CHAPTER 1

History, clinical aspects, epidemiology of plague and characteristics of *Y. pestis*

Abstract

DNA/DNA hybridization studies and 16SrDNA sequence analysis indicate that *Y. pestis* evolved from *Yersinia pseudotuberculosis* 1500 to 20,000 years ago. There are three primary clinical forms of *Y. pestis* infections in humans which without treatment exhibit a high incidence of death: bubonic plague, pneumonic plague, and septicemic plague. The designation "Black Plague" is derived from extensive gangrene of the extremities and other skin surfaces resulting from systemic infection. Present outbreaks are of the bubonic form, characterized by extensive swelling in one or several lymph nodes (buboes). The classical paradigm of infection involves fleas feeding on the blood of infected rodents and then transmitting the organism to humans during feeding. Approximately 3,000 human cases of plaque occur worldwide annually. Without treatment, fatalities can be as high as 57%. In the U.S.A. 12 to 15 cases of plague are reported each year in western U.S., primarily in the states of California, New Mexico, Arizona, and Colorado. Approximately 3,000 human cases of plaque occur worldwide annually. The polymerase chain reaction has allowed the detection of *Y. pestis* in archaeological skeletons dating to 600AD.

Introduction

Plague still occurs throughout the world today, although for reasons not fully understood plague epidemics do not develop from these outbreaks. Antibiotic treatment of bubonic plague is usually effective, but pneumonic plague is difficult to treat and even with antibiotic therapy death often occurs. A comparison of the genetic diversity of several housekeeping genes in *Y. pestis* and *Y. pseudotuberculosis*, a usually non lethal enteric pathogen, suggests that *Y. pestis* evolved from *Y. pseudotuberculosis* 1500 to 20,000 years ago (Achtman et al. 1999). In areas of the world where plague is endemic the organism appears to survive by causing chronic disease in animal reservoirs, such as rats, ground squirrels, gerbils, and marmots. Outbreaks of plague in humans are often associated with close contact with rodent reservoirs.
Characteristics of plague and *Y. pestis*

Characteristics of plague

Plague is a bacterial infection having a high level of fatality without treatment. The causative agent is *Y. pestis*, a gram-negative, nonmotile, nonsporforming coccobacillus that exhibits bipolar staining. The incubation period for plague is 1 to 6 days. There are three primary clinical forms of *Y. pestis* infections in humans: bubonic plague (Fig. 1A), primary pneumonic plague (Fig. 1B), and primary septicemic plague, (Fig. 1C). Septicemic plague usually leads to extensive gangrene of the extremities and other skin surfaces resulting in blackened necrotic tissue that led to the early designation of the "Black Plague" (Fig. 1C.).

Almost all present day outbreaks of plague occurring globally are the bubonic form of the disease. Bubonic plague is characterized by regional inflammation and swelling in one or several lymph nodes (buboes). The bubo can reach the size of a hen’s egg and is the classical feature of bubonic

Figure 1. Clinical manifestations of plague. A. Classic underarm bubo of bubonic plague, from U.S. Armed Forces Institute of Pathology. B. Chest radiograph of patient with primary pneumonic plague showing extensive lobar consolidation in left lower and left middle lung fields. B. Gangrene of the digits during the recovery phase of septicemic plague. Blackened areas of the skin are a typical manifestation resulting in the early designation of the "Black Plague" From the CDC, Division of Vector-Borne Infectious Diseases, Fort Collins, Colo.
plague. This type predominantly results from a flea bite or direct contamination of a skin lesion with contaminated material, with the infection spreading via the lymphatic system (Zeitz and Dunkelberg, 2004). Buboes can occur in any regional lymph nodes in the body. Early symptoms of bubonic plague include headache, shaking chills, fever, malaise, and pain in the affected regional lymph nodes with a sudden onset of the disease (WHO, 1999a; Perry and Fetherston, 1977). Primary septicemic plague is an overwhelming blood stream infection in the apparent absence of a primary lymphadenopathy or buboe with bacterial densities as high as $4 \times 10^7$ CFU/ml in the circulating blood (Titball and Williamson, 2001). An endotoxin is thought to initiate a sequence of immunologic responses leading to a spectrum of pathological events including disseminated intravascular coagulopathy multiple organ failure, and acute respiratory distress syndrome (WHO, 1999a; Perry and Fetherston, 1997).

Primary pneumonic plague caused by the respiratory droplet route is the most fulminating and fatal form of *Y. pestis* infection, leading to death in most cases. A rapid onset of symptoms occurs including chills and chest discomfort followed by cough, sputum production, difficulty in breathing, hypoxia and haemoptysis (WHO, 1999a; Perry and Fetherston, 1997). Death from pneumonic plague is thought to occur due to induction of the systemic inflammatory response (Perry et al., 1977).

Plague is still endemic in a number of countries. There are seven countries, Brazil, Democratic Republic of the Congo, Madagascar, Myanmar, Peru, the U.S.A., and Viet Nam which have encountered plague every year from 1954 to 1977. The total number of reported plague cases annually ranged from 200 to 6004 during that period (WHO, 2000).

**Characteristics of *Y. pestis***

During the Hong Kong epidemic of June 1894, Alexander Yersin (1894) and Shibasaburo Kitasato independently announced within a few days of one another the isolation of the causative organism of plague. The organism was initially called "Bacterium pestis" and was subsequently renamed *Pasteurella pestis* in 1900, and then in 1923 it became "Pasteurella pestis" and in 1944 it was named *Yersinia pestis*. The genus *Yersinia* presently consists of 11 species, three of which are pathogenic for humans and animals, *Y. pestis, Y. enterocolitica*, and *Y. pseudotuberculosis*. The latter two cause intestinal infections that are usually self-limiting and contain two of the three virulence related plasmids harbored by *Y. pestis*. Much of the work on pathogenicity associated with the plasmids of these two less infectious species is
therefore applicable to *Y. pestis*. *Y. pestis* is a Gram-negative, nonflagellated, coccoid, oval, or short rod. The organism is urease negative, esculin positive, sucrose negative, ornithine decarboxylase negative, citrate negative, and does not produce indole. Dextrin, galactose and xylose and d-tartrate are utilized with no gas produced from glucose. H₂S is produced. Some strains produce pesticins active on *Y. pestis* and other *Yersinia* species. β-galactosidase is produced but lactose is usually not fermented. Bacteriophage strains are frequently used to assist in confirming clinical identification of *Y. pestis* strains. A number of cell "envelope" antigens have been distinguished from somatic antigens. The "virulence" or VI antigen is plasmid encoded as is the protective capsular antigen F1 associated with increased resistance to phagocytosis.

An unusual strain of *Y. pestis* was reported by Winter et al. (1960). The culture was isolated at necropsy from a four-year old girl and designated the "Bryans" strain. The isolate was typical of *Y. pestis* regarding cultural, staining, and physiological characteristics, phage lysis, and susceptibility to antibiotics. However, serological investigations employing both agglutination and fluorescent antibody tests indicated that the Bryans strain was quite different from representative strains from other virulent cultures of *Y. pestis*. Four comparison strains were assessed for pathogenicity for mice, capsule formation, agglutination by several sera containing "whole cell-envelope" antibody, and agglutination by a *Y. pestis* commercial antiserum. The Bryans strain resembled the four reference strains only with respect to mouse pathogenicity. It failed to produce capsules invivo and invitro at 37 °C, and it was not stained by a fluorescence-labeled "envelope" antibody that brightly stained the cells of the four reference strains. Cell suspension of the Bryans strain underwent spontaneous agglutination in saline controls, which interfered with several agglutination assays, normally used to assist in identification of *Y. pestis* isolates. The F1 antigen was found to be present in reduced amounts compared to the four virulent reference strains which presumably accounted for the lack of staining by the fluorescently labeled "whole cell-envelope" antibody preparation. The Bryans strain also exhibited very low virulence for guinea-pigs.

