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Review Articles

A review of plague persistence with special emphasis on fleas

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Abstract

Sylvatic plague is highly prevalent during infrequent epizootics that ravage the landscape of western North America. During these periods, plague dissemination is very efficient. Epizootics end when rodent and flea populations are decimated and vectored transmission declines. A second phase (enzootic plague) ensues when plague is difficult to detect from fleas, hosts or the environment, and presents less of a threat to public health.

Recently, researchers have hypothesized that the bacterium (*Yersinia pestis*) responsible for plague maintains a continuous state of high virulence and thus only changes in transmission efficiency explain the shift between alternating enzootic and epizootic phases. However, if virulent transmission becomes too inefficient, strong selection might favor an alternate survival strategy. Another plausible non-exclusive hypothesis, best supported from Asian field studies, is that *Y. pestis* persists (locally) at foci by maintaining a more benign relationship within adapted rodents during the long expanses of time between outbreaks. From this vantage, it can revert to the epizootic (transmission efficient) form. Similarly, in the United States (US), enzootic plague persistence has been proposed to develop sequestered within New World rodent carriers. However, the absence of clear support for rodent carriers in North America has encouraged a broader search for alternative explanations. A telluric plague existence has been proposed. However, the availability of flea life stages and their hosts could critically supplement environmental plague sources, or fleas might directly represent a low-level plague reservoir.

Here, we note a potentially pivotal role for fleas. These epizootic plague vectors should be closely studied with newer more exacting methods to determine their potential to serve as participants in or accomplices to a plague persistence reservoir.

Key words Carrier – *Cynomys* – enzootic – epizootic – host – larvae – *Oropsylla* – plague flea – pupae – telluric – vector – *Yersinia pestis*

Introduction

Bubonic plague is caused by *Yersinia pestis*, a gram-negative coccobacillar bacterium that evolved within the last 1500–20,000 years by genetic divergence from the more ubiquitous rodent enteric pathogen *Yersinia pseudotuberculosis*[1]. Yersin was the first to characterize this member of the Enterobacteriaecae[2] during the third plague pandemic in the late 19th century while it ravaged the thriving port of Hong Kong. Flea infested peridomestic rodents emanating from that pandemic unleashed a strain of surprisingly homogeneous (clonal), highly virulent *Y. pestis orientalis*[3] into North America around 1900[4], and into South America about the same time. Since its arrival, sylvatic plague has readily adapted to and pro-

foundly influenced wild American rodent populations[5,6]. It has gradually migrated eastward over time, into the mid-western United States (US, ~102° west longitude)[4,7].

The American emergence of plague over 100 years ago reflects its versatility in adapting to a new set of vectors and hosts. We note some apparent limitations of popular hypotheses to explain plague persistence and revisit erstwhile explanations for plague persistence that deserve reconsideration. In particular, the potentially pivotal role of the flea as a vector and host-supported enzootic reservoir could help explain the persistence of sylvatic plague between infrequent epizootics.

In the classic description of sylvatic plague, some mammalian species, especially rodents, are believed to act as primary hosts, and to serve as persistent disease carriers[8,9]. Secondary hosts were described in the Old World and represent ancillary rodent sources of plague ultimately dependent on the primary host[10]. A third group consists of susceptible species that act as incidental bystanders. These include humans and selected carnivores (e.g. canids and felids). This latter group does not directly support long-term disease persistence (dead end hosts), but becomes infected principally during epizootics and may contribute to the environmental burden of infective organisms.

American rodents susceptible to epizootic plague include prairie dogs (*Cynomys*)[7,11–14], wood rats (*Neotoma*)[15], and some ground squirrels (*Spermophilus*)[16–18]. Moderately susceptible rodents include voles, many mice, other ground squirrel species, and rabbits (Lagomorpha). Relatively resistant species include kangaroo rats (*Dipodomys*), certain populations of mice, and selected carnivore species[19,20].

