circulate freely in the bloodstream. Once phagocytosis-resistant *Y. pestis* gain access to the bloodstream of susceptible hosts they begin to multiply rapidly and quickly reach densities ($>10^6$ bacteria/mL blood) sufficient to infect feeding fleas [37, 93].

3. FLEA-BORNE TRANSMISSION OF *Y. PESTIS*: THE BLOCKED FLEA PARADIGM

In 1914, Bacot and Martin described the blocked flea paradigm of *Y. pestis* transmission [4]. Under this scenario, a flea first consumes blood from a highly bacteremic host. Over time, the plague bacilli multiply within the proventriculus and midgut, eventually forming a blockage in the proventriculus, which is a globular structure lying between the flea's esophagus and midgut (stomach). The interior of the proventriculus is lined with a series of spines that prevent ingested blood cells from flowing back towards the mouth of the flea. In some species of fleas, such as the Oriental rat flea, *Xenopsylla cheopis*, the proventriculus also serves as a site for initial colonization and eventual blockage (occlusion) of the flea gut by multiplying *Y. pestis* [4, 37]. Recent molecular studies have demonstrated that proventricular block formation is regulated by a group of chromosomal genes called the hemin storage locus (hms), which is upregulated at ambient temperatures and not expressed at 37 °C. *hms* is required for successful colonization of the proventriculus, but not for colonization of the midgut [47]. In contrast, the *Yersinia* murine toxin (ymt), located on the newly acquired pMT1 plasmid, is a phospolipase required for survival in the midgut of the flea [49]. In addition to proventricular blockage, biofilm can cause a partial blockage, or may be useful for forming large bacterial aggregations that help prevent the bacteria from being passed in feces [50].

Although block formation can occur as early as 5 days post infection (d.p.i.), it is not typically observed until 2–3 weeks p.i. [17, 37, 39, 57, 58]. Blockage of the gut by the proliferating *Y. pestis* and its products (biofilm) prevents newly ingested blood from reaching the midgut, thus causing the flea to starve. It further disrupts proper function of the proventriculus, allowing blood to reflux from the midgut to the mouthparts. As a result of the proventricular blockage, the flea increases its feeding attempts but cannot satisfy its hunger because of its inability to pass the ingested blood through the foregut and into the midgut. In an effort to clear its gut blockage a flea will often regurgitate the contents of its foregut. Although these attempts to feed and then clear the blockage through regurgitation are unsuccessful, the vigorous movement of materials in the flea's foregut results in mixing of newly ingested blood with bits of *Y. pestis*-bearing material cleaved from the blockage.

Under the scenario of transmission of plague bacteria by blocked fleas, infection of the host results from regurgitation of this mixture of blood and *Y. pestis*-bearing blockage material back into the site of the initial flea bite.

Experimental evaluation of transmission efficiency by blocked rat fleas (*X. cheopis*), have revealed that the probability of a single bite resulting in transmission is quite low [17, 37,39,70,105]. Despite such low transmission efficiency, increased daily feeding rates by starving, blocked fleas, leads to an increase in estimates of the number of secondary infections arising from a focal infection (vectorial capacity) [30, 70]. Although Bacot and Martin [4] proposed that a partially blocked flea may be even more efficient as a vector, and that block formation is extremely rare in most species of fleas [17, 39, 42], the blocked-flea scenario has dominated the plague literature as the primary and only significant means of flea-borne transmission of *Y. pestis*. For example, vector efficiency is often equated with a flea's ability to block [39,50,51,64,67,70,88].

4. FLEA-BORNE TRANSMISSION OF *Y. PESTIS*: EARLY-PHASE TRANSMISSION BY UNBLOCKED FLEAS

While there is no doubt that the unique ability of *X. cheopis* to form a proventricular blockage which causes the flea to increase its daily biting rate enhances its ability to efficiently transmit *Y. pestis*, several observations have led to the need to identify an alternative flea-borne mechanism of transmission. First, most flea species, including those presumed to be important vectors of *Y. pestis*, do not readily form proventricular blockages [17, 39, 42]. Yet, ridding hosts of their fleas effectively halts pathogen transmission, thus implicating fleas in epizootic and epidemic transmission [33,45, 54, 88, 94]. Second, transmission by blocked fleas, which requires a long extrinsic incubation period prior to a short infectious window that is often followed by death of the flea due to starvation, cannot sufficiently explain the rapid rate of spread that typifies plague epidemics and epizootics [30, 70, 103]. Some have suggested that transmission by blocked fleas is important primarily during inter-epizootic transmission, but that mechanical transmission by unblocked fleas is significant during epizootics [17, 59, 60, 90]. Despite these assertions, true mechanical transmission, which would occur in fleas as a result of contamination of the vector's mouthparts by viable *Y. pestis*, has been discounted because bacteria survive on the mouth parts for less than 3 h [13]. As a result, early studies on flea-borne transmission typically focused on the period of time after which block formation was expected to occur [17, 37, 39, 57, 58]. In some instances, transmission by unblocked fleas was observed 1–4 d.p.i. [4, 17, 37, 52, 73, 88, 102, 105], but the results were often viewed as anomalous or attributed to occurring by mass action (i.e., unnaturally high flea loads).

Recently, several studies have explicitly evaluated the efficiency of several flea species to transmit *Y. pestis* during the first few days after being allowed to ingest a highly bacteremic blood meal but prior to the development of a proventricular blockage [30–35, 106, 107]. In these studies, fleas were exposed via an artifical feeding system to rat blood infected with a fully-virulent strain of *Y. pestis* at concentrations reflecting a terminal septicemia in the host. Fleas that consumed a bloodmeal were presumed to be infected and were held for 3 h to 4 d.p.i. before being allowed to feed on a naïve mouse. Studies with *X. cheopis* and *O. montana* revealed that, in contrast to the blocked flea model, these fleas were immediately infectious and could efficiently transmit *Y. pestis* for at least 4 d.p.i. [30, 32]. Thus far, each of the six species evaluated have demonstrated the ability to successfully transmit by this process, termed early-phase transmission [30], although efficiency differs among species [30–33, 106, 107].