When grown on agar containing hemin, *Y. pestis* colonies turn dark brown as a result of absorbing large quantities of the iron rich molecule (Burrows and Jackson, 1956). When media contain Congo red, a hemin analog, the colonies turns dark red (Hare and McDonough, 1999; Surgalla and Beesley, 1969). This phenotype has three designations: pigmentation (Pgm), hemin storage (Hms), and Congo red (CR). Interestingly, this phenotype is absent at 37 °C (Beardon et al., 1997).
Surgalla et al. (1964) reported that 0.1% NaHCO$_3$ stimulated the growth of a virulent *Y. pestis* strain while decreasing the growth of avirulent mutants in Brain Heart Infusion Broth supplemented with 0.2% glucose. This observation suggests that in vivo, where the organism may sense the presence of elevated CO$_2$ levels certain virulence genes may be turned on contributing to the stimulation of growth in vivo.

**Identification *Y. pestis* colonies**

Bhaduri and Sommers (2007) found that media such as CR-BHO (Brain Heart Infusion low calcium agarose plus 0.1% MgCl$_2$ plus 75 μg/ml of Congo red) and CR-MOX (a calcium-deficient magnesium oxalate agar containing 1% Congo red) designated for selective detection of *Y. pestis* in food are not satisfactory for that purpose. Expression of genetic determinants by pathogenic *Yersinia* species including low Ca$^{++}$ response (Lcr), colony size, crystal violet (CV) binding, Congo red (CR) uptake, autoaglutination (AA), and hydrophobicity (HP) were compared. Lcr and CV binding on Brain Heart Infusion Agar were detectable within 24 hrs. at 37 °C with *Y. enterocolitica* and *Y. pseudotuberculosis*, but only after 48 hrs. with *Y. pestis*. Distinguishable colony size, AA, and HP characteristics were expressed by *Y. pseudotuberculosis* and *Y. enterica*, but not by *Y. pestis*. pCD1$^{-}$ plasmid colonies of all three pathogenic *Yersinia* failed to bind crystal violet and remained white, while pCD1$^{+}$ strains yielded dark colonies from crystal violet contact. When pCD1$^{+}$ and pCD1$^{-}$ strains were cultivated at 37 °C on CR-BHO and CR-MOX agars, the pCD1$^{+}$ cells of *Y. enterocolitica* and *P. pseudotuberculosis* showed Congo red-uptake as red pinpoint colonies after 24-48 hrs, whereas Congo-red-uptake by *Y. pestis* on CR-BHO agar was insignificant. In contrast, colonies of *Y. pestis* incubated for 48 hrs. on CR-MOX agar were red due to expression of the low Ca$^{++}$ response as a result of oxalate in CR-MOX binding Ca$^{++}$ which did not occur in CR-BHO agar were Ca$^{++}$ is more abundant. The pCD1$^{-}$ strains of all three pathogenic *Yersinia* species failed to bind Congo red and formed much larger and white colonies compared to pCD1$^{+}$ strains. *Y. pestis* with chromosomally encoded pigmentation determinant (Pgm$^{+}$) but lacking the pCD1 plasmid failed to bind Congo red, clearly indicating that Congo-red-uptake is expressed by the pCD1 plasmid and is independent of chromosomally encoded pigmentation virulence determinants (Pgm$^{+}$). Congo red-uptake encoded by pCD1 is expressed only on a Ca$^{++}$-depleted medium. The authors recommended that for distinguishing colonies of *Y. pestis* from *Y. pseudotuberculosis* and *Y. enterocolitica* from foods, plating should be on CR-BHO and CR-MOX agars. If the colonies show Congo red uptake only on CR-MOX agar at 37 °C after 48 hrs. incubation, then those Congo red positive colonies can be tentatively
identified as *Y. pestis* and confirmed by PCR targeting the 9.5-kb pPCP1 plasmid encoded plasminogen activator gene (Loiez et al., 2003).

**Use of bacteriophage for identification of *Y. pestis***

Immunologically *Y. pestis* bacteriophage have been placed into four serovars. The most common and numerous of them belong to serovar 1, comprised of at least 33 *Y. pestis* lytic phages and is represented by nearly all known *Y. pestis* diagnostic phages. Serovar 2 includes L-413C and the majority of *Y. pestis* temperate phages. Serovar 3 consists of only one temperate phage designated P. Serovar 4 is represented by two additional temperate *Y. pestis* phages, Tal and 513. In 1933 Advier (1933) isolated a *Y. pestis* phage from the blood of a patient with clinical bubonic plague. This phage was subsequently adapted to the avirulent *Y. pestis* strain A1122 (Girard, 1943). Gunnison et al. (1951) reported that this phage designated “P phage” could be used to differentiate *Y. pestis* and *Y. pseudotuberculosis*. At 37 °C, the phage grows on both species, but at 20 °C – 27 °C it only grows on *Y. pestis*. Garcia et al. (2003) renamed this phage phiA1122 reflecting the *Y. pestis* host strain used for its routine propagation and also sequence the genome for this phage. The Centers for Disease Control and Prevention (CDC) have distributed and used phage lysates from this host since 1968 for identifying wild and clinical isolates of *Y. pestis*. Interestingly, early work by d’Herelle was successfully directed towards curing bubonic plague via phage therapy (Summers, 2001). Phage L-413 was originally isolated from the lysogenic virulent *Y. pestis* strain 413 which was obtained in 1961 from a red-tailed gerbil in a Central Asian Desert plague focus. The clear plague producing L-413 was selected after multiple passages in the *Y. pestis* host strain. L-413C is highly specific for *Y. pestis* and is inactive on other *Yersinia* species and the majority of enteric bacteria (Larina, 1976; Larina et al., 1970, 1981). In one study, among 103 *Escherichia coli* strains tested, only one was susceptible to L-413C (Larina, 1976). Further large-scale testing with ~7,000 strains of *Y. pestis* confirmed that L-413C has a unique specificity for *Y. pestis* and is a powerful tool for discriminating between *Y. pestis* and *Y. pseudotuberculosis*. L-413C has been shown to type 99.9% of *Y. pestis* strains and no *Y. pseudotuberculosis* isolate. There are only 10 known atypical *Y. pestis* strains resistant to L-413C (Imamaliev et al. 1986). Garcia et al. (2008) sequenced the entire genome of phage L-413C and characterized its molecular features. L-413C was found to be highly similar and collinear with enterobacteriophage P2, though important differences were found.