We derive an operational definition of sylvatic plague phrases more suited to describing its natural history as observed in the western US. There is considerable ambiguity from extensive use of these terms in the literature and because western plague might behave differently from its longer adapted Asian progenitor. An epizootic is the classic manifestation of plague where there is a progressive but rapid demise of large numbers of susceptible hosts and bystander species in large areas. This disease phenotype is often readily identified based on precipitous local declines in susceptible animal populations. Epizootic plague is relatively easily detected with standard diagnostic methods. A plague reservoir is a source of plague that nutritionally sustains *Y. pestis* to survive for extended periods without destroying its own food source. Such a reservoir in this context is not just a means of transport or source of infection (e.g. vector, environmental contaminant, etc.). Any organism can exist in a dormant state ("dormant plague") for some period without reproduction or significant growth, but this is an interim situation created by circumstances that do not further its long-term survival, reproduction or progressive dissemination. Similarly, overwintering is a typical dormant plague state, since infected rodents that arise in the spring become septic and die[21,22]. Enzootic plague as used here continues to rely on interactions with hosts and fleas, and refers to plague persistence (detected at the same location over time) that might be accompanied by low-level flea or host disease. However, sustainable populations of hosts and fleas are likely outcomes of this definition, when key nutrients, the organism and the flea need for growth and reproduction (dietary enrichment) are provided by successive hosts. Improved definitions should emerge when the mechanisms for the enduring success of plague are better understood.

The spread of epizootic plague

Because epizootic plague is the phase of greatest concern to human health, the easiest to detect, and the most visible part of the plague cycle, it is also the best studied. Precipitous host declines, plague detection from hosts and fleas (antibodies and culture), and evidence of flea blocking are signature events of epizootics[7,9,23]. Epizootics spread as *Y. pestis* is disseminated by blocked fleas as they regurgitate over-

whelming doses of organisms into the host which becomes septicemic and dies, forcing blocked starving fleas to seek the next available host. New fleas become blocked ("competent") from septicemic hosts, perpetuating the epizootic[24]. The block itself is a growing clot teeming with organisms that lodges primarily within the proventriculus obstructing upper gastrointestinal transit and intestinal alimentation, leading to regurgitation, starvation and repeated feeding attempts. Some flea species (e.g. rat fleas, *Xenopsylla cheopis*) block more readily than others. Even so, blocking appears to require critical numbers of bacteria. At sylvatic plague sites inhabited by prairie dogs, the dominant fleas (*Oropsylla* species) are inefficient blockers, and host blood concentrations of 10^{6-7}. *Y. pestis* organisms per milliliter (50–500 organisms in 50 μl) are required for progression of blocking. This appears to constrain block formation to bites from septic (moribund) hosts within 42 h of death[25–27]. Although blocked fleas do not always transmit *Y. pestis* from bites[26,28], competent flea transmission[29] at the population level effectively amplifies epizootics[28,30–32] most likely in a (vector, host) density-dependent manner.

The importance of understanding the enzootic phase

Diatlov[33] has recounted the failure of decades-long Soviet plague eradication initiatives based on host and flea elimination. Of all the strategies, only placing land into agricultural production stopped plague recrudescence from established foci. While host and flea control methods blunt or even prevent epizootics in the short-term[34], these methods are not practical to remove plague from the large expanses of remote land where it resides in the western US. In the face of threats of bioterrorism, a more virulent bioengineered variant of the plague bacillus could be introduced into natural foci. Thus, it is critical to know if vectored virulence is the primary overriding driver, possibly facilitating the spread of new genetic subvariants. In contrast, vectored virulence driver might be lost if it confers reduced fitness during the enzootic plague phase[35]. From an ecological standpoint, if low-level mortality is an attribute of enzootic plague, keystone species (e.g. *Cynomys*) could be impacted[36], distorting normal trophic relationships even without epizootics.

