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NATURAL HISTORY OF PLAGUE: Perspectives from More than a Century of Research*

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■ **Abstract** For more than a century, scientists have investigated the natural history of plague, a highly fatal disease caused by infection with the gram-negative bacterium *Yersinia pestis*. Among their most important discoveries were the zoonotic nature of the disease and that plague exists in natural cycles involving transmission between rodent hosts and flea vectors. Other significant findings include those on the evolution of *Y. pestis*; geographic variation among plague strains; the dynamics and maintenance of transmission cycles; mechanisms by which fleas transmit *Y. pestis*; resistance and susceptibility among plague hosts; the structure and typology of natural foci; and how landscape features influence the focality, maintenance, and spread of the disease. The knowledge gained from these studies is essential for the development of effective prevention and control strategies.

INTRODUCTION

Plague is a rodent-associated, flea-borne zoonosis caused by the gram-negative bacterium *Yersinia pestis* (48, 108, 113). The disease is often fatal in humans, particularly when antimicrobial treatment is delayed or inadequate. Although treatable, plague still causes fear and even mass hysteria, as demonstrated during a 1994 pneumonic plague outbreak in India. Plague's notoriety comes largely from its role as the cause of three massive pandemics, including the Black Death, a mid-fourteenth century calamity that killed nearly one third of Europe's population and remains the standard by which the effects of AIDS, SARS, or other new diseases are measured.

The perception that plague is only of historical interest has changed somewhat because of media reports that suggest *Y. pestis* could be a weapon of bioterrorism (70). Often lost in these messages, however, is that since the last pandemic began in

the late 1800s, plague's geographic range has expanded greatly, posing new threats in previously unaffected regions, including the western United States, portions of South America, southern Africa, and Madagascar, and certain regions of India and Southeast Asia (34, 48, 113) (Figure 1). Epidemics still occur frequently in developing countries where plague is endemic and persons live in unsanitary, rat-infested environments. Between 1987 and 2001, outbreaks involving hundreds of cases occurred in at least 14 countries, usually as a result of exposures to infectious rat fleas (143). From 1994 to 2003, only 61 cases (7 fatal) in the United States were identified (Centers for Disease Control, unpublished data). These cases and others in recent decades were acquired through exposures to wild rodent flea bites or handling infected mammals, including rodents, rabbits, wild carnivores, and domestic cats (48–52, 93).

Wildlife biologists have increasingly realized that certain wild mammal species also are highly susceptible to plague, a fact that can hamper recovery efforts for species such as prairie dogs or black-footed ferrets (17, 18). The black-footed ferret (*Mustela nigripes*), a highly endangered predator, is placed in double jeopardy because it preys almost entirely on prairie dogs and is itself highly susceptible to plague.

This article provides a selective review of research on plague and its natural history since Yersin's initial discovery of the plague bacillus in 1894. Because of space limitations, many topics, including most medical, microbiological, and public health aspects of plague, are neglected. Fortunately, these have been addressed in other reviews (5, 6, 10, 20, 23, 26, 34–36, 48–51, 60, 70, 93, 108, 111–114, 135).

THE BASIC TRANSMISSION CYCLE

Y. pestis is maintained in nature through transmission between hematophagous adult fleas and certain rodent hosts, with occasional involvement of some lagomorphs (48, 115) (Figure 2). Evidence of *Y. pestis* infection also has been identified among the Artiodactyla, Carnivora, Hyracoidea, Insectivora, Marsupialia, and Primates, which suggests that virtually all mammals can become infected with this agent (48, 49, 115). Susceptibility among these nonrodent, nonlagomorph species varies widely, but all are considered incidental hosts of plague, except perhaps the house or musk shrew (*Suncus murinus*) in Southeast Asia and Madagascar. Birds, reptiles, and amphibians are generally thought to be resistant to *Y. pestis* infection. Mammals or birds that prey on plague hosts might play an indirect role in the spread of plague by moving infectious fleas between areas (52).

Typically, plague is thought to exist indefinitely in so-called enzootic (maintenance) cycles that cause little obvious host mortality and involve transmission between partially resistant rodents (enzootic or maintenance hosts) and their fleas (51, 111, 112) (Figure 2). Occasionally, the disease spreads from enzootic hosts to more highly susceptible animals, termed epizootic or amplifying hosts, often causing rapidly spreading die-offs (epizootics). Although these concepts seem reasonable, the evidence for separate enzootic and epizootic cycles is often unconvincing, and epizootics might simply represent periods of greatly increased