Although the mechanism of early-phase transmission remains unclear, several pieces of evidence suggest that transmission efficiency is related to the location of the bacteria within the digestive tract at the time of transmission. First, in studies that provided previously

infected fleas with uninfected "maintenance" blood meals before their ability to transmit was measured, transmission efficiency waned following consumption of infectious blood [31, 106, 107]. Second, fleas that defecate copious amounts of partially digested blood during, or shortly after feeding (e.g., *Ctenocephalides felis*, *Aetheca wagneri*), appear to transmit infection less efficiently [33, 34]. Third, transmission efficiency is not significantly associated with bacterial loads in fleas [30–34, 106, 107]. Finally, the ability of fleas to remain infected with *Y. pestis* is affected by the type of host blood they consume. Infection prevalence and bacterial loads in *O. montana* and *X. cheopis* were significantly lower in fleas that consumed infected rabbit blood compared with infected mouse or rat blood [36]. It is possible that this is related to differences in the rates at which different blood types are digested and excreted by the flea but this has yet to be evaluated. The importance of host blood meal sources in natural *Y. pestis* transmission cycles also has yet to be demonstrated but could be an important factor in determining which hosts act as sources of infectious blood meals for vector fleas and, therefore, contribute to the maintenance of plague foci.

5. PERSISTENCE OF *Y. PESTIS* IN SOIL

Persistence of *Y. pestis* in soil has been suggested as a possible mechanism of inter-epizootic persistence, epizootic spread, and as a factor defining plague foci [6, 10, 28, 42, 88, 103]. Although *Y. pestis* recently evolved from an enteric bacterium, *Y. pseudotuberculosis*, that can survive for long periods in soil and water, it is believed that selection for vector-borne transmission has resulted in the loss of many of these survival mechanisms, suggesting that long-term persistence outside of the host or vector is unlikely [1, 16, 85]. Nonetheless, previous studies have demonstrated survival of *Y. pestis* in soil under experimental conditions [6, 28, 35, 42, 75–79] or under special circumstances (low temperature, protection from UV light) in natural systems [35, 56]. The mechanism by which *Y. pestis* is able to survive in the soil is unclear but is perhaps not surprising considering its ability to grow on many different types of media. Also, it should be noted that the soil samples used in most of these experiments were autoclaved and that the work of Eisen et al. [35] involved survival of *Y. pestis* in naturally occurring soil for only brief periods. Although the above experiments have assumed that if *Y. pestis* was to survive in soils it would do so as free-living, metabolically active and continually reproducing population of bacteria, others have proposed that *Y. pestis* may survive as an intracellular parasite of soil protozoa [26, 81, 89], in a latent form in the soil [100], within a biofilm on the surface of nematodes [21], or within host tissue [35]. If an infectious host dies before infecting fleas, survival in soil could provide a second chance to infect a new host, provided that contaminated soil can be equated with infectious soil. Although the suggestion of survival in soil as a possible maintenance mechanism is intriguing, much work needs to be done to determine whether currently published laboratory experiments or the short-term survival of *Y. pestis* in naturally occurring soil samples with known history of *Y. pestis* contamination have relevance for the long-term survival and maintenance of plague in natural foci.

6. MECHANISMS OF EPIZOOTIC SPREAD

Plague epizootics represent periods when *Y. pestis* can spread rapidly across landscapes. During such periods of time, an average focal infection gives rise to more than 1 secondary infection ($R_0 > 1$) [62]. Understanding the factors leading to and supporting plague epizootics is important because human infections are typically associated with such periods of amplification [8, 42]. In part, it is for this reason that the majority of field and modeling studies have focused on understanding factors that lead to and drive epizootics.

Several studies have identified associations between plague epizootics and climatic conditions [38, 84, 96, 97, 99], host abundance [23, 25], or flea infestation rates [51]. These factors are probably not mutually exclusive.

As others have summarized in trophic cascade models [38, 42, 84, 96], that elevated precipitation rates lead to increases in primary vegetative production which serves to increase the abundance of food sources for rodents and thus results in increases in rodent abundance. Such an increase in rodent abundance likely results in amplification of the populations of fleas infesting rodents. Indeed, Hirst noted that rat and flea densities are closely correlated (so much so that he proposed that flea indices are predictive of rat abundance) [51]. Increased precipitation also is likely to result in increased soil moisture which could increase humidity levels in burrows. Increased burrow humidity should in turn improve flea survival because these insects are quite susceptible to desiccation [18]. Also, Krasnov et al. [64] noted that flea species with high vector potential tend to infest their hosts more abundantly than those that were classified as having lower vector potential. In many mathematical models, increasing contact rates between infectious (including vectors) and susceptible individuals typically results in an increase in the number of secondary infections arising from a focal infection [3, 23, 30, 40, 43, 70, 71]. Thus it is logical that elevated rodent densities coupled with increased flea loads per rodent would improve the likelihood of infectious fleas finding new susceptible hosts, resulting in increased numbers of secondary infections arising from a single focal infection ($R_0 > 1$). Indeed, a recent mathematical model of plague transmission [25] indicates that spatial structure and host abundance are critical to predicting spatial epidemics. However, although $R_0 > 1$ remains a prerequisite for epizootic spread, this value may need to be much larger in order for sustained spread across a landscape.

The rate of spread, however, should be strongly dependent on the extrinsic incubation period (the period of time from a flea becoming infected until it is able to transmit). As described above, the extrinsic incubation period for blocked fleas is quite long relative to early-phase transmission and thus would result in a relatively slow progression of the pathogen within a susceptible population [30]. In fact, a recent mathematical model used a novel approach to investigate the relative importance of transmission by blocked fleas, compared with airborne transmission via infectious respiratory droplets and direct contact with a "short term reservoir" [103]. The model suggests that blocked fleas cannot drive plague epizootics in black-tailed prairie dogs. The findings are well supported by empirical data from the field which show that prairie dog populations frequently experience plague epizootics in the absence of flea species that are prone to forming proventricular blockages [39, 97]. The authors of this same model concluded that perhaps fleas are important during the start of an epizootic, but that a short-term reservoir that persists for at least 2–3 weeks is necessary to drive epizootics. The proposed short-term reservoirs cited in this study echo common themes proposed previously. These include (1) resistant hosts that could serve directly as reservoirs or provide maintenance feeds for infected fleas that would later transmit *Y. pestis* to other animals, (2) soil or recently dead and decaying carcasses could serve as sources of infection by direct contact with contaminated soils or decaying tissues or through ingestion of infectious tissues, or (3) unblocked fleas that are capable of transmitting *Y. pestis*.