Ypfphi is a filamentous phage infectious for *Y. pestis*. Chouikha et al., (2010) found that this phage is able to infect with variable efficiencies, strains
of all three pathogenic Yersinia species in addition to E. coli. This variable efficiency of infection most probably reflects host controlled modification. Upon infection, Ypfphi was found to integrate into the chromosomal dif site, but extrachromosomal forms were also frequently observed. A resident Ypfphi was found to confer some protection against superinfection. In contrast to other filamentous phages, the incoming Ypfphi genome was found to insert itself between two copies of the resident prophage. The lack of species and genus specificity precludes this phage for use in identifying Y. pestis.

Early history of the plague and historical origins of the three pandemics

Early history

The oldest account of the plague is in the bible where the book of Samuel described an outbreak with buboes among the Philistines in 1320 BC (I Samuel, V and VI). The first recorded outbreak of an epidemic consistent with plague was in Athens in the summer of 430 BC. This occurred at the outbreak of the Peleponnesian war resulting in an estimated 300,000 deaths (~30% of the population of Athens). Thucydides, who survived the epidemic, described the symptomology which included high fever, blistered skin, bilious vomiting, intestinal ulcerations, and diarrhea. He also noted that animals were affected. The first pandemic or the "Justinian" plague occurred between 542 and 546 AD and is thought to have arisen in Ethiopia spreading rapidly through the Middle East to the Mediterranean basin and to a limited extent, to Mediterranean Europe, reaching Constantinople (now Istanbul, Turkey) and Greece, Asia, and Africa claiming nearly 100,000,000 victims. Early chroniclers indicate a 50% mortality of the Byzantine empire due to the disease. After an absence of plague for 600 years in Europe, the second pandemic known as the "Black Death" was initiated in 1330 and persisted to 1352, originating presumably from the steppes of Central Asia where an epidemic among rodents was noted. It then spread westward along the trade routes entering Europe in 1347 where it was chronicled in Sicily and Marseilles and resulted in a total of 50,000,000 deaths (WHO, 2002). Introduction of plague into Europe in 1347 initiated the second pandemic which persisted until 1352 and affected all of the known world (North Africa, Europe, Central and Southern Asia, and Africa). Mortality levels of 15 to 40% were commonly recorded. It has been estimated that 50 to 60% of the worlds human population was lost between AD 551 and 700 (Drancourt and Raoul, 2002) due to plague. The extensive depopulation as a result of deaths and flight resulted in severe social disruption. It has been estimated that 15 to
23.5 million Europeans or about one-fourth to one-third of the European population succumbed to the plague (Polzer, 1982). The plague then recurred in two to five year cycles until the beginning of the 18th century.

The third and current pandemic is thought to have started in Canton and Hong Kong in 1894 and then rapidly spread to all continents (WHO, 2002). Periodic outbreaks have occurred since, with e.g., nearly 13 million in India dying of the plague between 1898 and 1918 (Perry and Fetherston, 1997).

**Evolutionary history of Y. pestis**

Bercovier et al. (1980) compared the biochemical characteristics of *Y. pestis* and *Y. pseudotuberculosis*. Motility at 28 °C, urease production, fermentation of rhamnose, and growth rate on Nutrient Agar were found to be the best phenotypic means of separating these two organisms (Table 1). DNA hybridization studies indicated that *Y. pestis* strains are 90% or more interrelated and that *Y. pestis* and *Y. pseudotuberculosis* are indistinguishable by DNA relatedness. The authors recommended that on the basis of DNA hybridization data and biochemical and antigenic similarity, both organisms should be treated as two separate subspecies of *Y. pseudotuberculosis* and that for medical purposes, *Y. pestis* and *Y. pseudotuberculosis* designations should continue to be used.

Sequence comparisons of rRNA genes has confirmed their close relationship in that 16S rRNA is identical between the two species and 23S rRNA is almost identical (Trebsius et al., 1998). After Achtman et al. (1999) applied multilocus sequence typing (MLST) to *Y. pestis* and *Y. pseudotuberculosis* strains it became apparent that *Y. pestis* is simply a clone of *Y. pseudotuberculosis* that has evolved during the past few millennia. Fragments of five housekeeping genes plus a sixth gene involved in lipopolysaccharide (LPS) biosynthesis were sequenced from 36 isolates of *Y. pestis* that had been isolated from humans, rodents, and fleas from diverse global sources. The same gene fragments were sequenced from 12 isolates of *Y. pseudotuberculosis*. Only one allele was found for each of the gene fragments in *Y. pestis*, i.e., all strains possessed identical sequences. In contrast, 2-5 alleles were found for each gene fragment in *Y. pseudotuberculosis*. In addition, each of the *Y. pestis* alleles was either identical or almost identical to an allele within *Y. pseudotuberculosis*. Age calculations indicated that *Y. pestis* has evolved from *Y. pseudotuberculosis* in the last 10,000 to 40,000 years (Achtman et al., 2004).

During the evolution of *Y. pestis* from *Y. pseudotuberculosis* the acquired genes include one plasmid, a filamentous phage, and a few chromosomal
loci. Derbise et al. (2010) after screening 98 strains of *Y. pestis* and *Y. pseudotuberculosis* found that eight chromosomal loci (six regions and two coding sequences) specified *Y. pestis*. Results suggested that acquisition of new chromosomal material has not been of major importance in the emergence of *Y. pestis*.

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<th>Table 1. Differential characteristics of <em>Y. pestis</em> and <em>Y. pseudotuberculosis</em>.a</th>
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<td>Characteristic</td>
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<td>Motility at 28 °C</td>
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*Y. pestis* is nonureolytic, while all other *Yersinia* species are strong urease producers. Sebbane et al. (2001) reported that *Y. pestis* harbors a complete urease locus composed of three structural (*ureABC*) and four accessory (*ureEFGD*) genes. The absence of urease activity was found due to the presence of one additional G residue in a poly (G) stretch, which introduces a premature stop codon in *ureD*. Spontaneous excision of the extra G was found to occur at a frequency of $10^{-4}$ to $10^{-5}$ and resulted in a urease positive phenotype. The presence or absence of the extra G was found to have no influence on virulence for mice. Urease has been shown to be important for *Y. enterocolitica* survival during passage through the acidic environment of the stomach (Koning-Ward and Robins-Browne, 1995; Gripenberg-Lerch et al. 2000). Since *Y. pestis* is not commonly transmitted by the oral route, the absence of urease activity would not be expected to affect its dissemination from the site of a flea bite.

The classical paradigm of flea-borne transmission of plague

Flea-borne transmission

Under the classical paradigm of flea-borne transmission of plague (Bacot and Martin, 1914) plague bacilli multiply, and block the proventriculus, the
valve-like organ between the esophagus and midgut, thereby blocking the oriental rat fleas (*Xenopsylla cheopis*) flea from feeding. In some fleas, a solid culture of *Y. pestis* was found to form in the proventriculus. Blockage is usually observed 2 to 3 weeks post infection thus constituting a lengthy extrinsic incubation period and prevents newly ingested blood from reaching the midgut, thus causing the fleas to starve, thereby increasing feeding frequency. Newly ingested blood then mixes with bits of *Y. pestis*-bearing material cleaved from the blockage. Regurgitation of this mixture of blood and *Y. pestis*-bearing blockage material back into the site of the mammalian host then resulting in low transmission frequency, and is usually followed by death of the flea. Mechanical transmission by unblocked fleas is also viewed as a transmission mechanism during epizootics (Eisen et al., 2006).