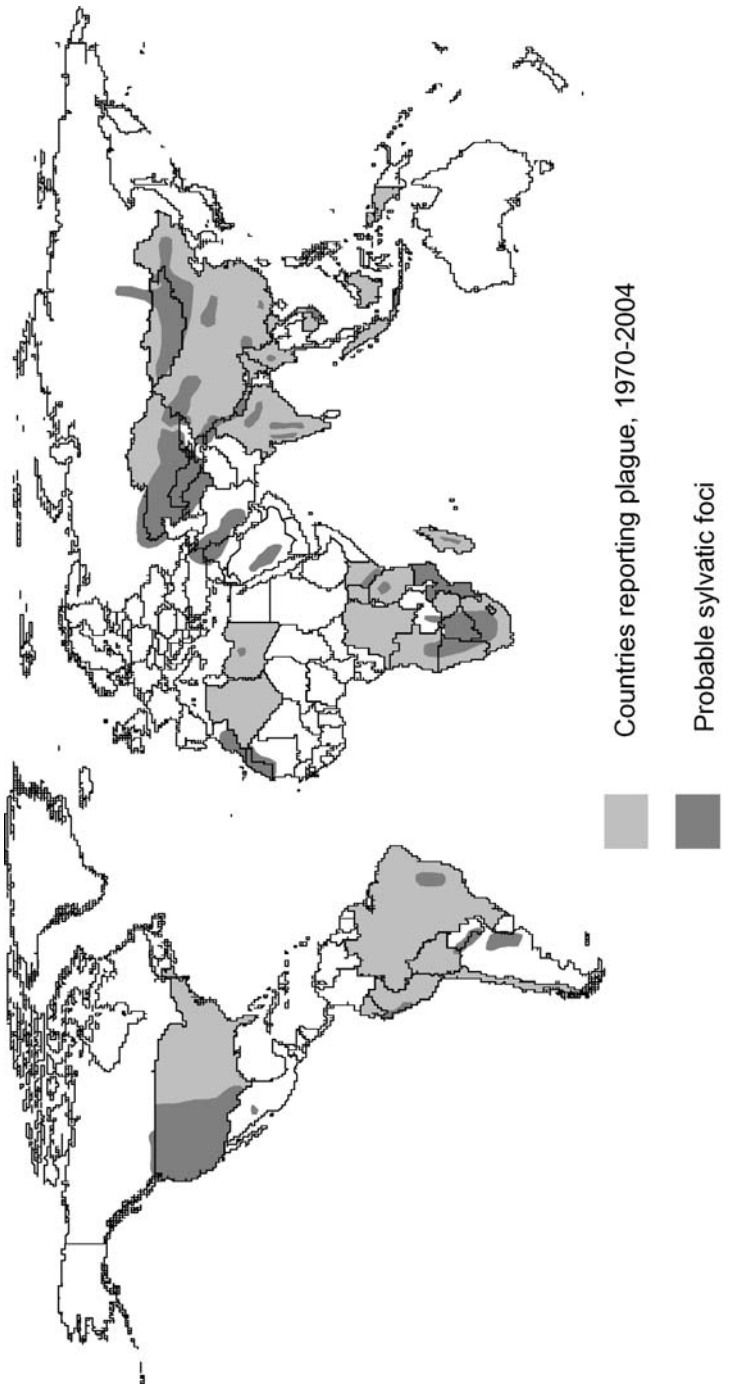

Figure 1 Distribution of plague foci and countries reporting plague.

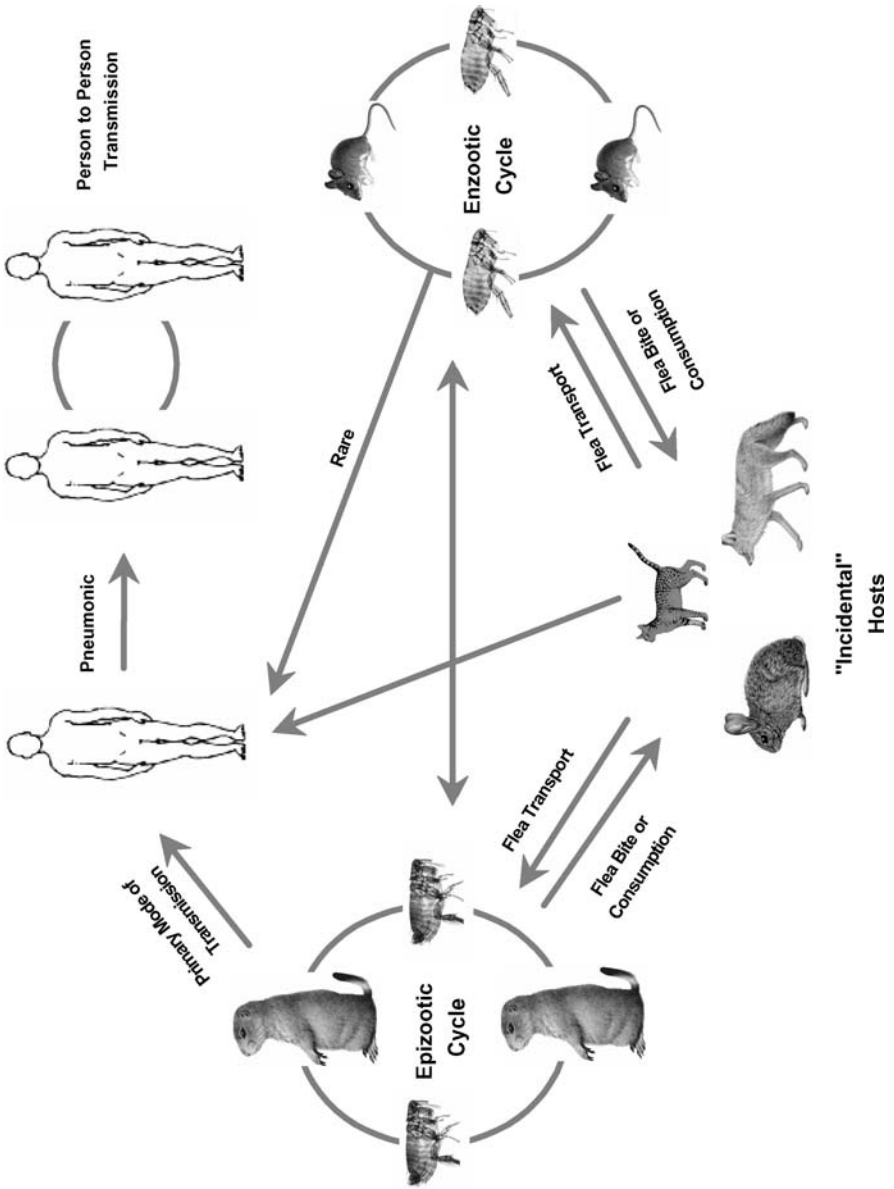

Figure 2 Natural cycles of plague.

transmission among the same hosts and fleas that support *Y. pestis* infection during interepizootic periods.