Among the above three possibilities, it is unlikely that resistant hosts, such as grasshopper mice [98, 103] or deer mice [2, 74, 80], could drive plague epizootics. In this context, use of the term "resistant" host applies to the population level, such that some individuals are highly susceptible, succumbing to infection, while other individuals are resistant and recover from challenge with *Y. pesti*s. If the Webb et al. [103] model is correct, a short term reservoir that is infectious for 2–3 weeks is required to drive epizootics. However, laboratory studies have demonstrated that to reliably infect feeding fleas, bacteremia must meet or exceed 10^6 cfu per mL of blood [37, 70]. Typically, most rodents that achieve this level of infection survive for only 2 days or less after exceeding this threshold [70, 93], thus limiting the infectious period. In addition, flea loads on

host may be too low to overcome low vector efficiency. For example, transmission efficiency by *Aetheca wagneri*, the primary flea infesting deer mice, is very low [34, 39, 57]. A recent study determined that at least 68 fleas per host would be required to maintain mouse to mouse transmission by this flea [34]. However, natural infestation levels are typically below three per mouse [2, 7, 20, 53, 66, 68, 92].

Alternatively, it has been proposed that immune individuals could provide uninfected "maintenance" blood meals to keep infected fleas alive and maintain infection (thus making fleas, rather than immune rodents, short-term reservoirs) [98, 103]. Such a mechanism might allow *Y. pestis* to temporarily persist in the wake of an epizootic when most susceptible hosts have died of infection and the likelihood of an infectious flea finding a susceptible host upon which to feed is quite low, but strong evidence for this requires further evaluation. It is possible that fleas ingesting blood from immune hosts could become cleared of infection. Bell [12] noted that previous work indicated that fleas will lose infection with *Y. pestis* more quickly after consuming a bloodmeal from an immune host. Unfortunately, supporting data or references were not provided. A recent study comparing infection prevalence and bacterial loads in fleas fed on different types of blood (rat versus rabbit versus mouse) showed that the species of host blood significantly affects the ability of fleas to retain plague bacteria in their guts, with some *X. cheopis* and *O. montana* fleas that were fed rabbit blood containing high numbers of *Y. pestis* becoming cleared of *Y. pestis* infection in as little as 2 d.p.i. [36]. These results imply that not all blood meals should be considered to have the same effect on maintaining *Y. pestis* infection in fleas.

The role of soil as a temporary reservoir and source of infection during epizootics is at this point largely hypothetical. As described above, several studies have demonstrated that *Y. pestis* can survive in autoclaved soil for many months. Recently, Eisen et al. [35] also demonstrated that plague bacteria could remain viable for at least three weeks in naturally occurring soil contaminated by blood from a dying mountain lion, a period of time that would at first glance appear to meet the criterion of Webb et al. [103] for a 2–3 weeks reservoir of *Y. pestis* to sustain epizootic transmission. However, mere contamination of soil with viable *Y. pestis* does not indicate that these soils will be infectious to rodents or that *Y. pestis* could be acquired from them by these animals at rates sufficient to maintain epizootic transmission.

In contrast to the above two proposed short-term reservoirs, considerable evidence exists to suggest that transmission of plague by unblocked fleas could represent a significant short-term reservoir supporting the epizootic spread of plague. As early as 1947, Burroughs proposed that mechanical transmission (contamination of flea mouthparts with viable *Y. pestis*) could be important during epizootic periods [17]. The short extrinsic incubation period provided by this mode of transmission clearly could help to explain the rapid rate of spread that typifies plague epizootics. However, mechanical transmission has been discounted because plague bacteria are believed to be short-lived on the external mouthparts [13]. Like mechanical transmission, early-phase transmission requires a very short extrinsic incubation period [30], but appears to be quite common and efficient in many flea species. For example, efficient early-phase transmission has been demonstrated for several flea species that commonly infest epizootic hosts. These include *Xenopsylla cheopis* [32], which commonly infest *Rattus rattus* [42, 51, 88]; *Oropsylla montana* [30], which occur commonly on California ground squirrels and rock squirrels [22, 39, 52]; *Oropsylla hirsuta* [106] and *Oropsylla tuberculata cynomuris* [107] which frequently infest prairie dogs [15, 39, 106, 107]. Interestingly, cat fleas (*Ctenocephalides felis*) are not typically encountered on epizootic hosts [55], and although they are capable of transmitting *Y. pestis* during the early-phase, exhibit a vector efficiency that is quite low compared to such species as *X. cheopis* [33]. Similarly, *Aetheca wagneri*, a flea commonly associated with deer mice (*Peromyscus*

maniculatus), is a very inefficient vector during the early-phase [34]. Interestingly, deer mice are not commonly associated with plague epizootics, but instead have been implicated as enzootic reservoirs [2, 68]. Although the sample size is limited, available data suggest that flea species that are commonly found on certain host species known to experience high mortality during plague epizootics are efficient early-phase vectors. In contrast, those fleas that are typically associated with other host species that rarely become involved in epizootics or show elevated mortality during these events appear to be inefficient vectors during the early-phase. These data suggest that early-phase transmission may be important for driving plague epizootics. Although transmission efficiency may wane after an initial highly infectious period, it is possible for individuals to remain infected long-term by infecting a secondary host and then reacquiring infection from that host as it becomes bacteremic [31]. Furthermore, it is possible that infected host-seeking fleas remain highly infectious for long periods until they consume their first uninfected blood meal. Such data provide a mechanism for unblocked fleas to fulfill the 2–3 weeks infectious window that was suggested to be important for driving epizootics [103].