*Y. pestis* cells in the blocked proventriculus of the flea are surrounded by extracellular material that is positive for polysaccharide (Jarett et al., 2004). *Y. pestis* lacking the hmsHFRS operon, which encodes polysaccharide biosynthesis proteins, fails to block fleas (Hinnebusch et al., 1996). *Y. pestis* produces biofilms on the cuticle of the nematode *Ceanorhabditis elegans* that covers the mouth and blocks the worm from feeding on bacteria (Darby et al., 2002; Joshua et al., 2003). Like flea blockage, this process requires *hmSHFR* (Darby et al., 2002). Darby et al. (2005) identified *gmhA* as a gene required for normal biofilm formation. *gmhA* encodes phosphoheptose isomerase, an enzyme required for the synthesis of heptose, a conserved component of lipopolysaccharides and lipooligosaccharides in *Y. pestis*. A *Y. pestis* *gmhA*-mutant was constructed by Darby et al. (2005) and was found to be severely defective for biofilm formation on the surface of *C. elegans* and for flea blockage but only moderately defective in an invivo biofilm assay.

Blockage of the proventriculus is regulated by a group of chromosomal genes called the hemin storage locus (HMS), which is upregulated at ambient temperatures and is not expressed at 37 °C (Eisen and Gage, 2009). HMS is required for successful colonization of the proventriculus, but not for colonization of the flea midgut (Hinnebusch et al., 1996). In contrast, the *Yersinia* murine toxin (YMT), located on the pFra (pMt1) plasmid is a phospholipase required for survival of *Y. pestis* in the midgut of the flea (Hinnebusch et al. 2002). Mechanical transmission by unblocked fleas is also viewed as a transmission mechanism during epizootics (Eisen et al. 2006).

Recent mathematical models have demonstrated that the dynamics of blocked flea transmission are not sufficient to explain the rapid spread of plague epidemics and epizootics (Lorange et al. 2005; Eisen et al., 2006; Webb et al. 2006). Eisen et al., (2007a) found that the rat flea *Oropophylla*
Montana, the primary vector of *Y. pestis* to humans in North America, feeds readily on a daily basis, has a short extrinsic incubation period, and efficiently transmits *Y. pestis* for at least 4 days p.i. Transmission of *Y. pestis* was not observed beyond 7 days after initial exposure in fleas that received a single infectious blood meal, whereas fleas given a booster infectious blood meal 5 days p.i. could transmit throughout the 9 day period of the study.

Lorange et al. (2005) found that the infectivity of *Y. pestis* for the rat flea *Xenopsylla cheopsis* and subsequent transmission efficiency to shaved areas of mice are both low. The authors speculated that poor vector competence of fleas likely imposed selective pressure that favored the emergence and continued maintenance of a hypervirulent *Y. pestis* clone. The authors experimentally determined that the 50% infective dose (ID₅₀) for *X. cheopsis* is 4.8 x 10³ CFU of *Y. pestis*. The average volume of blood ingested by *X. cheopsis* is ~0.12 microliters, and the ID₅₀ of *Y. pestis* for susceptible animals is <10 CFU. Therefore, the threshold septicemic level in a mammal that would provide one ID₅₀ for *X. cheopsis* is 4.8 x 10³ CFU of *Y. pestis* in the 0.12 microliters of blood ingested, which is equivalent to ~4.0 x 10⁷ CFU/ml of peripheral mammalian blood. The authors stated that epidemiological modeling predicts that to compensate for a relatively short period of infectivity of the mammalian host for the arthropod vector, plague epizootics require a high flea burden per host of 9.4, even when the susceptible host population is high.

Eisen et al. (2007b) found that early phase transmission by unblocked oriental rat fleas (*Xenopsylla cheopsis*) was at least as efficient as transmission of blocked fleas, and that an unblocked flea can transmit *Y. pestis* to a susceptible host as early as one day p.i.

In recent decades, the majority of human plague cases have been reported from Africa. In the West Nile region of Uganda, Arua and Nebbi districts have consistently reported human cases of plague. From 1999 though 2007, approximately 223 suspect human cases per year with an annual range of 76 - 467 have been reported from these districts (Eisen et al. 2008a). Cat fleas (*Ctenocephalides felis*) have been reported to be the most common flea in the homes of these districts and occasionally infect potential rodent reservoirs of *Y. pestis* such as the roof rat (*Rattus ratus*) or the Nile rat (*Arvicantis niloticus*). Eisen et al. (2008a) reported that *C. felis* is a competent vector for transmission of plague to laboratory Swiss mice, but that the efficiency of transmission is notably low compared to the oriental rat flea (*Xenopsylla cheopsin*) collected in the same area. The authors concluded that plague control programs should remain focused on reducing rat flea populations, although cat fleas should not be ignored as they could play a significant role as secondary vectors.
Direct examination of infected fleas, in addition to invitro studies with the nematode *Caenorhabditis elegans* has established that *Y. pestis* forms a biofilm in the insect (Darby, 2008). *Y. pestis* was found to form large aggregates on the outer surface of *C. elegans* (Darby, 2002), particularly on the head, covering the mouth which prevented feeding, a functional similarity to *Y. pestis* in fleas. The extracellular matrix of the biofilm appears to contain a homopolymer of N-acetyl-D-glucosamine which is a constituent of many bacterial biofilms. Scanning electron microscopy showed wild-type *Y. pestis* in a matrix attached to spines in the proventriculus (Darby, 2008). In contrast, cells of an *hms* mutant infecting fleas, were absent from the spines.

Hinnebusch et al. (1996) found that the binding of *Y. pestis* to the dissected proventriculus was no different between *Y. pestis* with and without *hms* and that *hms* containing bacteria aggregate in culture and proposed that aggregation is sufficient to plug the proventriculus without specific binding to the flea tissue. Darby (2008) therefore concluded that it is questionable as to whether *Y. pestis* actually results in specific biofilm-mediated adherence to the proventriculus.

Hinnebusch et al. (2002) presented evidence that a plasmid (pFra) encoded phospholipase D (PLD) earlier characterized as *Yersinia* murine toxin (Ymt) was required for survival of *Y. pestis* in the midgut of its principal vector the rat flea (*Xenopsylla cheopis*). Intracellular PLD activity appeared to protect *Y. pestis* from cytotoxic digestion products of blood plasma in the flea gut.

### Recent outbreaks of plague

#### Global incidence

Approximately 3,000 human cases of plague occur worldwide annually. Statistical analysis has revealed that from 1954 to 1998 the largest proportion of reported cases (58.4%) was from Asia. For Africa and the Americas the percentages were 27.8 and 13.8 respectively. Mortality was 54.6% for Asia, 34.4% for Africa and 11.0% for the Americas. There were 47,091 cases and 3,595 deaths in 10 Asian countries during this 44 year period. In 1967–1971, the period of the highest incidence of the disease in the world from 1954 to 1998, the plague situation in the world at large (not solely in Asia) was determined by plague outbreaks in Viet Nam. There were 21,716 human plague cases reported from Viet Nam, comprising 97.2% of cases in Asia and 89.2% of the global total. Increased plague morbidity in Viet Nam was presumably due to disruption of the economy, ecosystem and infrastructure as a result of prolonged armed conflict. A considerable proportion of human
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plague cases occurred in southern Viet Nam where the defoliation of vast areas during military operations is thought to have been one cause of the high incidence (WHO, 1999). During the 44 year period of 1954 to 1998, the total of worldwide cases of plague reported was 80,613 and 6,587 deaths (WHO, 2001). The maximum number of reported plague cases (6004) occurred in 1967 and the minimum (200) occurred in 1981.