Understanding the factors that lead to epizootics is important because it is during these events that the disease spreads rapidly. Humans and other highly susceptible mammals also experience their greatest exposure risks during epizootics. Recent modeling studies have suggested that the abundance of susceptible hosts must exceed certain limits for plague to invade and persist in new areas (32a). Climatic factors appear to be important in many but perhaps not all areas (24, 25, 41, 44, 105, 113, 129). Recently, a trophic cascade hypothesis was proposed whereby increased precipitation results in greater plant growth and rodent food production, leading to increased host populations and a greater likelihood of epizootics and human cases (105). Others developed models that incorporated temperature and precipitation effects and noted that increased precipitation likely leads to increased host and flea populations and heightened plague risk, whereas high threshold temperature ($>$32.2–35°C) values should decrease flea survival and lower this risk (44). Some have proposed that epizootic activity decreases during hot weather ($\geq$27.5°C) because high temperatures adversely affect blockage of fleas by *Y. pestis* (24).

Different factors purportedly influence the dynamics of enzootic plague cycles through their effects on interactions between *Y. pestis* and its hosts and vectors (7, 87). These factors include heterogeneity among *Y. pestis* strains, density and diversity of rodent communities, host immune status, genetic structure of host populations, physiologic status of hosts or vectors, species of flea vector, mechanisms of transmission, mutagenic effects of phagocytic cells on *Y. pestis*, bacteriophage activity, and interactions between *Y. pestis* and other bacteria, (3, 7, 45, 48, 71, 87, 102, 104, 115).

THE PLAGUE BACTERIUM

Y. pestis is a gram-negative coccobacillus that belongs to the Enterobacteriaceae, a family that includes *Escherichia coli*, *Salmonella typhi*, and other enteric pathogens typically transmitted through contaminated food and water (108). Among the Enterobacteriaceae, *Y. pestis* is unique in both its choice of host habitat (blood, lymphoid system, reticuloendothelial system) and primary mode of transmission (flea vectors). To exist as a vector-borne pathogen, the plague bacterium must not only survive in its vertebrate host, but also disseminate from the site of inoculation, usually a flea bite, and proliferate, causing a high bacteremia that can serve as a source of infection for feeding fleas (5, 22, 43). Dispersal of *Y. pestis* within the host is enhanced by a plasminogen activator (Pla) that has fibrinolytic activity (108). Other virulence factors include an array of *Yersinia* outer proteins (Yops) and the pH 6 antigen, which are involved in cytotoxic processes, immune suppression, or survival of *Y. pestis* within naïve host phagocytes in the initial stages of infection. Upon exiting the vector and entering the higher-temperature environment of the vertebrate host, *Y. pestis* begins to express the F1 capsular antigen (Caf1), thereby enabling it to resist phagocytosis and killing by potentially

activated monocytes later in the course of infection. The yersiniabactin siderophore system and other iron uptake systems enable *Y. pestis* to acquire this essential nutrient in blood or other environments where its availability is limited by host iron-binding molecules (107). Rough *Y. pestis* lipopolysaccharides probably confer resistance to compliment mediated lysis. *Y. pestis* endotoxin also presumably causes the signs and symptoms associated with septic shock, systemic inflammatory response syndrome, and other serious conditions likely to cause death (36, 108, 111). Two additional virulence factors, murine toxin (Ymt) and the *pgm* locus, not only are associated with pathogenicity in mammals but also affect the survival of *Y. pestis* in fleas (Ymt) or its transmission by these vectors (*pgm* locus) (63, 64). The *pgm* locus consists of two linked regions (107). The first is a high pathogenicity island that bears the genes for the yersiniabactin siderophore system (see above), and the other, which is called the *hms* locus, contains genes required for *Y. pestis* to bind hemin or Congo red (pigmented phenotype) and form blockages in the foreguts of vector fleas.

Although the closely related *Y. pseudotuberculosis* can survive for relatively long periods in water or soils, most researchers believe the plague bacterium perishes quickly outside its normal hosts or vectors (20, 108). Nevertheless, some have reported that *Y. pestis* can survive for many days to weeks in flea feces, tissues of dead animals, and a few other naturally occurring substrates, including soils. Survival in soils has been proposed as a possible mechanism for plague persistence during interepizootic periods (9, 98). According to this scenario, *Y. pestis* cycles between a short and unstable parasitic phase associated with rodents and fleas and a more stable soil (saprophytic) phase that allows for survival between epizootics (9). Others have proposed that *Y. pestis* might survive as an intracellular parasite of soil protozoa (38, 103, 117) or exist in a latent (nonculturable) form in soils (131). Some have even speculated that *Y. pestis* might undergo prolonged survival in plants (94, 124). If plague bacteria do indeed survive in soils, soil protozoans, or plants, rodents presumably could reacquire infection while burrowing or foraging. Although such hypotheses deserve further investigation, they do not seem, at least at first glance, to agree with observed patterns of transmission and have been received with much skepticism.

EVOLUTION OF PLAGUE

Early concepts about plague evolution concentrated on the types and distribution of hosts utilized by *Y. pestis*. Kucheruk (89, 90) concluded that the origin of plague coincided most closely with the appearance of burrow-dwelling rodents that were abundant in dry unwooded steppe and desert regions. Contrary to previous opinions, he decided that *Rattus* spp. were important only in secondary foci that arose as a result of human activity and therefore played no role in the early evolution of *Y. pestis*. He also noted that although other murids, along with sciurids and caviids, were important hosts in some foci, the distribution of plague in Eurasia,

Indo-Malaya, Africa, and the Americas corresponded closely only with that of the Cricetidae, a group that included gerbillines, microtines, sigmodontines, and cricetines. Notably, cricetids represented 82 of 183 mammalian species reported to be naturally infected with *Y. pestis*. On the basis of these observations, Kucheruk concluded that plague first evolved in the Cricetidae, perhaps appearing during the Oligocene (23–38 mya)–Miocene (5–23 mya) era, with the upper Oligocene or lower Miocene periods considered most likely. North America and Eurasia were considered likely sites of origin for plague, although the Cricetidae probably originated in northern Asia. Historical evidence also indicates that plague was first introduced into North America around 1900, when rat-infested ships brought the disease from Asia, a finding that is supported by molecular studies indicating that plague strains from this continent are similar to others identified from areas affected by the last pandemic (48, 59, 113). Most evidence suggests plague arose in Asia, although *Y. pestis* has been present in east-central Africa for most of the past two millennia and probably longer. Recently, Suntsov & Suntsova (132) proposed an alternative hypothesis for the evolution of plague that involves marmots, their hibernation behaviors, and larval parasitism by one of their fleas.