7. MECHANISMS OF INTER-EPIZOOTIC MAINTENANCE

Understanding how *Y. pestis* is maintained during inter-epizootic periods and the factors responsible for transitioning to epizootics is important for preventing and controlling pathogen transmission and ultimately reducing the burden of human disease. Within plague-endemic areas, some have proposed that *Y. pestis* is maintained in transmission cycles involving "enzootic" hosts [8, 41, 42, 80, 91]. Under this scenario enzootic host populations display a heterogeneous response to infection such that some hosts are highly susceptible to *Y. pestis*, developing the very high bacteremia required to infect feeding fleas before dying of infection, while other individuals are resistant to infection and may serve to provide maintenance blood meals to infectious fleas that eventually infest susceptible hosts. Essentially, immune hosts serve to dilute the contact rate between infectious fleas and susceptible hosts, which ultimately reduces the force of infection (R_0) and is likely to result in very low rates of transmission and the threat of local extirpation of *Y. pestis* unless other reservoir mechanisms are available, including survival of the plague bacterium in off-host flea populations. The species typically proposed to act as enzootic reservoirs are western deer mice and their allies (*Peromyscus* spp.) or voles (*Microtus californicus* and perhaps other microtines) [42].

There is very little empirical evidence supporting plague persistence in independent enzootic cycles. First, as described above, it is unlikely that flea loads are sufficiently high during inter-epizootic periods to maintain host to host transmission given the low vector efficiency described for fleas associated with proposed enzootic hosts. Furthermore, presumed "enzootic" reservoirs such as deer mice or grasshopper mice are seldomly infected prior to outbreaks in susceptible "epizootic" host populations. Recently, grasshopper mice were collected from prairie dog towns before, during and for up to two years after plague epizootics. Serological evidence of exposure to *Y. pestis* in grasshopper mice was not observed prior to or two years after observed epizootic events in prairie dogs, but during epizootics, grasshopper mice were consistently seropositive [98]. Similar results were reported for deer mice from the same area [92]. These data suggest that although grasshopper mice and deer mice display a heterogeneous response to infection, they are probably not maintaining infection during inter-epizootic periods. Instead, susceptible hosts that succumb to infection may help to drive plague epizootics in prairie dogs. Additional studies combining field- and laboratory-derived data on flea infestation rates, host immunity, vector efficiency and extrinsic incubation periods for fleas associated with enzootic host species are required to evaluate the likelihood of independent enzootic transmission cycles.

It is possible that within populations of epizootic hosts (e.g., ground squirrels, rock

squirrels), *Y. pestis* could persist during inter-epizootic periods without the need for secondary or "enzootic" hosts, providing that the host population is sufficiently large and consists of a metapopulation with a number of distinct subpopulations, each of which are spaced sufficiently far apart or separated by landscape features that allow some populations to escape infection or the epizootic to proceed slowly enough so that at least some of the affected subpopulations would recover before being subjected to another epizootic [19, 24, 42, 63, 96]. A recent study described plague transmission as a percolation phenomenon, which emphasizes the importance of spatial structure of host populations and host abundance for predicting epizootic transmission [25]. Future modeling efforts are required to evaluate how changes in flea abundance and transmission efficiency affect such model predictions.

Consistent with this notion, Krasnov et al. [64] noted that flea species with high vector potential infest a narrow taxonomic host range and are abundant on their hosts. Narrow host specificity increases the likelihood of vectors feeding on other susceptible hosts, rather than bridging to host species that display a heterogeneous response to infection. If highly efficient fleas consistently feed on susceptible hosts, perhaps secondary hosts are not required for inter-epizootic maintenance and R_0 is regulated instead by changes in flea loads on susceptible hosts, which is likely related to climatic changes. Using standard vectorial capacity models [30, 70, 107], if all factors are held constant (daily biting rate, infectious period of the host, vector efficiency, extrinsic incubation period), changing the flea-host contact rates would result in local extinction ($R_0 < 1$), enzootic maintenance ($R_0 = 1$) or epizootic spread ($R_0 > 1$). The rate of spread could be regulated by the duration of host-seeking time. If the host population density is low, it would likely take longer for an infectious flea to find its next bloodmeal. However, empirical studies suggest that infectiousness of the first bloodmeal after infection remains high throughout the duration tested, but declines after feeding on an uninfected host [30–32, 106, 107]. In some instances, when the contact rates between fleas and susceptible hosts are high, epizootics would be expected. This is consistent with the trophic cascade model described above. Of course, not all susceptible host populations experience plague epizootics following conducive climatic events, suggesting that *Y. pestis* does not remain at low levels in all host populations. In many instances, contact rates between infectious fleas and susceptible hosts likely drops below levels needed to maintain infection and local extinctions occur. Appropriate spatial structuring could allow for local persistence within plague endemic foci. Numerous verbal and quantitative models have suggested that metapopulation dynamics allow *Y. pestis* to persist in relatively small, connected rodent populations that experience local extinction and recolonization [19, 42, 51, 63, 88, 96]. The ability of *Y. pestis* to infect a broad array of hosts and vectors and perhaps its ability to persist outside of the host may allow the bacteria to hopscotch across the landscape causing long periods of quiescence followed by short, explosive epizootics.

8. CONCLUSIONS

– Because flea-borne transmission requires very high bacteremia to infect the fleas and death of the host increases the likelihood of infected vectors to seek new hosts upon which to feed, there appears to be strong selection for virulence.

– Early-phase transmission coupled with the use of hosts that are highly susceptible and carry high flea loads helps explain the rapid spread that typifies plague epizootics and may play a role in "reseeding" new areas, including those that might become new foci.

– The role of blocked fleas in epidemic or epizootic spread depends on the flea species involved, infestation rates, and host-seeking flea abundance. *X. cheopis* blocks quickly compared to most fleas and has been the standard model, but probably represents an exception, rather than the norm. A combination of early-phase transmission

and blocking probably helps to explain why this flea is so dangerous but other fleas that are important in epizootic spread probably rely mainly on early-phase transmission.