Quantitative multi-locus analyses support the existence of two *Y. pestis* identities corresponding to the two dominant phases. These consist of a rapidly dispersed epizootic form, and a more resilient, but genetically diverse, persistence form[37]. *Yersinia pestis* mutability is also well-recognized[38,39]. Strains with varied virulences are documented from native Asian sources[40] suggesting that resident forms with alternate transmission strategies might exist at these foci. In the western US, even starting with a limited repertoire of strains, given the short generation times exhibited by most microbes, a century of evolution acting on *Y. pestis* provides ample time to resurrect or introduce new capabilities, especially in a complex natural environment[41]. In fact, a recent study comparing *Y. pestis* and *E. coli* found that they mutated and introduced diversity at similar rates[42]. The process of incorporating of favorable mutations under selection has even been used to hypothesize reduced virulence and host adaptation[43] suggesting likely *Y. pestis* population heterogeneity. Previously, American studies have emphasized research on destructive epizootics, and may in part reflect the practical limitations imposed when collecting quality enzootic field isolates. Sometimes the distinction between enzootic and epizootic plague is difficult to make. For example, Webb *et al*[44], studying epizootic plague, reviewed literature and field data and concluded that a temporal reservoir is required to model this disease. Based on their description, they modeled recoveries from epizootics (epizootic behaviour).

Explanations for the almost coincidental appearance of plague epizootics over large areas seems to implicate simultaneous eruptions from multiple sources, perhaps facilitated by favourable weather. On a smaller scale, spread from initial foci might involve the movement of plague-infected fleas by "transporter species" such as carnivores or ungulates[45], or by sequential host ferrying. For example, transfer of

Y. pestis up to 120 m was shown from labeled flea studies[46] while comparable rodent movements covered only half this distance, irrespective of habitat. In the western US, plague resistant carnivores such as coyotes (*Canis latrans*) frequently seroconvert to plague[47–54] indicating exposure, making them excellent plague sentinels. Likewise, the swift fox (*Vulpes velox*) was demonstrated to transit infected fleas and possessed high *Y. pestis* seroconversion rates[55–57]. However, how long fleas survive on foraging carnivores, the resistance of these predators to disease, their contact rates with prey, their territoriality, and flea densities might be considered factors in evaluating their contribution to plague spread and persistence.

In the summer of 2005, simultaneous outbreaks of epizootic plague occurred in several western states (e.g. Utah, Colorado and Montana), where outbreaks were separated by hundreds of kilometers and encompassed a geographical area extending from Montana to Texas (unpublished data). The timing and the distances between sites made flea transfer between sites unlikely while long distance and short time separations precluded movement of *Y. pestis* by even the most mobile of native vertebrates. Large-scale models have been used to explain epizootic cycles of plague within a region and trophic relationships are a possible explanation[58,59]. However, these landscape models[60,61] provide little direct insight into local events, how epizootics start, where the organism originates, and how it survives. The presumed density-dependency of epizootics on participants (e.g. rodents and fleas) suggests that *Y. pestis* might benefit from trophic cascades mediated by weather cycles.

Is plague always directing virulence toward hosts and fleas?

Ewald[62] has argued that pathogen virulence optimizes to assure efficient transmission. Therefore, efficient vectors require less host-virulent plague[63,64], whereas inefficient vectors further increasing host virulence[62] to infect yet more fleas. Lorange *et al*[32] argued that inefficient plague transmission (e.g. including blocking rates) would be sufficient to favor the *Y. pestis* virulence toward the host, while virulence against the flea vector would be counterproductive unless virulence depends on blockage to make plague transmission efficient. Presumably, such blockage is a sufficient trade-off to warrant flea mortality (i.e. a virulence maintenance hypothesis). Applied to western plague foci, the limited number of flea species shown to block and low rate of blocking in such species might favor increased virulence. Bacot[24] observed that partial blockage led to more efficient transmission and prolonged flea survival. This latter suggestion makes the flea a potential reservoir, not just as a vector. Eisen *et al*[65] demonstrated in the laboratory that *Oropsylla* efficiently transfers *Y. pestis* mechanically, without flea blockage, to hosts. However, their model system still requires access to septic hosts[66].