Kucheruk and others probably were mistaken in their belief that plague arose many millions of years ago. DNA-DNA hybridization assays and sequencing of the 16S rRNA gene indicate a close relationship between *Y. pestis* and *Y. pseudotuberculosis* (14, 136). The chromosomal DNA of these bacteria are similar and have an approximately 70-kb plasmid (pLcr) that carries genes for Yops found in both species (20, 108). Recently, Achtman et al. (1) analyzed 6 genes from each of the 36 plague strains that were selected to include representatives from each of the three plague biotypes. Their results suggested that *Y. pestis* is a recently evolved clone of *Y. pseudotuberculosis*, appearing as recently as 1500 to 20,000 years ago.

Considering their genetic similarity, the marked differences in pathogenicity between *Y. pestis* and *Y. pseudotuberculosis* are striking. The most obvious genetic differences are two plasmids (pPCP1 and pFra, also called pMT in some strains) found in *Y. pestis* but absent in *Y. pseudotuberculosis* (108). pPCP1 contains the plasminogen activator noted above, a bacteriocin (pesticin), and a pesticin immunity protein; pFra contains the genes for Ymt and the fraction 1 capsular antigen (Caf1). The origins of these plasmids remain uncertain but approximately 50% of the *Y. pestis* pFra (or pMT) DNA sequences share a high degree of similarity (over 90% of the similar regions exhibited >96% DNA identity) to those on a *Salmonella enterica* serovar *typhi* plasmid (116).

CLASSIFICATION OF STRAINS AND THEIR ASSOCIATION WITH MAMMALIAN HOSTS

Variation among *Y. pestis* strains is associated with geographic origins, host associations, virulence, or possible links to past epidemics, including the three great pandemics (6, 37, 56, 59, 86). Unlike many bacterial pathogens, *Y. pestis* has only

one serotype, but typing schemes have been developed on the basis of variations in biochemical reactivity for certain substrates, primary host associations, ribotypes, and other molecular markers (2, 59, 68, 82, 99).

Expanding on earlier work by Berlin & Borzenkov (15), Devignat (37) identified three *Y. pestis* biotypes (antiqua, mediaevalis, and orientalis) that differed in their abilities to acidify glycerol and reduce nitrates. The distributions of these biotypes corresponded reasonably well with the sites of origin and areas affected by the three pandemics [Justinian's Plague, Black Death, and Modern (Third) Pandemic]. Later ribotyping studies provided support for Devignat's scheme (59).

Tumanskii (137, 138) proposed three host-related *Y. pestis* varieties: *ratti* (rat-borne), *marmotae* (marmot-borne), and *citelli* (suslik-borne), and Levi (92) identified another vole-specific variety (*microti*). Stepanov (130) later suggested that these varieties should be recognized as *Y. pestis* subspecies because of their specific characteristics, geographic distributions, adaptations to particular rodents, and level of virulence for animals, a proposal that was further modified by Kozlov (86), who listed biochemical reactivities and virulence characteristics that distinguished separate biotypes among Stepanov's subspecies. Recently, Gorschkov et al. (56) used a DNA probe to fingerprint 85 *Y. pestis* strains from different natural foci. The seven genetic variants identified were associated with certain rodent species and geographic regions. Although the above studies indicate that *Y. pestis* strains vary among regions in a manner that correlates well with the predominant host found in each region, it is not clear whether these correlations represent evidence of adaptation of the strains to these host species or simply geographic variation that has arisen over time.

In China, investigators identified 17 distinct *Y. pestis* types on the basis of variations in biochemical properties, nutritional requirements, virulence factor expression, and other features (95). Additional studies documented variations among plasmid profiles and ribotypes of Chinese strains, including one that describes an interesting 6-kb plasmid of uncertain function and origin (39). *Y. pestis* strains bearing this plasmid have been spreading among rats and fleas in southwestern China, where the last pandemic arose.

North American strains of *Y. pestis* are less diverse than those found in Asia, which is hardly surprising considering that only a limited number of orientalis biotype strains were likely to have been introduced into this continent during the last pandemic. Interestingly, plague strains from North America have a distinctive 19-kb dimer of the 9.5-kb plasmid (pPCP1) that does not appear in other orientalis isolates collected from various areas affected by the above pandemic (27). Recently, multiple-locus variable number tandem repeat analysis, PCR-based IS100 genotyping, and pulsed-field gel electrophoresis have been used to analyze variation among North American and other strains (2, 68, 82, 99). The above methodologies are still under evaluation but undoubtedly will be useful for epidemiological and ecological studies, as demonstrated in a study that investigated *Y. pestis* population genetic structure and transmission patterns during an epizootic in prairie dogs in Arizona (54a).

MAJOR MAMMALIAN HOSTS OF PLAGUE

Among the 203 rodent species or subspecies and 14 lagomorph species reported to be naturally infected with *Y. pestis* (114), only a small proportion are actually significant hosts. Although certain lagomorphs, such as pikas in central Asia, appear to be important, rodents are the major host taxon (48, 57). Each of these small mammals possesses unique characteristics that influence its ability to serve as a host of plague, although most have certain features in common. Many members of important host populations, probably 40% or more, not only become infected with *Y. pestis* but also circulate sufficient numbers of bacteria in their blood ($>10^6$ *Y. pestis* ml^{-1} blood) to serve as reliable sources of infection for feeding fleas (22, 43, 115). Few, if any, hosts that become so heavily bacteremic survive, but more resistant members of the same population might develop less severe illness and live to reproduce. Major plague hosts also are often heavily infested with one or more important flea vectors, a trait that obviously promotes the spread of the disease. Finally, many significant hosts live in burrows that support large flea populations, and those that dwell elsewhere, such as wood rats (*Neotoma* spp.), often have complex nests that are also heavily flea infested (101, 106, 111).