– Mechanisms of inter-epizootic maintenance of plague is not well-understood. Although fleas are generally short-lived, there is evidence of fleas surviving and maintaining infection for many months to over a year [9, 44, 61, 95]. This might be the situation in which biofilm production is most important because it could allow maintenance of the bacterium in the flea and, if the blocked flea survives long-term, would then enable the flea to have a reasonably good chance of transmitting *Y. pestis* if it finds another host weeks to months later. Thus, fleas could serve as a reservoir of infection and may act as a spark that initiates epizootics when flea loads and host densities are sufficiently high to support epizootics. Because this mechanism is expected to be fairly inefficient, it could explain the sporadic epizootic patterns that are typically observed. Alternatively, chronic infections in rodents, or persistence in soil could play a similar role in local persistence of *Y. pestis* in the absence of host to host transmission.

– Patchy landscapes leading to metapopulation structuring of susceptible host populations, coupled with the ability of *Y. pestis* to infect a broad array of hosts and vectors may contribute to the ability to be maintained within plague foci.

REFERENCES

[1] Achtman M., Zurth K., Morelli G., Torrea G., Guiyoule A., Carniel E., *Yersinia pestis*, the cause of plague, is a recently emerged clone of *Yersinia pseudotuberculosis*, Proc. Natl. Acad. Sci. USA (1999) 96:14043–14048.

[2] Anderson S.H., Williams E.S., Plague in a complex of white-tailed prairie dogs and associated small mammals in Wyoming, J. Wildl. Dis. (1997) 33:720–732.

[3] Anonymous, Ecology of infectious diseases in natural populations, Cambridge, Cambridge University Press, 1995.

[4] Bacot A.W., Martin C.J., Observations on the mechanism of the transmission of plague by fleas, J. Hyg. (Plague Suppl. III) (1914) 13:423–439.

[5] Baltazard M., Bahmanyar M., Mofidi C., Seydian B., Kurdistan plague focus, Bull. World Health Organ. (1952) 5:441–472.

[6] Baltazard M., Karimi Y., Eftekhari M., Chamsa M., Mollaret H.H., La conservation interépizootique de la peste en foyer invétéré hypothèses de travail, Bull. Soc. Pathol. Exot. (1963) 56:1230–1241.

[7] Barnes A.M., Ogden L.J., Archibald W.S., Campos E., Control of plague vectors on *Peromyscus maniculatus* by use of 2% carbaryl dust in bait stations, J. Med. Entomol. (1974) 11:83–87.

[8] Barnes A.M., Surveillance and control of bubonic plague in the United States, Symp. Zool. Soc. Lond. (1982) 50:237–270.

[9] Bazanova L.P., Maevskii M.P., The duration of the persistence of plague microbe in the body of flea *Citellophilus tesquorum altaicus*, Med. Parazitol. (Mosk.) (1996) 45–48 (in Russian).

[10] Bazanova L.P., Maevskii M.P., Khabarov A.V., An experimental study of the possibility for the preservation of the causative agent of plague in the nest substrate of the long-tailed suslik, Med.Parazitol.(Mosk.) (1997) 37–39 (in Russian).

[11] Bazanova L.P., Nikitin A., Maevskii M.P., Conservation of *Yersinia pestis* in winter by *Citellophilus tesquorum altaicus* females and males, Med. Parazitol. (Mosk.) (2007) 34–36 (in Russian).

[12] Bell J.F., The infection of ticks (*Dermacentor variabilis*) with *Pasteurella tularensis*, J. Infect. Dis. (1941) 76:83–95.

[13] Bibikova V.A., Contemporary views on the interrelationships between fleas and the pathogens of human and animal diseases, Annu. Rev. Entomol. (1977) 22:23–32.

[14] Bizanov G., Dobrokhotova N.D., Experimental infection of ground squirrels (*Citellus pygmaeus* Pallas) with *Yersinia pestis* during hibernation, J. Infect. (2007) 54:198–203.

[15] Brinkerhoff R.J., Markeson A.B., Knouft J.H., Gage K.L., Montenieri J.A., Abundance patterns of two *Oropsylla* (Certatophyllidae: Siphonaptera) species on black-tailed prairie dog (*Cynomys ludovicianus*) hosts, J. Vector Ecol. (2006) 31:355–363.

[16] Brubaker R.R., The recent emergence of plague: a process of felonious evolution, Microb. Ecol. (2004) 47:293–299.

[17] Burroughs A.L., Sylvatic plague studies: The vector efficiency of nine species of fleas compared with *Xenopsylla cheopis*, J. Hyg. (1947) 43:371–396.

[18] Cavanaugh D.C., Marshall J.D., The influence of climate on the seasonal prevalence of platue in the Republic of Vietnam, J. Wildl. Dis. (1972) 8:85–93.

[19] Collinge S.K., Johnson W.C., Ray C., Matchett R., Grensten J., Cully J.F., et al., Landscape structure and plague occurrence in black-tailed prairie dogs on grasslands of the western USA, Landscape Ecol. (2005) 20:941–955.

[20] Cully J.F., Barnes A.M., Quan T.J., Maupin G., Dynamics of plague in a Gunnison's prairie dog colony complex from New Mexico, J. Wildl. Dis. (1997) 33:706–719.

[21] Darby C., Hsu J.W., Ghori N., Falkow S., *Caenorhabditis elegans*: plague bacteria biofilm blocks food intake, Nature (2002) 417:243–244.

[22] Davis R.M., Smith R.T., Madon M.B., Sitko-Cleugh E., Flea, rodent, and plague ecology at Chuchupate campground, Ventura County, California, J. Vector Ecol. (2002) 27:107–127.

[23] Davis S., Begon M., De Bruyn L., Ageyev V.S., Klassovskiy N.L., Pole S.B., et al., Predictive thresholds for plague in Kazakhstan, Science (2004) 304:736–738.

[24] Davis S., Klassovskiy N., Ageyev V., Suleimenov B., Atshabar B., Klassovskaya A., et al., Plague metapopulation dynamics in a natural reservoir: the burrow system as the unit of study, Epidemiol. Infect. (2007) 135:740–748.

[25] Davis S., Trapman P., Leirs H., Begon M., Heesterbeek J.A., The abundance threshold for plague as a critical percolation phenomenon, Nature (2008) 454:634–637.

[26] Domaradsky I.V., Is not plague a "protonosis"?, Med. Parazitol. (Mosk.) (1999) 2:10–13.

[27] Douglas J.R., Wheeler C.M., Sylvatic plague studies. II. The fate of *Pasteurella pestis* in the flea, J. Infect. Dis. (1943) 72:18–30.