One consequence of a penurious ever-virulent-rolling-plague model is that *Y. pestis* exists largely at the mercy of events that determine flea and host population numbers, such that epizootics would directly couple to population dynamics. If alternatively, there is some phase or form change where the organism can persist in fleas or the environment, this would provide an alternative to continued high host and flea mortality between epizootic outbreaks, allowing flea and host populations to recover. The resulting dynamics would be less driven by host and flea population increases that permit epizootics.

The ability of *Y. pestis* to adhere within the flea's gastrointestinal tract has been used to explain its relatively recent divergence from *Y. pseudotuberculosis*[67]. While *Y. pestis* is tolerated by fleas, *Y. pseudotuberculosis* causes fatal diarrhoea in them[68]. One key to this adhesion appears to be the production of *Ymt*, a flea intestinal adhesion factor (i.e. phospholipase D—common in function to other bacteria)[28]. For example, *Ymt* inserted *Y. pseudotuberculosis* resided within *X. cheopis* up to 1 month longer[69]. Similarly, the hemin storage gene is responsible for proventricular adhesion. If mutated, it al-

lows (*hms*$^-$) *Y. pestis* to reside for extended periods in the flea midgut[70]. As a strategy of the organism, repressing the hms$^+$ phenotype might allow *Y. pestis* retention within the flea as a commensal where nutrition is available and host virulence could be retained in the absence of flea mortality. Temperature responsive genes[71,72] can be unleashed by the organism, and in turn, toxin is released within the flea, converting it to a vector. Plague mediated changes include biofilm elaboration, mucosal spine stickiness, and proventricular blockage[25,73,74].

The number of plague organisms increase within fleas in response to feeding, and numbers of *Y. pestis* temporarily decrease during digestion, then increase again[75]. Retention of bacteria was largest in the proventricular area, although mid-gut organisms were noted, as was *Y. pestis* excretion[76]. Vectored transmission might not assure persistence of plague by itself, especially during the enzootic phase. For example, *Salmonella* was effectively transmitted by fleas[77], but this does not suggest *Salmonella* exhibits a higher fitness from vectored over enteric transmission.

Four major hypotheses of plague persistence

Gage and Kosoy[3] have described four hypotheses to explain the continued existence of sylvatic plague. None of these hypotheses can be viewed as mutually exclusive; one or more might be occurring at one or multiple sites simultaneously or in succession. One hypothesis suggests *Y. pestis* exerts its influence largely as a continuous propagation event of varying velocity (enzootic periods punctuated by irregular brief epizootic periods), dependent on a continuous supply of naive hosts and vector fleas. This hypothesis is largely congruent with the virulence maintenance hypothesis mentioned earlier.

The carrier host hypothesis: The second hypothesis suggests epizootics die out and become separated by long periods when the plague organism resides sequestered within carrier hosts. This carrier state has been attributed to at least one rodent species at each plague focus. Moderate host susceptibility coupled with field identification of host seroconversion in the wake of outbreaks led to the suspected role of carrier rodents as a key plague reservoir. Carrier hosts would exhibit a propensity to harbor the organism in a sequestered location within their bodies[7,78,79], and later become septicemic in response to some stressor; their fleas, imbibing the organism under septic conditions, would initiate a new epizootic. If rodent carriers reside at American plague foci, their existence has yet to be established[80]. However, at least one Asian species (the great gerbil, *Rhombomys opimus*) from plague endemic areas has attributes that are reputed to allow it to be a carrier as defined earlier[81].

The telluric hypothesis: Another of Gage and Kosoy's hypotheses was recently revisited by French researchers and emphasized the capability of *Y. pestis* to survive for extended periods in the burrow soil or substrate during the largely occult inter-epizootic period[82,83]. Indeed, Yersin[2] reports in his seminal work characterizing the etiology of plague his ability to culture the organism from deep within contaminated soil. Mollaret[82] and Drancourt *et al*[83] emphasized the importance of distinguishing fortuitous soil contamination and short-term survival from long-term plague persistence, in which the organism reproduces and completes its life cycle. A recent study[84] involving epizootic plague inoculation of sterile soil showed the potential durability of the organism in soil substrate. A long duration of survival as a soil contaminant could indirectly support the virulence maintenance. In culture, the growth optimum for *Y. pestis* is 28ºC, close to ambient temperatures of many environments[85]. Studies of susceptible prairie dogs from plague foci reveal little evidence of seroconversion in the period between epizootics[86]. Susceptible animals succumb to plague at relatively low doses. However, if their carcasses remain underground, relatively large amounts of organism could contaminate the environment. Because proliferation of *Y. pestis* in the carcass is all but completed by death, this retention of plague is better described as dormant (contaminant) plague, than as a reservoir form. Mechanistic details underlying *Y. pestis* reten-