RESISTANCE TO PLAGUE IN MAMMALIAN HOST POPULATIONS

Host susceptibility to *Y. pestis*–induced mortality depends on many factors, including host species, genetic differences among individuals and populations, age, breeding status, immune and physiologic status, and season of the year (69, 85, 115). Allegedly resistant host populations typically consist of a mixture of highly susceptible individuals that usually die following infection and more resistant animals that become infected but eventually recover, as indicated by the detection of specific antibodies or resolving *Y. pestis* lesions in their tissues (49, 79, 113). Although the evidence is limited, some researchers have proposed that recovered hosts might become chronic carriers of plague, thereby acting as reservoirs for maintaining the disease between transmission seasons or epizootics (113, 141, 142). Others believe that normally susceptible animals might become infected shortly before entering hibernation, maintain a latent infection while hibernating, and then succumb to plague upon reawakening in the spring (54, 115).

Species that exhibit heterogeneous responses to infection include gerbils (*Meriones* spp. and *Rhombomys opimus*) and marmots in Asia (*Marmota* spp.), and deer mice (*Peromyscus maniculatus*) and California voles (*Microtus californicus*) in North America (51, 79, 86, 110–112). Certain other hosts are more susceptible and typically undergo devastating die-offs when infected with *Y. pestis*. Black-tailed prairie dogs (*Cynomys ludovicianus*) experience almost 100% mortality during epizootics (17, 18, 111), and other sciurid species, including other prairie dog species, are nearly as susceptible (18, 30, 88, 121, 134). Recent

evidence suggests that plague not only causes high mortality among prairie dogs but also reduces genetic variability within their populations (136a).

The occurrence of significant resistance among a rodent population might indicate past contact with plague and selection for resistant individuals (47, 96, 113, 115, 122), and some have proposed that the only true hosts of plague are those that have survived repeated contact with the disease. It has been further suggested that the role a particular host population plays in maintaining plague is determined by the ratio of resistant (survives infection) to susceptible (dies following infection) individuals within that population. The great gerbil (*R. opimus*) was considered the most important host in central Asian desert foci because 40% to 60% were resistant to *Y. pestis*–induced mortality, a level higher than that observed for other sympatric gerbils of the genus *Meriones* (110, 123). Some Asian marmots, including certain populations of *Marmota sibirica*, also exhibit relatively high resistance (54). In other instances no particular host stands out as unusually resistant. For example, resistance in the Ural steppe focus of Kazakhstan was about equal for great gerbils (50%–80%), little susliks (*Spermophilus pygmaeus*) (50%–70%), and midday gerbils (*Meriones meridianus*) (44%–60%) (7).

Relatively few studies have addressed resistance among North American rodents. Thomas et al. (133) compared *Y. pestis* infections in northern grasshopper mice (*Onychomys leucogaster*) collected from a plague-free area in Oklahoma with those from a plague-affected region of Colorado and found that the plague-naïve mice were much less resistant than the Colorado population. Variations in plague resistance among populations of deer mice (*P. maniculatus*) and California voles (*M. californicus*) also have been reported (119, 120), and this resistance is known to be genetic in *M. californicus* (69). Kangaroo rats (*Dipodomys* spp.) are considered highly resistant (67). Although some studies suggest that repeated plague exposures result in the appearance of at least partial resistance among rodents, populations of California ground squirrels (*Spermophilus beecheyi*), rock squirrels (*S. variegatus*), and prairie dogs (*Cynomys* spp.) have remained highly susceptible (111, 112, 121, 131), and epizootics still cause high mortality among some suslik populations (*Spermophilus* spp.) in Asia (88).

TRANSMISSION OF *YERSINIA PESTIS* BY FLEAS

In 1897 Ogata (113) suggested that fleas might transmit *Y. pestis*, and succeeded in infecting mice by injecting them with ground suspensions prepared from fleas that had fed on infected rats. One year later Simond demonstrated that rat fleas previously fed on infected rats could transmit *Y. pestis* to uninfected rats. In 1913 Swellengrebel demonstrated that transmission by *Xenopsylla cheopis* occurs through the rat flea's mouthparts rather than through contamination of the feeding site with infectious flea feces.

A classic study by Bacot & Martin (8) describes the development of *Y. pestis* infections in *X. cheopis* and the blocking process that enables these fleas to be efficient vectors. They found that after *X. cheopis* feeds on an infected rat, the ingested

plague bacteria multiply rapidly in the flea's midgut and spine-filled proventriculus and form noticeable colonies in just a few days. As these colonies grow and coalesce, they eventually become large enough to occlude the proventriculus, effectively blocking the movement of blood from the foregut to the midgut, causing the flea to starve. Blocked and starving fleas repeatedly attempt to feed by using their pharyngeal muscles to draw blood into the foregut, which causes distension of the esophagus but no movement of blood past the proventricular block. Eventually the flea is forced to relax its pharyngeal muscles, which results in *Y. pestis*–contaminated blood being flushed from the distended esophagus back into the feeding site, thereby causing infection of the vertebrate host.

Many factors influence blocking and transmission, including *Y. pestis* strain differences and transmission factors, flea species, proventricular morphology, and temperature (16, 22, 24, 32, 43, 46, 60, 61, 63, 64, 71a, 73, 75, 76, 79, 115). The ability of *Y. pestis* to form blockages in fleas is associated with its ability to bind Congo red (CR) or absorb exogenous hemin when grown on media containing these compounds (16, 108). Strains that fail to bind hemin or CR are less "sticky" than pigment-positive strains, which provides a likely explanation for their inability to form blocks. To bind hemin or CR, *Y. pestis* must possess a group of chromosomal genes called the hemin storage (*hms*) locus. Hinnebusch et al. (63) demonstrated that a *Y. pestis hms* mutant containing a deletion in the *hmsR* gene of the *hmsHFR* region of the *hms* locus was incapable of forming blocks in fleas, as was a polar mutant with a single transposon insertion in *hmsH*. The ability of both mutant strains to cause blockages could be restored by complementing the bacteria with a plasmid that bears functional *hmsHFR* genes.