[28] Drancourt M., Houhamdi L., Raoult D., *Yersinia pestis* as a telluric, human ectoparasite-borne organism, Lancet Infect. Dis. (2006) 6:234–241.

[29] Du Y., Rosqvist R., Forsberg A., Role of fraction 1 antigen of *Yersinia pestis* in inhibition of phagocytosis, Infect. Immun. (2002) 70:1453–1460.

[30] Eisen R.J., Bearden S.W., Wilder A.P., Montenieri J.A., Antolin M.F., Gage K.L., Early-phase transmission of *Yersinia pestis* by unblocked fleas as a mechanism explaining rapidly spreading plague epizootics, Proc. Natl. Acad. Sci. USA (2006) 103:15380–15385.

[31] Eisen R.J., Lowell J.L., Montenieri J.A., Bearden S.W., Gage K.L., Temporal dynamics of early-phase transmission of *Yersinia pestis* by unblocked fleas: secondary infectious feeds prolong efficient transmission by *Oropsylla montana* (Siphonaptera: Ceratophyllidae), J. Med. Entomol. (2007) 44:672–677.

[32] Eisen R.J., Wilder A.P., Bearden S.W., Montenieri J.A., Gage K.L., Early-phase transmission of *Yersinia pestis* by unblocked *Xenoopsylla cheopis* (Siphonaptera: Pulicidae) is as efficient as transmission by blocked fleas, J. Med. Entomol. (2007) 44: 678–682.

[33] Eisen R.J., Borchert J.N., Holmes J.L., Amatre G., Van Wyk K., Enscore R.E., et al., Early-phase transmission of *Yersinia pestis* by cat fleas (*Ctenocephalides felis*) and their potential rolse as vectors in a plague endemic region of Uganda, Am. J. Trop. Med. Hyg. (2008) 949–956.

[34] Eisen R.J., Holmes J.L., Schotthoefer A.M., Vetter S.M., Montenieri J.A., Gage K.L., Demonstration of early-phase transmission of *Yersinia pestis* by the mouse flea, Aetheca wagneri, and implications for the role of deer mice as enzootic reservoirs, J. Med. Entomol. (2008) in press.

[35] Eisen R.J., Petersen J.M., Higgins C.L., Wong D., Levy C.E., Mead P.S., et al., Persistence of *Yersinia pestis* in soil under natural conditions, Emerg. Infect. Dis. (2008) 14:941–943.

[36] Eisen R.J., Vetter S.M., Holmes J.L., Bearden S.W., Montenieri J.A., Gage K.L., Source of host blood affectes prevalence of infection and bacterial loads of *Yersinia pestis* in fleas, J. Med. Entomol. (2008) 45:933–938.

[37] Engelthaler D.M., Hinnebusch B.J., Rittner C.M., Gage K.L., Quantitative competitive PCR as a technique for exploring flea-*Yersina pestis* dynamics, Am. J. Trop. Med. Hyg. (2000) 62:552–560.

[38] Enscore R.E., Biggerstaff B.J., Brown T.L., Fulgham R.E., Reynolds P.J., Engelthaler D.M., et al., Modeling relationships between climate and the frequency of human plague cases in the southwestern United States, 1960-1997, Am. J. Trop. Med. Hyg. (2002) 66:186–196.

[39] Eskey C.R., Haas V.H., Plague in the western part of the United States, Bull. Publ. Health Soc. (Kuala Lumpur) (1940) 254:1–83.

[40] Fine P.E.M., Epidemiological principles of vector-mediated transmission, In: McKelvey J.J., Eldridge B.F., Maramorosch K. (Eds.), Vectors of disease agents: Interactions with plants, animals, and man, Praeger Publishers, New York, 1981.

[41] Gage K.L., Ostfeld R.S., Olson J.G., Nonviral vector-borne zoonoses associated with mammals in the United States, J. Mammal. (1995) 76:695–715.

[42] Gage K.L., Kosoy M.Y., Natural history of plague: Perspectives from more than a century of research, Annu. Rev. Entomol. (2005) 50:505–528.

[43] Garrett-Jones C., Schidrawi G.R., Malaria vectorial capacity of a population of *Anopheles gambiae*: An

exercise in epidemiological entomology, Bull. World Health Organ. (1969) 40:531–545.

[44] Golov D.A., Ioff A.G., On the question of the role of rodent fleas in the southeastern part of the USSR and the epidemiology of plague, Proceedings of the first All-Russian Anti-Plague Conference Saratov (1928) 102–105.

[45] Gratz N., Rodent reservoirs and flea vectors of natural foci of plague, In: Plague manual: epidemiology, distribution, surveillance and control, World Health Organization, Geneva, 1999.

[46] Guinet F., Ave P., Jones L., Huerre M., Carniel E., Defective innate cell response and lymph node infiltration specify *Yersinia pestis* infection, PLoS ONE (2008) 3:e1688.

[47] Hinnebusch B.J., Perry R.D., Schwan T.G., Role of the *Yersinia pestis* hemin storage (hms) locus in the transmission of plague by fleas, Science (1996) 273:367–370.

[48] Hinnebusch B.J., Bubonic plague: a molecular genetic case history of the emergence of an infectious disease, J. Mol. Med. (1997) 75:645–652.

[49] Hinnebusch B.J., Rudolph A.E., Cherepanov P., Dixon J.E., Schwan T.G., Forsberg A., Role of *Yersinia murine* toxin in survival of *Yersinia pestis* in the midgut of the flea vector, Science (2002) 296:733–735.

[50] Hinnebusch B.J., The evolution of flea-borne transmission in *Yersinia pestis*, Curr. Issues Mol. Biol. (2005) 7:197–212.

[51] Hirst L.F., The Conquest of Plague, Claredon Press, Oxford, 1953, 478 p.

[52] Holdenried R., Sylvatic plague studies: VII. Plague transmission potentials for the fleas *Diamanus montanus* and *Polygnis gwyni* compared with *Xenopsylla cheopis*, J. Infect. Dis. (1952) 90:131–140.

[53] Holdenried R., Morlan H.B., Plague-infected fleas from northern New Mexico wild rodents, J. Infect. Dis. (1955) 96:133–137.