tion (if it exists), and its means for re-entry into the active plague cycle remain speculative at best.

The flea reservoir hypothesis: The remaining hypothesis of Gage and Kosoy will be the focus of the rest of this review. We consider adult and larval fleas as potential reservoirs, and we discuss flea relationships that could support the telluric hypothesis. If fleas invariably died from blocking, their vector role would seem to dominate any putative role as a reservoir. Lorange *et al*[32] revisited the blocking propensity of *Xenopsylla cheopis*, and reported a maximum rate of 38% in this model species while many other species block less readily. Bacot[24], one of the original discoverers of flea blocking, and others[25,75,87,88] have observed protracted periods where infected fleas did not block. Some fleas were capable of harboring the organism for up to 130 days[25]. Pollitzer[89] suspected avirulent *Y. pestis* strains were responsible. Indeed, high levels of host bacteremia alone do not assure flea blockage[25]. Other factors such as temperature[90], flea age[91] and flea gender[92] influenced blocking efficiency.

Traditional diagnostic results have not provided data supportive of fleas as a definitive reservoir. For example, fleas sampled inter-epizootically were largely negative[93], while plague is readily detected during epizootics[7,29,94–96]. However, a recent study has identified *Y. pestis* in fleas at relatively high frequencies from potential enzootic plague foci using a more sensitive polymerase chain reaction (PCR) technique[97]. While other methods should be applied to assure the specificity of this finding, limited sensitivity of past assays could have profoundly biased previous results. For example, there is little data on the ability of fleas to harbor small numbers of *Y. pestis* below the detection limits of traditional diagnostic techniques (i.e. at low copy numbers).

A final possibility is that the adult flea directly or indirectly through larval provisioning provides another enduring reservoir for *Y. pestis*. If as Lorange *et al*[32] found, *Xenopsylla cheopis* has a median survival post-infection of 14 days before blocking but the host only survives for two days, fleas might be better reservoirs than their mammalian hosts. Thus, even in the penurious virulence maintenance transmission model mentioned above, the flea might serve in a reservoir capacity to some degree to keep epizootics rolling forward. For fleas to serve as a reservoir during the enzootic phase, the lack of seroconversion in susceptible hosts has to be explained while these fleas continue to feed on them. First, bacteria above the intestine might be few in number or rarely regurgitated. One might envision the organism behaving as a typical enteric microbe of the mid-gut or proventriculus of the flea under these circumstances, where it is maintained in low numbers as it competes with other resident flora for nutrients and attachment sites. In this case, living in the proventriculus at low levels could reduce competition, and could help explain why *Y. pestis* prefers to colonize this site. Second, the adult flea might not reliably harbor the organism for extended periods, but when feeding might support the alimentation of *Y. pestis* in the host burrow substrate (telluric) or as it settles in the intestinal tract of flea larvae feeding on adult excreta and the contaminated substrate. In either case, the reasons for low host seroconversion rates would be similar to those proposed for telluric plague.

Adult fleas as candidate plague carriers

The flea genus *Oropsylla*[7,78,98] is commonly associated with American rodents (especially *Cynomys*) involved in plague outbreaks and especially in human plague infections[99]. Kartman *et al*[100] observed that *Oropsylla* harbored *Y. pestis* for extended periods, based on the observation that plague was isolated one year after an epizootic decimated the host population. However, there was no definitive evidence that epizootic transmission of *Y. pestis* was no longer occurring. Several researchers working in Asia have provided similar accounts of prolonged infected flea longevity[3]. Fleas of the genera *Catallagia*, *Echidnophaga*, *Hystrichopsylla*, *Malareus* and *Thrassis* (now *Oropsylla*) found in the western US appear to block[25,26,78,89,101–104] but a putative role for them as a plague reservoir has not

been studied using refined techniques, nor has their response to plague been as carefully investigated as the case for rat fleas[26,28,105–107].