If fleas are to become blocked, *Y. pestis* must grow in their guts. Hinnebusch et al. demonstrated that for *Y. pestis* to colonize the flea midgut, the 110-kb plasmid (pMT or pFra) must be present and express murine toxin (Ymt) (61, 64). Others have suggested that the ability of *Y. pestis* to form biofilms in flea guts might be critical for colonization and blocking (31, 71a). The fleas themselves also must provide a suitable environment for *Y. pestis* growth. Engelthaler et al. (43) compared *Y. pestis* infections in *X. cheopis* and *Oropsylla montana* and found that early in the course of infection *Y. pestis* colonies often appeared simultaneously in the proventriculus and midgut of *X. cheopis*, but only in the midguts of *O. montana*. This could explain why the latter requires much longer blocking times, has lower blocking rates, clears itself of infection more frequently, and is generally a much less efficient vector than *X. cheopis* (22, 43, 65).

Differences in blocking rates also might be explained by the structure of the proventriculus (46). It was reported that blocking in *Citellophilus tesquorum* occurred in those fleas that had high levels of fluctuating asymmetry among their proventricular spines (84). Strictly morphological explanations, however, are unlikely to explain why certain flea species not only fail to become blocked but also are highly resistant to *Y. pestis* colonization in their guts.

Temperature obviously affects transmission (24, 44, 113). Block formation in two rodent fleas, *C. tesquorum* and *Neopsylla setosa*, was optimal at 16 to

22°C (58). Blocking rates and times differed between these fleas, however, with 53.3% to 55.1% of *N. setosa* becoming blocked within 3 to 4 days after taking an infectious blood meal, whereas 10 to 14 days were required for 28.0% to 42.7% of *C. tesquorum* to do so. Interestingly, blocking in *C. tesquorum* depended mainly on the frequency of feeding, whereas duration of feeding was more important in *N. setosa*. Cavanaugh (24) reported that infected fleas held at temperatures above 27.5°C had decreased transmission rates compared with those held at lower temperatures. Kartman (74) noted that clearance of infection increased more than 10-fold and blocking rates decreased by slightly more than half for *X. cheopis* held at 29.5°C rather than 23.5°C. These effects were attributed to the temperature-dependent fibrinolytic activities of the *Y. pestis* plasminogen activator (Pla) (24), a hypothesis that was later refuted by Hinnebusch et al. (61), who found that blocking rates decreased with temperature even among fleas that had been infected with *Y. pestis* strains lacking the plasmid (pPCP) that carries the gene for Pla.

Compared with most other fleas, *X. cheopis* is an unusually effective and dangerous vector, particularly in plague-endemic areas that are heavily rat infested. This flea is remarkable in its ability to become blocked, and therefore infectious, within as few as 5 days after imbibing *Y. pestis*–infected blood (46). Other common rat fleas, with the probable exception of *Xenopsylla brasiliensis*, are less effective vectors. *X. astia*, a flea that occurs in southern Asia, is a relatively inefficient transmitter of plague, which suggests that phylogenetic relationships among vectors are an unreliable indicator of likely vector competency (113). The northern rat flea, *Nosopsyllus fasciatus*, also transmits *Y. pestis* but much less efficiently than *X. cheopis*. Although it is generally accepted that wild rodent fleas are important vectors of *Y. pestis*, they typically transmit at lower rates than *X. cheopis* (22, 43, 46, 65, 66, 113). Some even clear themselves of infection soon after ingesting a presumably infectious blood meal (40, 46). Other wild rodent fleas support *Y. pestis* infections in their midguts but fail to become blocked, or do so only rarely, while others become blocked only after extrinsic incubation periods of many weeks to a few months. Recently developed quantitative competitive PCR techniques can be used to estimate the numbers of *Y. pestis* found in field-collected fleas, thereby providing useful information on whether individual fleas are likely to be blocked or merely infected (42, 62).

The roles played by most wild rodent fleas in plague transmission are poorly known (111). The number of potential vectors is great (114). Serzhan & Ageyev (127) analyzed a list prepared by Goncharov of 263 fleas reported to be naturally infected with *Y. pestis* and found that 223 of these (85%) were specific parasites of rodents and 145 (55.0%) were further specific for cricetids. Most of the infected fleas (57%) came from the Palearctic region, 22.9% occurred in North America, and the remainder originated from foci in southern Asia, Africa, and South America. Despite the large number of species potentially involved, only those fleas that are found on key hosts and able to transmit under natural circumstances are likely to be important.

That many wild rodent fleas are considered important vectors but block at much lower rates than *X. cheopis* raises interesting questions about the relative

roles of blocked, partially blocked, and infected but completely unblocked fleas in the transmission of plague. Relatively little data exist on this subject, but some researchers have reported experimental transmission by infected and unblocked or partially blocked fleas (13, 22, 33, 53, 140). In separate studies, individual, block-free *O. montana* succeeded in transmitting *Y. pestis* four days after feeding on infectious hosts (22, 43). Despite being an important vector in southern Siberia, the suslik flea *C. tesquorum altaicus* blocked at low rates (5.8%), an apparent discrepancy that could be explained by the fact that 40% to 50% of infected but block-free fleas experimentally transmitted *Y. pestis* to susceptible animals (13, 140). Similarly, infected *Rhadinopsylla rothschildi* and *Rh. daurica* exhibited low blocking rates (2.1%–12.5% and 7.2%–10.5%, respectively), but block-free individuals of both species succeeded in transmitting *Y. pestis* to *Microtus brandti* and to *Spermophilus daurica* (53). In yet another study, Degtyareva et al. (33) reported that block-free fleas from the Dagestan alpine focus could transmit plague to common voles.