There are seven countries, namely Brazil, Democratic Republic of the Congo, Madagascar, Myanmar, Peru, United States of America, and Viet Nam which have been affected by plague virtually every year from 1954 to 1998 (WHO, 2001).

Outbreaks of plague have reappeared in 1994 in Malawi, Mozambique, and India where the disease was not encountered during the previous 15 to 30 years (Drancourt and Raoult, 2002). A global map of recent plague outbreaks and probable loci is presented in Figure 2.

In the U.S., 390 cases of plague were reported from 1947 to 1996, 84% of which were bubonic, 13% septicemic, and 2% pneumonic. Fatality rates were 14%, 22%, and 57% respectively. Most cases occurred in New Mexico, Arizona, Colorado, and California. Among the 15 cases following exposure to domestic cats with plague, 4 were primary pneumonic plague (CDC, 1994).

On July, 30 2010, a total of 17 cases of plague were confirmed in one province of Peru for that year. Among these, four were pneumonic plague, 12 bubonic plague and one was septicemic plague. On August 11, 2009, China reported a cluster outbreak of plague in a remote town of Qinghai.

![Figure 2. Distribution of natural foci of plague. From CDC (1996).](image-url)
province involving 12 patients. The source of this outbreak was reportedly a wild marmot, which had contact with the dog of the index case. As of September 29, 2006, a suspected outbreak of plague was reported by the Democratic Republic of the Congo involving four health zones in a single district. A total of 1174 suspected cases with 50 deaths were involved. As of 9th July 9, 2003 a total of 10 laboratory confirmed cases and 1 probable case of plague in a district of Algeria were reported. In Malawi, 71 cases of bubonic plague were reported as of May 27, 2002 since the onset of an outbreak on April 16, 2002. The outbreak affected 26 villages. In India, 30 cases of pneumonic plague including 4 deaths were reported from a single village in February of 2002 (Joshi et al. 2008; WHO, 2010).

Fever with cough were the most common symptoms. Diagnosis of pneumonic plague was based on conventional biochemical tests on blood agar isolates, phage susceptibility of the isolates, F1 antigen ELISA, and PCR for the pla gene. The index case was a 35-year-old male who had gone hunting in a nearby forest and reportedly killed a wild animal which was then skinned and on the same day developed fever and chills leading to haemoptysis (spitting up of blood or blood tinged mucus). Zambia on March 26, 2001 reported 23 hospitalized cases of plague from a single district, including 3 deaths (WHO, 2010). During February and March of 1998, 12 sudden deaths were reported among residents of a high Andean community in Ecuador (Gabastou et al. 2000). Symptoms included fever, headache, sore throat, abdominal pain, myalgias, and cough progressing to haemoptysis, symptomatic of pneumonic plague. All 12 fatalities were members of the same extended family and some had apparent exposure to a sick guinea-pig. Guinea-pigs are a common food source in the central Andean region and are raised freely in the homes. Five of 14 dogs in the community were seropositive for plague antibody, providing evidence of a recent epizootic plague in the area.

On August 11, 2009, China reported a cluster outbreak of plague in a remote town of Qinghai province involving 12 patients. The source of this outbreak was reportedly a wild marmot, which had contact with the dog of the index case. From 1987 to 1997 the annual reported incidence of human plague in China has varied from 6 to 98 (WHO, 2000). Zou et al. (2004) have reviewed in considerable detail the status of plague in China. From 1644 to 1899, human plague epidemics spread in 13 provinces involving 202 counties. From 1900 to 1949, another seven provinces were added to the list of foci. According to available records, the total number of patients exceeded
2.5 million, with about 2.2 million deaths. Human plague has reportedly been well controlled in China since the 1950s. However, as of 2004, 11 kinds of natural plaque foci still remained, encompassing 277 counties in 19 provinces. By the end of 2001, 87 species of mammals were identified to be naturally infected with *Y. pestis* and 13 (II rodents) were determined to be primarily reservoirs. Forty-one species of fleas were found to be infected with *Y. pestis* in nature and 14 were determined to be primary vectors. A unique eco-typing system which utilized several biochemical features, including glycerin, rhamnose, maltose, melibiose, and arabinose fermentation, nitrate reduction, amino acid utilization, mutation rate from Pgm\(^+\) to Pgm\(^-\), and water soluble protein patterns on SDS-PAGE of presumably cell lysates was used to establish 18 ecotypes. Each of these ecotypes is located in a particular geographic region and is identifiable on the basis of these phenotypic properties. This unique eco-typing system has served as a framework for ecological and epidemiological analysis of plague in China with respect to environmental factors, reservoirs of infection, and vectors. DNA microarray-based genomic comparisons revealed 18 islands are commonly harbored in both *Y. pestis* and *Y. pseudotuberculosis* with an additional three present only in *Y. pestis* (Zou et al., 2004). Interestingly, all three of these islands were found to encode prophages. Most of the geographic regions with different primary reservoirs in China have their unique genovars, with each of the genovars corresponding to a unique set of natural environmental factors and primary vector(s).

The three classes of biovars of *Y. pestis* are *Antiqua*, *Orientalis*, and *Medievalis*. In China, a fourth biovar designated *Microtus* has emerged. *Microtus* strains belong to biovar *Medievalis* according to glycerol and nitrate metabolism. In addition, all *Microtus* strains are unable to utilize arabinose while all other *Y. pestis* strains can. Table 2 presents the differential phenotypic distinctions between the four biovars. These biovars can be distinguished depending on their abilities to ferment glycerol and reduce nitrate. The Medievalis biovar is unable to reduce nitrate due to a G to T mutation that results in a stop codon in the *napA* gene (Achtman et al., 2004), while the Orientalis biovar cannot ferment glycerol because of a 93-bp deletion in the *glpD* gene (Achtman et al., 2004; Motin et al., 2002). Conversely, the Antiqua biovar is capable of performing both reactions (Devignat, 1951). Devignat (1951) proposed that each of the three plague pandemics have been caused by a different biovar. However, recent ancient DNA analysis of samples from the 7th, 9th, and 18th centuries yielded Orientalis-specific microsatellites (Drancourt et al., 2004) and that the characteristic 93-bp *glpD* deletion (Drancourt et al., 2007) thus suggesting that the Orientalis biovar also caused Justinian’s plague and the second pandemic. Despite these results, a debate continues regarding whether *Y.
pestis really was the causative agent of the “Black death”. Biovar Medievalis is believed to be linked with the Back Death pandemic. There is no evidence that human plaque can result from Microtus strains, although Microtus plague epizotic outbreaks occur annually in the two Microtus-foci in China. In addition, it has been demonstrated that strains from one Microtus focus were avirulent to humans by s.c. inoculation (Zhou et al., 2004). Microarray analysis by Zhou et al. (2004) identified five deletions of genomic regions specific to Microtus gene loss, unique to Microtus strains. In addition, microarray analysis revealed that all Microtus strains tested have almost identical genomic contents. PCR and gene sequencing of 12 genes indicated that all the Microtus strains tested, possessed the same mutations, while all other (non Microtus) strains of Y. pestis have these genes intact. The Microtus strains have therefore accumulated massive unique mutations resulting in the inactivation of functional genes, reflecting the process of reductive evolution.