[54] Hoogland J.L., Davis S., Benson-Amram S., Labruna D., Goossens B., Hoogland M.A., Pyraperm kills fleas and halts plague among Utah prairie dogs, Southwest. Nat. (2004) 49:376–383.

[55] Hubbard C.A., Fleas of Western North America: Their relation to public health, The Iowa State College Press, Ames, Iowa, 1947, 533 p.

[56] Karimi P.Y., Conservation naturelle de la peste dans le sol, Bull. Soc. Pathol. Exot. Filiales (1963) 56:1183–1186.

[57] Kartman L., Prince F.M., Studies on *Pasteurella pestis* in fleas. V. The experimental plague-vector efficiency of wild rodent fleas compared with *Xenopsylla cheopis*, together with observations on the influence of temperature, Am. J. Trop. Med. Hyg. (1956) 5:1058–1070.

[58] Kartman L., Quan S.F., McManus A.G., Studies on Pasteurella pestis in Fleas. IV. Experimental blocking of *Xenopsylla vexabilis hawaiiensis* and *Xenopsylla cheopis* with an avirulent strain, Exp. Parasitol. (1956) 5:435–440.

[59] Kartman L., Prince F.M., Quan S.F., Studies on Pasteurella pestis in fleas. VII. The plague-vector efficiency of *Hystrichopsylla linsdalei* compared with *Xenopsylla cheopis* under experimental conditions, Am. J. Trop. Med. Hyg. (1958) 7:317–322.

[60] Kartman L., Prince F.M., Quan S.F., Stark H.E., New knowledge on the ecology of sylvatic plague, Ann. NY Acad. Sci. (1958) 70:668–711.

[61] Kartman L., Quan S.F., Lechleitner R.R., Die-off of a Gunnison's prairie dog colony in central Colorado II. Retrospective determination of plague infection in flea vectors, rodents and man, Zoonoses Res. (1962) 1:201–224.

[62] Keeling M.J., Gilligan C.A., Bubonic plague: a metapopulation model of a zoonosis, Proc. Biol. Sci. (2000) 267:2219–2230.

[63] Keeling M.J., Gilligan C.A., Metapopulation dynamics of bubonic plague, Nature (2000) 407:903–906.

[64] Krasnov B.R., Shenbrot G.I., Mouillot D., Khokhlova I.S., Poulin R., Ecological characteristics of flea species relate to their suitability as plague vectors, Oecologia (2006) 149:474–481.

[65] Lahteenmaki K., Kukkonen M., Jaatinen S., Suomalainen M., Soranummi H., Virkola R., et al., *Yersinia pestis* Pla has multiple virulence-associated functions, Adv. Exp. Med. Biol. (2003) 529:141–145.

[66] Lang J.D., Rodent-flea-plague relationships at the higher elevations of San Diego County, California, J. Vector Ecol. (2004) 29:236–247.

[67] Laudisoit A., Leirs H., Makundi R.H., Van Dongen S., Davis S., Neerinckx S., et al., Plague and the human flea, Tanzania, Emerg. Infect. Dis. (2007) 13:687–693.

[68] Lechleitner R.R., Kartman L., Goldenberg M.I., Hudson B.W., An epizootic of plague in Gunnison's prairie dogs (*Cynomys gunnisoni*) in south-central Colorado, Ecology (1968) 49:734–743.

[69] Levy C.E., Gage K.L., Plague in the United States, 1995–1997, Infect. Med. (1999) 16:54–64.

[70] Lorange E.A., Race B.L., Sebbane F., Hinnebusch B.J., Poor vector competence of fleas and the evolution of hypervirulence in *Yersinia pestis*, J. Infect. Dis. (2005) 191:1907–1912.

[71] Macdonald G., Epidemiologic models in studies of vector-borne diseases, Public Health Rep. (1961) 76:753–764.

[72] Maevskii M.P., Bazanova L.P., Popkov A.F., The survival of the causative agent of plaque in the long-tailed suslik from a Tuva natural focus in wintertime, Med. Parazitol. (Mosk.) (1999) 55–58 (in Russian).

[73] McCoy G.W., A note on squirrel fleas as plague carriers, Public Health Rep. (1910) 25:465.

[74] Meyer K.F., The modern outlook on plague in California, Bureau Vector Control, State Dept Public Health Calif. Vector Views (1955) 2:41–43.

[75] Mollaret H., Conservation du bacille de la peste durant 28 mois en terrier artificiel: demonstration experimentale de la conservation interepizootique de las peste dans ses foyers inveteres, C. R. Acad. Sci. Paris (1968) 267:972–973.

[76] Mollaret H., The causes of plague inveteration in its natural foci, Bull. Soc. Pathol. Exot. Filiales (1971) 64:713–717 (in French).

[77] Mollaret H.H., Experimental Preservation of Plague in Soil, Bull. Soc. Pathol. Exot. Filiales (1963) 56:1168–1182 (in French).

[78] Mollaret H.H., Remarks on the report of Messieurs Brygoo and Dodin apropos of telluric plague and of burrowing plague. Madagascan data, Bull. Soc. Pathol. Exot. Filiales (1965) 58:140–154 (in French).

[79] Mollaret H.H., New knowledge in the field of plague epidemiology, Med. Monatsschr. (1969) 23:338–344 (in German).

[80] Nelson B.C., Smith C.R., Ecological effects of a plague epizootic on the activities of rodents inhabiting caves a Lava Beds National Monument, California, J. Med. Entomol. (1976) 13:51–61.

[81] Nikul'shin S.V., Onatskaia T.G., Lukanina L.M., Bondarenko A.I., Associations of the soil amoeba Hartmannella rhysodes with the bacterial causative agents of plague and pseudotuberculosis in an experiment, Zh. Mikrobiol. Epidemiol. Immunobiol. (1992) 2–5 (in Russian).

[82] Oyston P.C., Isherwood K.E., The many and varied niches occupied by *Yersinia pestis* as an arthropod-vectored zoonotic pathogen, Antonie van Leeuwenhoek (2005) 87:171–177.