The importance of mechanical transmission also should be further investigated. Bibikova (16) discounted its importance, noting that *Y. pestis* survived for only 3 h on flea mouthparts. Others, however, have taken a more moderate view. Kartman et al. (76, 77) and Burroughs (22) suggested that transmission by blocked vectors is important in enzootic cycles and that mechanical transmission is most significant during epizootics. Indeed, such transmission might explain how die-offs can spread so rapidly among prairie dogs, ground squirrels, and other highly susceptible species that harbor fleas that apparently take weeks to months to become blocked. Although the mouse and vole flea *Malaraeus telchinum* rarely becomes blocked, it can transmit plague when large numbers of fleas are placed on susceptible animals soon after they have taken an infectious blood meal, a finding strongly suggestive of mechanical transmission (21, 22, 77). Another sympatric flea, *Hystrichopsylla linsdalei*, was far less abundant than *M. telchinum* but blocked efficiently and was thought to be important in enzootic transmission of *Y. pestis* (76). Others have suggested that even *X. cheopis* might transmit mechanically during plague epizootics (118). *Pulex irritans* is an exceptionally poor biological vector of plague but can transmit the disease mechanically, a fact that may explain why some regions in developing countries, or perhaps parts of Europe during the Black Death, suffered outbreaks of bubonic plague in the apparent absence of *X. cheopis* (19).

The ability to experimentally transmit *Y. pestis* is likely to provide some indication of whether a particular flea species will be an important vector; however, other factors must be considered. Fleas that are highly host specific might be suitable vectors for transmitting plague among a particular host species but are less likely to spread the infection to other species. Otherwise suitable vectors might also occur in plague-free regions or appear as adults during a time of the year when few host animals are bacteremic, making it unlikely that these fleas will become infected and transmit plague. The ability of *Y. pestis*–infected fleas to survive in off-host environments should also be considered, as this provides a means for maintaining *Y. pestis* over extended periods, including from one transmission season to the next.

FLEAS AS RESERVOIRS OF *YERSINIA PESTIS*

Although fleas often die relatively quickly after becoming blocked, infected but unblocked fleas, and occasionally even blocked fleas, can survive for many months. Infected *Ctenophthalmus breviatus*, *C. tesquorum*, and *N. setosa* survived for up to 220 days when held at 14 to 27°C and *Ct. breviatus* survived for 396 days at 0 to 15°C (55). In another experiment, more than half the *C. tesquorum altaicus* that fed on infected long-tailed susliks (*Spermophilus undulatus*) maintained *Y. pestis* from mid-September to mid-June, thus allowing the plague bacterium to survive during hibernation of their hosts (11). A few fleas (0.7% to 19.4%) survived from one summer to the next and 30% of these remained infected. A single female survived through two winters and lived for 411 days after feeding on an infected suslik. Most importantly, infected fleas that survived through the winter transmitted plague to healthy susliks. In a field study, Sharets et al. (128) plugged the openings of marmot burrows and found that infected *Rhadinopsylla li ventricosa* were still present after nearly 14 months. Infected prairie dog fleas (*Oropsylla labis* and *O. tuberculata cynomuris*) also were recovered from burrows in Colorado for more than one year after their hosts had perished from plague (78).

CHARACTERIZING PLAGUE FOCI AND HOSTS

Zabolotny (144) proposed that the 1910–1911 Manchurian epidemic, which killed 50,000 to 60,000 people, was caused not by the introduction of plague through movement of infected rats or people, but rather by a spillover of infection from a natural focus that existed in native marmots. Zabolotny's recognition of the link between this human outbreak and disease observed among marmots represents one of the first discussions of a natural plague focus. Since that time many authors have proposed various concepts on the hosts, landscape characteristics, structure, and typology of these foci.

PRIMARY AND SECONDARY HOSTS

Fenyuk (47) proposed classifying hosts as either primary or secondary carriers of plague. According to this concept, primary hosts and their fleas maintain plague in natural foci without the involvement of other potential hosts. Conversely, secondary hosts and their fleas cannot maintain *Y. pestis* in the absence of primary hosts but might assist in disseminating the disesase. Rall (122) later proposed that the ability of an animal to act as a primary host depends on its susceptibility, abundance, distribution, and behavior. Other proposed factors include coloniality, proximity of colonies to one another, use of complex burrow systems, resistance to a specific biotype of *Y. pestis*, ability of resistant animals to produce high antibody titers and levels of phagocytic activity, and occurrence of prolonged bacteremias in susceptible animals (B. Suleimenov & N. Klassovskii, personal communication).

Opinions differ on whether a single primary host and its fleas actually can maintain plague within a focus without the involvement of other hosts. Rall (122) believed that this was indeed true in central Asia and supported the related concept of monohostality (72), wherein *Y. pestis* can be maintained in a particular focus through infection of a single host, such as the great gerbil (*R. opimus*) in central Asian desert foci. Other investigators (72, 100) believed that these foci are supported by multiple hosts (polyhostality). Siberian susliks (*S. undulatus*) and Pallas' pikas (*Ochotona pallasi*) were considered important in the same Mongolian focus, as were Daurian susliks (*S. dauricus*) and rats (*Rattus* spp.) in northeastern China; Siberian marmots (*Marmota sibirica*), Daurian susliks (*S. dauricus*), pikas (*Ochotona* spp.), and voles (*Microtus* spp.) in the Daurian enzootic area; and little susliks (*Spermophilus pygmaeus*), gerbils, and jerboas in central Asia. Although Petrov (109) and many others proposed that the great gerbil and its fleas support monohostal foci in the deserts of central Asia, others believed that maintenance in these areas depends on the presence of additional rodent species. In the foothills of Kopet-Dag, Mangishlack, and western and northwestern Turkmenia, *R. opimus* and the Libyan jird (*Meriones libycus*) were considered essential for maintaining plague, whereas *R. opimus* was joined by the midday gerbil (*M. meridianus*) in the Aral Karakum and northwestern Kizilkum deserts, the little suslik north of the Aral Sea, and the Aral yellow suslik (*Spermophilus fulvus*) on the Krasnovodsky Peninsula (91). One of the most interesting questions is whether commensal rats can maintain plague in the absence of other rodent hosts. Although some have doubted whether this is possible, *Y. pestis* circulates for decades in some areas without the apparent involvement of other hosts (115), and rats are considered the primary hosts of plague in Vietnam and Madagascar (57). Recent modeling studies have suggested that plague can persist in small rat subpopulations for indefinite periods and serve as sources of infection for later outbreaks (80, 81).