Plague was originally introduced into Madagascar in 1898, during the third pandemic and still remains an acute public health problem for this African nation. From 1996 to 1998, 5,965 patients with suspected plague were identified in 38 districts (WHO, 2000; Chanteau et al. 2000). Standard bacteriological procedures yielded 917 as confirmed or presumptive. More than 2,000 plague cases were confirmed using an F1 antigen assay. Bubonic plague accounted for 97.2% of the cases. Two of the 711 Y. pestis isolates were resistant to chloramphenicol and ampicillin respectively. Lethality was 20% among the confirmed and presumptive patients and was 63.6% among unknown clinical forms, comprised presumably of septicemic cases or unexamined deaths. The explosive increase of over 10-fold compared to previous years is thought to be due to a reduction in resources devoted to

<table>
<thead>
<tr>
<th>Biovar</th>
<th>Glycerol fermentation</th>
<th>Nitrate reduction</th>
<th>Arabinose fermentation</th>
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</thead>
<tbody>
<tr>
<td>Medievalis</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Orientalis</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Antiqua</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Microtus</td>
<td>+</td>
<td>-</td>
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public hygiene and the low socioeconomic conditions of the majority of the 5 – 6 million inhabitants.

In Madagascar, among the ~2,000 cases reported each year, very few are of the pneumonic type, which usually arises from direct transmission of Y. pestis through infective cough droplets. Ratsitorahina et al. (2000) reported the details of an exceptional outbreak of pneumonic plague that occurred in a remote village of Madagascar, where plague has been endemic for many decades. On October 30, 1997, eight patients with pneumonic symptoms suspected of having pneumonic plague were hospitalized. Sputum samples were positive by the F1 dipstick assay. Treatment was with 0.5g of streptomycin every 3 hrs for 2 days, followed by 0.5g every 4 hrs for 2 days, and 1 g twice a day for 4 days. The index case, a woodcutter, on October 14 complained of fever, tender axillary adenitis, chest pain, and exhibited cough and blood-stained sputum (clinical signs of pneumonic plague). A local healer on October 19 incised the patients epigastric region and sucked out some blood, the patient died early the next morning. The healer succumbed from pneumonic plague on October 26. The eight treated patients, consisted of the healer's brother (who succumbed shortly after admission) and 7 other local villagers who recovered. A total of 18 patients were identified.

In the U.S.A. 12 to 15 cases of plague are reported each year in western U.S., primarily in the states of California, New Mexico, Arizona, and Colorado (WHO, 1999b). In August of 2012 a 7 year old girl was stricken with bubonic plague after going camping in southwest Colorado. She had encountered a dead squirrel. Insect bites were present on her torso. Initial symptoms were flue-like leading to an elevated heart rate and a swollen lymph node in the groin. Antibiotic therapy was successful. Two other confirmed cases, one in New Mexico and one in Oregon and one probable case in Oregon were reported as of September 6, 2012.

In early June of 2012, a 59 year old man in Oregon attempted to remove a rodent from the mouth of his cat and subsequently developed plague resulting in extensive gangrene of the fingers and toes with marked necrotic blackening of the tissue which required multiple digit amputation.

The plague has been reported to be endemic among rodents inhabiting lava caves in California (Nelson and Smith, 1976).

**Ecology and transmission of Y. Pestis by rodents other than rats**

**Soil and rodent burrows**

For many decades there has been much speculation as to how Y. pestis can persist quiescently in the environment and periodically give rise to
outbreaks of plague. The classic concept of plague transmission involves human infection transmitted by fleas from infected rats. Transmission of plague by cloths was chronicled in 1665, with body lice or human fleas suspected as the cause of this transmission (Baltazard, 1956). In the 1940's, human infected body lice were collected from septicemic patients during familial plague in south Morocco and remained infectious for 7 to 10 days, (Blanc and Baltazar, 1941a; Blanc and Baltazard, 1941b; Blanc and Baltazard, 1942; Blanc and Baltazard, 1943).

The possibility that \textit{Y. pestis} can persist in the soil and rodent burrows for prolonged periods of time has given rise to a number of studies in this area. Karimi (1963) reported that \textit{Y. pestis} could be isolated from a chamber in a gerbil burrow in which an animal had died of plague 11 months earlier after closure of the burrow. In addition, rodents have been shown to acquire plague by burrowing into soil experimentally infected several days earlier (Mollaret et al., 1963). The possibility that \textit{Y. pestis} may survive intracellularly in soil protozoa has also been proposed (Nikul'shin et al., 1992; Pushkarva, 2003, Baltazard, 1971).

Mollaret (1963) found that \textit{Y. Pestis} survives in the litter and soil of the burrows of infected animals for years which can serve as reservoirs for future rodent infections. He also reported survival of \textit{Y. pestis} for 16 months in autoclaved soil and indicated that hydration of soil before experimental inoculation was necessary for \textit{Y. pestis} survival (Mollaret, 1965).

Suchkov et al. (1997) observed that \textit{Y. pestis} inoculated into sterile soil in association with protozoa, algae, and euglena entered into the nonculturable state within 7 days. Few CFU were detected after 7 days, while the PCR detected $10^4$ to $10^5$ CFU/ml of soil at the end of 30 days. After nutritional enrichment of the nutrient medium with fetal serum the complete reversal of \textit{Y. pestis} nonculturable forms occurred yielding $10^5$ CFU/ml of soil.

These reports led Drancourt, Houhamdi, and Raoult (2006) to hypothesize that soil may be the ultimate \textit{Y. pestis} reservoir and that human ectoparasites (fleas and lice) may be one of the driving forces in huge epidemics. Such a concept is viewed as supplementing rat plague in the presence of a human population heavily infested by ectoparasites, resulting in the large pandemics of the past.

Kuske et al. (2006) reported on the results of a soil survey for detection of \textit{Y. pestis}. Extracts of 129 soil samples from 32 states and the district of Columbia in the U.S.A. from a wide variety of terrains (urban, suburban, agricultural, desert, forests) were subjected to the PCR. The primers GA-YP2F/GA-YP1R (Table 1, chapter 3) amplified a 704-bp sequence of the 16s rRNA gene for detection of members of the genus \textit{Yersinia}. Primers Caf1-f/Caf1-R (Table 1, chapter 3) amplified a 388-bp sequence of the caf1 gene encoding the F1 capsular antigen. Three of the soil samples produced the
704-bp amplicon using the 16S rRNA gene primers specific for the genus \textit{Yersinia}. Sequence analysis confirmed nonpathogenic \textit{Yersinia} species. One soil sample produced the 388-bp amplicon using the Caf1 primer pair. However, this soil sample failed to produce an amplicon with the \textit{Yersinia} specific 16S rRNA gene primers, suggesting that \textit{Yersinia} species were not present or were below detection limits. These results also suggest that the F1 capsule protein of \textit{Y. pestis} may be related to capsule proteins of other bacteria. Unfortunately, no soil samples were knowingly obtained from known geographic areas of the U.S.A. where outbreaks of plague have occurred over the past 20 years.