If fleas provision plague without vectoring, then the size of flea populations and increased host transfer rates could impact plague prevalence, thus, degree of flea (and indirectly host) density dependence would likely be observed, as for the virulence maintenance model. For example, if plague carrying fleas decreased in number, provisioning of flea nests would diminish the number of *Y. pestis* shed and available and harbored in the nest substrate. In time, a disruption in the flea life cycle could allow plague to die out until a new colonization event occurred. Flea host shifts have been observed in response to plague epizootics[102], but loss of host fidelity might adversely impart burrow dependent plague survival. Similarly, host-to-host compatibility might constrain social contacts that could dampen plague transfer[108]. The energetic costs of feeding on the wrong host might influence host fidelity[109]. Host deaths or increased flea fecundity create local population booms that could necessitate a loss of host and nest fidelity and cause increased larval mortality[110].

Flea life stages as candidates for plague persistence

Russian investigators have hypothesized that fleas imbibing from the mucous membranes of hibernating marmots provided the necessary conditions for the genesis of *Y. pestis*[111,112]. In their opinion, the organism evolved and survived in close association with fleas. Reminiscent of this coexistence, the longevity of both *Y. pestis* infected fleas estimated from laboratory studies[26,113–119] suggest that with sufficient high quality food provisioning, fleas might live to 220[120], 396, or 411[121] days (*Ctenophthalmus breviatus*, *Citellophilus tesquorum*, *Neopsylla setosa* respectively). Unfortunately, little field longevity data exist[21], and even less is known about the longevity of infected or uninfected fleas from the western US.

Once feeding[122] and reproduction begins[123–125], adult fleas require frequent meals[126,127] to avoid desiccation, malnutrition and death[128]. Adult fleas liberally pass partially digested host blood to their brood larvae[129,130]. Dependence of early life stages on host nests is typical of fleas that spread plague[127]. Compared to ticks, fleas produce considerably fewer eggs at a time[130–133], possibly suggesting that in fleas, parental provisioning may significantly improve larvae survival to adulthood. As might be expected, young adults produce more robust offspring[125] and lay more eggs[134] than older fleas. Larvae grow rapidly, going through several instars before pupating. From the resting imago (pupal) stage, adult emergence is triggered by host cues, assuring food is nearby[129,131,135]. The intensity of host effects on flea reproduction seems to vary with the host and their differential tolerance of flea densities[136]. Finally, the rapacious larvae and adult fleas each are suspected to compete with others in their cohort[137] and this in turn likely impacts adult survival[123].

Several investigators[104,138–140] have shown that adult fleas excrete *Y. pestis*, and that even parenterally infected fleas harbor the organism for extended periods without apparent ill-effects[141, 142]. Flea larvae are indiscriminate consumers that supplement the blood they imbibe from adult excreta. For example, cat fleas grow more rapidly on mixed diets than on host blood alone[143]. Larval fleas of various species may indiscriminately consume plague from the nest environment[87,113,131], dead animal carcasses, injured adult fleas and other arthropods[87,144]. Dead fleas and burrow substrate sustained *Y. pestis* (typically L-forms) for up to 427 days[145], making consumption of *Y. pestis* by flea larvae likely.

Certain growth factors are auxotrophic to *Y. pestis* when added to its growth medium (e.g. thiamin, pantothenic acid[79,85,146,147]); and many such nutrients can be derived from host blood[147,148]. Thus, even if *Y. pestis* is genetically depauperate[149] and behaves as an obligate host parasite, as some have suggested[79,147], the dependence of larvae on adult flea sanguineous excreta could significantly support *Y. pestis* in the environment. Finally, a flea and host

recapture study performed under semi-natural conditions suggested strong flea to host fidelity[150]. Flea to host preferences in part reflect flea nutritional requirements[113,151] and host blood digestibility[152].