The concepts of primary and secondary hosts, as well as monohostality and polyhostality, have not appeared extensively in the American literature. Instead, important rodent species have been characterized as enzootic (maintenance) or epizootic (amplifying) hosts (111, 112). According to this scheme, plague is maintained primarily in so-called enzootic cycles that involve transmission among presumed enzootic hosts, namely various species of *Peromyscus* and *Microtus*, and their fleas. As noted above, *Y. pestis* is believed to occasionally spread from its enzootic hosts to other highly susceptible epizootic hosts, often causing widespread die-offs among these animals. Commonly proposed epizootic hosts include various prairie dogs, ground squirrels, chipmunks, and wood rats.

Although the occurrence of epizootic cycles is suggested by obvious die-offs among presumed epizootic hosts, little evidence exists to indicate that supposed enzootic hosts (*Peromyscus* spp. and *Microtus* spp.) are truly essential for the interepizootic maintenance of plague. Alternatively, plague might be maintained during interepizootic periods through low-level transmission among a number of potential host species and their fleas, causing epizootics only when environmental

conditions are favorable and host populations are high. The long-term maintenance of plague in such a polyhostal system would be favored not only by the presence of multiple host and flea species but also by the patchy distribution of these hosts within multiple habitats, thus ensuring that all populations of these animals are never completely wiped out and that low levels of transmission can occur as plague slowly passes from patch to patch and as host populations recover from previous epizootics. Pollitzer & Meyer (115) also cautioned that the importance of supposedly resistant hosts, such as *P. maniculatus*, for maintaining plague foci between epizootics might be overrated, noting that various mechanisms in addition to spatial isolation could ensure plague persistence even among highly susceptible host populations, including age-related or seasonal variations in susceptibility and the possibility that hosts could maintain latent infections while hibernating and thus carry *Y. pestis* from one transmission season to the next.

TYPOLOGY OF PLAGUE ENZOOTIC TERRITORY

Several scientists developed systems for typing natural plague foci in the former Soviet Union, China, and neighboring regions (89, 109, 122). Kucheruk (89) proposed five types of plague foci for the Palearctic region on the basis of primary mammalian hosts, flea vectors, *Y. pestis* biotypes, and landscape characteristics. These included a suslik type in the steppes of the Pre-Caspian Sea region, central Caucasus mountains, Transbaikalia, and northeastern China; a marmot type in meadow belts of the Gissarski Hrebet, Alaj, and Pamir mountains; a pika type in the steppe belt of the Altay mountains; a vole alpine type in the Caucasus and Gissarski Hrebet mountains; and a gerbil type spread across the vast deserts that occur from the western Sahara to the eastern Gobi. Chinese scientists also developed a system for typing plague foci on the basis of the occurrence of particular landscapes, the presence and characteristics of principal hosts and fleas, and the existence of *Y. pestis* infections in these same hosts and fleas (95).

In North America, Barnes (10) identified a series of epizootic host-flea complexes that involved ground squirrel, prairie dog, chipmunk, and wood rat species, and their fleas. Unlike Kucheruk's system, however, Barnes' system was intended to identify those rodent and flea species most likely involved in the amplification and geographic spread of plague during epizootics.

LANDSCAPE ECOLOGY STUDIES

Pavlovsky and other Soviet scientists first brought attention to the relationships between landscapes and the distribution and occurrence of various diseases, including plague (89, 106, 125). These landscape ecology studies continue to provide insights into the focality of plague and how its spread can be affected by landscape features (4, 95, 126). One recent study hypothesized that the occurrence of plague epizootics in the Altay mountains, Tuva (Trans-Baikal), Kyzyl Kum desert

in Uzbekistan, and Caspian lowlands is correlated with medium or high concentration of iron, cobalt, and titanium, and low concentrations of copper, nickel, and vanadium (126). These field observations were supported by laboratory studies indicating that manganese, iron, cobalt, nickel, copper, and zinc influenced the course of *Y. pestis* infections in three gerbil species (*R. opimus*, *M. meridianus*, and *M. tamariscinus*) (97). The distribution of foci in China also is reported to be correlated with calcium- and iron-enriched environments (95).

CONCLUSIONS AND FUTURE DIRECTIONS

In many respects, research on the natural history of plague presents an excellent example of how multidisciplinary investigations can enhance our knowledge of the natural cycles, maintenance, transmission, and focality of zoonotic diseases. The importance of this research can hardly be questioned, as it has provided critical information for the development of effective plague prevention and control techniques. And yet this same body of research often has yielded contradictory results and interpretations that make it difficult to evaluate the relative merits of different concepts on the dynamics of plague cycles, the occurrence of epizootics and spread of plague, the mechanisms for the interepizootic maintenance of *Y. pestis*, the roles of fleas as biological and mechanical vectors, and the distribution and structure of natural foci. Although such problems are not entirely unexpected, they point out the need for studies designed to test specific hypotheses on the above topics. The success of such research likely depends on ongoing support for long-term studies and collaborations between scientists from many disciplines, including entomology, ecology, microbiology, molecular biology, mathematical modeling, geographic information systems, and remote sensing.

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