Ayyadurai et al. (2008) investigated the ability of a virulent \textit{Y. pestis} Orientalis strain to persist alive and virulent in soil. Sterilized soil was inoculated with $10^6$ CFU/g and held at ambient temperature. After 40 weeks in humidified soil, \textit{Y. pestis} was extracted from the soil and injected intraperitoneally (i.p.) into mice, all of which succumbed from systemic infection within 72 hrs. postinfection (p.i.) with extensive internal organ necrosis. Control mice inoculated with a similar extract from unseeded steam sterilized soil survived. \textit{Y. pestis} was in turn isolated from the blood of systemically infected and dead mice and PCR assays targeting the \textit{pla}, \textit{ureD}, and \textit{caF1} genes of \textit{Y. pestis} used to confirm the identity of the reisolated organism. The PCR primers \textit{pla-F/pla-R}, amplified a 480-bp sequence of the plasminogen activator gene \textit{pla}; the primers \textit{ureD-F/ureD-R}, amplified a 402-bp sequence of the urease gene; and the primers \textit{caF1-F/caF1-R} amplified a 388-bp sequence of the F1 capsular antigen gene \textit{caF1} (Table 1, chapter 3). In Madagascar, anecdotal evidence has reported observations of pneumonic plague cases among persons attending ritual excavation of plague corpses in the absence of direct contact with the dead (anonymous, 1906).

Eisen et al. (2008b) reported that the death of a mountain lion from plague in Grand Canyon National Park in 2007 and the subsequent death of a wild-life biologist from plague who came into contact with the carcass. A large pool of blood in the soil under the animals mouth and nose was observed. Three weeks later, about 200 ml of soil from this location at a depth up to ~15 cm from the surface was obtained. The soil was found positive for the presence of \textit{Y. pestis} and resulted in mouse lethality, F1 antigen detection of isolates. Biotyping indicated the responsible strain was an Orientalis biotype. Pulsed-field gel electrophoresis indicated that the same strain was responsible for both deaths. Details regarding the quantitative presence of blood in the soil and CFU counts of \textit{Y. pestis} per gram of soil were not presented, and at best this was a limited study and short term study of persistence of \textit{Y. pestis} in soil. It does however indicate that \textit{Y. pestis} can persist in soil in a viable state at least for several weeks.
Alternate rodent hosts other than rats

Plague was first discovered in New Mexico, USA in ground squirrels known as Gunnison's prairie dogs in 1938. Plague is found throughout the state, and by 1982 had been reported in 31 of 33 counties. In New Mexico, 21 species of rodents and 16 species of fleas have been found infected with *Y. pestis* (Weber, 1978). Plague was first recorded in the Moreno Valley, in 1949 when it was found in prairie dogs. There were no further observations of plague until 1983 when a teenage boy became ill with plague and died. Fleas collected at the time from prairie dog and ground squirrel burrows indicated *Y. pestis* positive fleas associated with thirteen-lined ground squirrels, Gunnison's prairie dogs, and a deer mouse.

A plague epizootic spread through Gunnison's prairie dogs and possibly other rodent species, in the Moreno valley (100 km²) in North-Central New Mexico between winter 1984 - 1985 and autumn 1987. At two towns, the prairie dogs were trapped, marked, and released prior to the epizootic (Cully et al. 1997). At two additional towns, prairie dogs were marked following the epizootic. In 1988, a second plague epizootic occurred in one town. One hundred thirty-nine serum samples were collected from prairie dogs and other rodents and 1,750 fleas were collected from animals and burrows. Fleas infected with *Y. pestis* were collected from prairie dogs, deer mice, and ground squirrels. A total of six species of fleas were collected from prairie dog burrows, several of which are normally associated with other rodents. Among serum samples collected from Gunnison's prairie dogs, ground squirrels, deer mice and meadow voles, elevated serum titers against *Y. pestis* were found only in prairie dogs. Between September 1984 and May 1987, the Gunnison's prairie dog population in the Moreno Valley was reduced from more than 100,000 animals to between 250 - 500, representing prairie dog mortality at all four towns 99.5%. Serum antibody titers indicated that more than 40% of the few surviving prairie dogs left to establish new colonies following epizootics survived infection with plague. The presence of *Y. pestis* antibodies in a large proportion of survivors of the epizootics indicates that these animals were exposed to *Y. pestis*, survived, and presumably were immune to plague. The authors concluded that with the high rates of mortality among Gunnison's prairie dogs in the Moreno Valley, it is highly unlikely that this rodent serves as a maintenance host species for plague between epizootics. Seventy-three genera and more than 200 species of rodents worldwide have been found naturally infected with *Y. pestis* (Poland and Barnes, 1979). Weber (1978) identified 30 rodent species, two rabbit species, and six wild carnivorous species implicated by infection or antibodies to *Y. pestis* in New Mexico.
Collinge et al. (2005) reported on a study that attempted to correlate landscape structure and the occurrence of plague in black-tailed prairie dogs on grasslands in Boulder County, Colorado, and Phillips County, Montana. Roads, streams, and lakes were found to serve as barriers to plague among black-tailed prairie dog colonies. Colorado prairie dog density was found to increase as surrounding urbanization increased (Johnson and Collinge, 2004), due presumably to urban obstacles such as roads preventing the spread of plague.

Anderson and Williams (1997) documented plague in a complex of white-tailed prairie dogs and other small mammals trapped during a field study near Meeteetse, Wyoming, USA in 1989 and 1990 to investigate the dynamics of plague in this rodent population. Fleas were identified and tested for \textit{Y. pestis} by mouse inoculation. \textit{Y. pestis}-positive fleas were found on prairie dogs and in their burrows. Flea species on prairie dogs changed from spring to late summer. White-tailed prairie dog numbers were significantly lower in the presence of \textit{Y. pestis} positive fleas. However, affected populations generally recovered 1 to 2 years following the absence of detectable plague.

Grids where recovery from plague occurred had a high proportion of juvenile male prairie dogs. Eighteen flea species were identified on small mammals, six of which were infected with \textit{Y. pestis}. Each summer the complex was found to be a mixture of prairie dog colonies variously impacted by plague, with some declining, some free of plague, and others recovering from population declines due to plague. \textit{Y. pestis} positive fleas were also found on dessert cotton tail rabbits, ground squirrels, deer mice, and were collected from white-tailed prairie dog burrows. In 1990, \textit{Y. pestis} positive fleas were collected from the burrows of five towns on the Meeteetse complex. Deer mice were the most numerous associated mammal in the study. Fitzgerald (1970) suspected that both deer mice and ground squirrels were serving as reservoir species in a prairie dog epizootic. Karami (1981) viewed deer mice as maintenance hosts in many prairie dog plague epizootics due to their abundance and resistance to plague mortality compared to many other rodents. It is clear that in western areas of the USA, plague is endemic among rodents other than rats which directly or indirectly may account for the annual incidence of human plague afflicting several dozen individuals yearly.

Snäll et al. (2008) reported on the climate-driven spatial dynamics of plague among black-tailed prairie dog colonies in Phillips County, Montana, USA. The rate of plague transmission among prairie dogs was found to increase with increasing precipitation, while the rate of infection from unknown sources decreased in response to hot weather.