[83] Parkhill J., Wren B.W., Thomson N.R., Titball R.W., Holden M.T., Prentice M.B., et al., Genome sequence of *Yersinia pestis*, the causative agent of plague, Nature (2001) 413:523–527.

[84] Parmenter R.R., Yadav E.P., Parmenter C.A., Ettestad P., Gage K.L., Incidence of plague associated with increased winter-spring precipitation in New Mexico, Am. J. Trop. Med. Hyg. (1999) 61: 814–821.

[85] Perry R.D., Fetherston J.D., *Yersinia pestis* – etiologic agent of plague, Clin. Microbiol. Rev. (1997) 10:35–66.

[86] Poland J.D., Barnes A.M., Plague, In: Steele J.H. (Ed.), CRC Handbook Series in Zoonoses, Section A: Bacterial, rickettsial and mycotic diseases, Volume I, CRC Press Inc., Boca Raton, FL, 1979, pp. 515–559.

[87] Poland J.D., Quan T.J., Barnes A.M., Plague, In: Beran G.W. (Ed.), CRC Handbook Series in Zoonoses, Section A: Bacterial, Rickettsial, and Mycotic Diseases, CRC Press, Boca Raton, FL, 1994, pp. 93–112.

[88] Pollitzer R., Plague, World Health Organization Monograph Series No. 22, Geneva, Switzerland, 1954, 698 p.

[89] Pushkareva V.I., Experimental evaluation of interaction between *Yersinia pestis* and soil infusoria and possibility of prolonged preservation of bacteria in the protozoan oocysts, Zh. Mikrobiol. Epidemiol. Immunobiol. (2003) 40–44 (in Russian).

[90] Quan S.F., Burroughs A.L., Holdenried R., Meyer K.F., Studies on the prevention of experimental plague epizootics instituted among mice by infected fleas, Estratto dagli atti del VI Congresso Internazionale di Microbiologia (1953) 5:1–4.

[91] Quan S.F., Kartman L., Ecological studies of wild rodent plague in the San Franciso Bay Area of California VIII. Susceptibility of wild rodents to experimental plague infection, Zoonoses Res. (1962) 1:121–144.

[92] Salkeld D.J., Stapp P., No evidence of deer mouse involvement in plague (*Yersinia pestis*) epizootics of prairie dogs, Vector Borne Zoonotic Dis. (2008) 8:331–337.

[93] Sebbane F., Gardner D., Long D., Gowen B.B., Hinnebusch B.J., Kinetics of disease progression and host response in a rat model of bubonic plague, Am. J. Pathol. (2005) 166:1427–1439.

[94] Seery D.B., Biggins D.E., Montenieri J.A., Enscore R.E., Tanda D.T., Gage K.L., Treatment of black-tailed prairie dog burrows with deltamethrin to control fleas (Insecta: Siphonaptera) and plague, J. Med. Entomol. (2003) 40:718–722.

[95] Sharets A.S., Berendyev S.A., Krasnikova L.V., Tristan D.F., Effectiveness of the one shot marmot control, Trudy Sredneaziatskogo Protivochumnogo Instituta Monograph, Almaty, Kazakhstan, 1958, pp. 145–147.

[96] Snall T., O'Hara R.B., Ray C., Collinge S.K., Climate-driven spatial dynamics of plague among prairie dog colonies, Am. Nat. (2008) 171:238–248.

[97] Stapp P., Antolin M.F., Ball M., Patterns of extinction in prairie dog metapopulations: plague outbreaks follow El Nino events, Frontiers in Ecology and the Environment (2004) 2:235–240.

[98] Stapp P., Salkeld D.J., Eisen R.J., Pappert R., Young J., Carter L.G., et al., Exposure of small rodents to plague during epizootics in black-tailed prairie dogs, J. Wildl. Dis. (2008) 44:724–730.

[99] Stenseth N.C., Samia N.I., Viljugrein H., Kausrud K.L., Begon M., Davis S., et al., Plague dynamics are driven by climate variation, Proc. Natl. Acad. Sci. USA (2006) 103:13110–13115.

[100] Suchkov Iu G., Khudiakov I.V., Emel'ianenko E.N., Levi M.I., Pushkareva V.I., Suchko I., et al., The possibility of preserving the causative agent of plague in soil in resting (nonculturable) form, Zh. Mikrobiol. Epidemiol. Immunobiol. (1997) 42–46 (in Russian).

[101] Titball R.W., Hill J., Lawton D.G., Brown K.A., *Yersinia pestis* and plague, Biochem. Soc. Trans. (2003) 31:104–107.

[102] Verjbitski D.T., The part played by insects in the epidemiology of plague, J. Hyg. (1908) 8:162–208.

[103] Webb C.T., Brooks C.P., Gage K.L., Antolin M.F., Classic flea-borne transmission does not drive plague epizootics in prairie dogs, Proc. Natl. Acad. Sci. USA (2006) 103:6236–6241.

[104] Welkos S.L., Friedlander A.M., Davis K.J., Studies on the role of plasminogen activator in systemic infection by virulent *Yersinia pestis* strain C092, Microb. Pathog. (1997) 23:211–223.

[105] Wheeler C.M., Douglas J.R., Sylvatic plague studies. V. The determination of vector efficiency, J. Infect. Dis. (1945) 77:1–12.

[106] Wilder A.P., Eisen R.J., Bearden S.W., Montenieri J.A., Gage K.L., Antolin M.F., *Oropsylla hirsuta* (Siphonaptera: Ceratophyllidae) can support plague epizootics in black-tailed prairie dogs (*Cynomys ludovicianus*) by early-phase transmission of *Yersinia pestis*, Vector Borne Zoonotic Dis. (2008) 8:359–367.

[107] Wilder A.P., Eisen R.J., Bearden S.W., Montenieri J.A., Tripp D.T., Brinkerhoff R.J., Gage K.L., Antolin M.F., Transmission efficiency of two flea species (*Oropsylla tuberculata cynomuris* and *Oropsylla hirsuta*) involved in plague epizootics among prairie dogs, EcoHealth (2008) 5:205–212.

[108] Wren B.W., The Yersiniae – a model genus to study the rapid evolution of bacterial pathogens, Nat. Rev. Microbiol. (2003) 1:55–64.