The needs of immature fleas and abiotic influences

The role of early flea life stages in plague persistence has not beeninvestigated for sometime, systematic study is required especially in sylvatic fleas. This hypothetical persistence strategy is appealing because pupating larvae could passively harbor small numbers of *Y. pestis* that are then retained within environmentally durable pupae, until adult forms emerge. Molyneux[87] and Bacot[153] observed that larval rat fleas (*X. cheopis*, *N. fasciatus*) passed *Y. pestis* by defecation, and using culture methods on triturated fleas, found that plague was undetectable after two days. If the hosts used as a blood source to rear the larvae possessed antibodies against *Y. pestis*, this could conceivably contribute to the rapid clearances observed. In addition, these older studies relied principally on culture media without antibiotics to control the growth of contaminants, and the methods employed could have lacked sufficient sensitivity to detect low-grade infections. Larval plague isolates, like substrate-adapted forms[121], might be challenging to culture[154, 155]. Similarly, they might be challenging to amplify if PCR reaction inhibitors are retained in the sample matrix. At present, definitive identification of the plague organism still relies heavily on orthogonal testing employing some combination of bioassay, PCR, culture, and host challenge, supplemented with biochemical testing, direct immunofluorescence, bacteriophage typing or host serological screening[156]. However, applying these rigorous criteria for all potential isolates, and particularly for low-level, contaminated, or fastidious (atypical) plague samples has become a major challenge limiting the detection of novel forms. Capturing these novel isolates if they exist may require the development of more specialized high throughput PCR methods[97], unique sequence targeting, the collection of inaccessible fresh field samples, or the development of new culture enrichment techniques to produce sufficient quantities for further assessment.

In general, plague foci in the western US exist in xeric semi-desert environments, and plague recrudesces in epizootic form in response to moderately dry seasons and temperatures[157–159], but with sufficient moisture to sustain hosts, fleas and the plague bacillus below ground. On the other hand, excessive moisture[160] adversely affects fleas. When coupled with a high organic load, excessively wet conditions nurture molds (and possibly other flea pathogens[161]) that diminish larval survival in rodent nests[162], a matter requiring some vigilance during captive flea colony management[126]. Numbers of predaceous mesostigmatid mites were inversely related to flea numbers[161]. Not surprisingly, since *Y. pestis* does not form spores, it favors life underground[163] over inhabiting surface soils[164]. A recent microbiological survey recovered a very low prevalence of *Yersinia* species from surface soils[165], yet burrow contamination and retention appears a likely source[166].

Flea abundance relies on the effect of both abiotic (e.g. moisture, seasonality, darkness, environmental stability etc.) and biotic (e.g. organic load) inputs[167]. Flea larvae are highly susceptible to desiccation[168], acquiring water principally from adult excreta, but also reduce detrimental moisture losses and temperature swings by living deep underground[169,170]. Fine sandy nesting substrates have the potential to abrade the waxy epicuticle of flea larvae, increasing water loss and larval mortality[123]. Soil composition likely represents a series of trade-offs depending on the flea species in question. For example, desert-adapted fleas fared better in sand substrate than in loess-like sediments[171]. Cocooned flea stages are more resistant to desiccation[124]; cocooned imagoes are viable for many months, and more resistant to freezing, under conditions where larvae are killed[131].

Conclusions

While transmission efficiency and differential flea

blocking might help to explain shifts from enzootic to epizootic plague, we hypothesize that fleas potentially offer some important advantages for provisioning and preserving a plague reservoir that extends beyond their well-studied, and established role as plague vectors. Fleas in this context would enhance plague survival during enzootic periods by non-vectored and vectored provisioning, while also serving as key transmission amplifying vectors during epizootics. Fleas living on animals, within rodent burrows or while completing their growth stages, might significantly contribute to plague persistence.

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