Hoogland et al., (2004) reported that the infusion of prairie dog burrows with Pyraperm (an insecticidal dust) killed fleas and immediately halted the spread of plague within colonies.
An extensive study of the effect of climate variation on plague dynamics of great gerbils was undertaken by Stenseth et al. (2006) in an area of Kazakhstan utilizing data from 1949 to 1995. The study was based on the fact that desert regions of Central Asia are known to contain natural foci of plague where the great gerbil is the primary host. The spread of plague in these areas is known to require both a high abundance of hosts and a sufficient number of active fleas as vectors transmitting *Y. pestis* between hosts. Davis et al. (2004) documented the presence of an abundance threshold of hosts in this system, below which plague is unable to invade or persist. From the analysis of field data collected between 1955 and 1996 in Kazakhstan they found that plague invades, fades out, and reinvades in response to fluctuations in the abundance of its main reservoir host, the great gerbil (*Romboymys opinus*). Data analyzed by Stenseth et al. (2006) indicated that when gerbil abundance is above the threshold, increasing spring temperature leads to an increased prevalence of plague in the spring. In addition, increased summer precipitation was correlated with the fall prevalence of plague. Additional data indicated that this climate forcing effect on prevalence of plague is mediated through fleas. The flea burden of gerbils was found to correlate with climatic variables in that spring flea burden negatively correlated with the number of days with frost on the soil in spring and positively correlated with spring temperature. The fall flea burden was found to be positively correlated with summer relative humidity. Spring temperature is relevant because fleas are only active when the air temperature is above ~10 °C. Increased host attack rate, migration to burrow entrance, egg maturity, and egg production by adults can thus start earlier and may last longer when spring warmth comes early. Increased humidity during the summer months results in more fleas and the enhanced transmission of plague. Data allowed prediction that an increase of 1 °C will result in an average increase in plague prevalence of 59%. Interestingly, an increase in summer rainfall of 10% was predicted to increase prevalence of plague by only 7%. Changes in spring temperature were therefore found to be the most important environmental factor influencing prevalence of plague among great gerbils. The data suggested that warmer spring and wetter summers can trigger a cascade effect on the occurrence and level of plague prevalence in years with above-threshold levels of great gerbil abundance during the fall two calendar years earlier in a region that is itself dry with hot summers and cold winters. Data also indicated that enhanced flea survival and reproduction are also critical factors.

In Kazakhstan, *Y. pestis* circulates in natural populations of gerbils, which are the source of human cases of bubonic plague. Eisen et al. (2008c) evaluated the likelihood that *Y. pestis* could be spread from mouse to mouse by the flea *Aetheca wagneri*, a common flea species
infesting deer mice. *A. wagneri* was found capable of transmitting *Y. pestis* to Swiss laboratory mice as early as 3 days p.i., but transmission efficiency was notably low (1.0%). A predictive model estimated that at least 68 *A. wagneri* per deer mouse would be required to support levels of transmission adequate for enzootic maintenance of plague. Because deer mice typically harbor fewer than three *A. wagneri* per host Eisen et al. (2008c) concluded that an independent deer mouse-*A. wagneri* transmission cycle is unlikely. In addition, laboratory challenge with up to $10^7$ CFU of *Y. pestis* inoculated into deer mice i.c. revealed that 82% of the hosts were resistant to infection (Quan and Kartman, 1962). Deer mice are therefore able to survive infection and presumably limit any bacteremia to a level below $1 \times 10^6$/μl of blood which is required to infect fleas. Also, seropositive mice were observed only after, rather than preceding plaque epizootics in prairie dogs (Salkeld and Stapp, 2008). In contrast, the flea species *Oropsylla montana* vector efficiency has been shown to be sufficient to maintain enzootic transmission of plague in ground squirrels or rock squirrels (Eisen et al., 2006).

**Food, pet and large animal borne plague**

On rare occasions, food borne oropharangeal plague has resulted from handling or consumption of inadequately cooked goat and camel meat (Arbaji et al., 2005; Bin Saed et al., 2005; Christie et al, 1980). The consumption of meat contaminated with *Y. pestis* can cause oropharangeal plague in humans meat (Arbaji et al., 2005; Bin Saed et al., 2005; Christie et al, 1980). The occurrence of multidrug resistant strains Gailmand et al., 1997) and the potential for deliberate contamination of food with *Y. pestis* prompted Bhaduri (2010) to study the stability of the virulence plasmid pYV in ground beef at varying temperatures and fat content. *Y. pestis* strain KIM5 was used, and is a derivative of a fully virulent strain KIM which lacks the chromosomally-encoded pigmentation (Pgm') locus, but contains all three virulence plasmids (Beardon and Perry, 2008). The KIM5 strain is conditionally virulent in that it is infectious only when inoculated intravenously and can be used in a BL2 laboratory facility. The KIM5 strain was inoculated at a level of ~$10^3$ CFU/g into ground beef previously sterilized by irradiation containing 7, 15, and 25% fat. Inoculated samples were then incubated at 0, 4, 10, and 25°C. Results indicated that the KIM5 strain did not grow in raw ground beef at 0 and 4°C but did survive during a two month period and did grow at 10 and 25°C.

In February of 1976, in a small remote village in Libya, an apparently sick camel was slaughtered and skinned, and camel meat distributed for human consumption. A few days later, 13 villagers suffered severe febrile
illness. All five individuals involved in the slaughter and dispensing of the camel were dead within four days (Christie et al. (1980). One additional individual succumbed from consuming the camel meat. Serum from the 7 surviving patients were all positive for *Y. Pestis* as determined by F1 antigen detection and by passive hemagglutination assay. The seven seropositive patients and the one who was not serologically tested and who succumbed, all had buboes in the neck, axilla, and groin. The 7 surviving patients were satisfactorily treated with tetracycline, streptomycin, or both. In late June of 1976, a 12 year-old boy in Libya contracted plague in a village three kilometers distant from the first outbreak. He had skinned a sick goat, developed an axillary bubo and recovered with treatment. Sera from four goats were positive for *Y. pestis*. In early June of 1976, five individuals from a single household in a village in Libya 100 kilometers from the first outbreak presented with symptoms of severe plague and recovered after treatment. A day or two before the onset of symptoms the father had killed and skinned a sick goat, after which the skin was treated by the women of the household and kept in the house. Sera of four of the patients were positive for *Y. pestis*. Recommended antibiotics and dosages are given in Table 3.

Bin Saeed et al. (2005) reported on the investigation of a cluster of plague cases in a town of Saudi Arabia. The patients included a nine year old girl and three adults with severe pharyngitis and submandible lymphadenitis. These patients had eaten raw camel liver. The young girl and the index patient succumbed. A fifth patient without pharyngitis had lymphadenitis and cellulitis of his right arm as a result of cutting his arm while killing the sick camel. *Y. pestis* was isolated from the bone marrow of the leftover camel bones. *Jirds (Meriones libycus)*, rodent burrows, and rodent excreta were found at the camel corral. *Y. pestis* was isolated from the blood and liver of live jirds collected from the camel corral and from fleas combed from the jirds.

In January and February of 1997, an outbreak of pharangeal plague occurred in a Jordanian village Arbaji et al. (2005). Eleven of the 12 patients were hospitalized and found to have bilateral cervical lymphadenopathy, with each enlarged lymph reaching the size of a small hens egg. All 12 cases were successfully treated with gentamycin and were found to have sera that tested positive for *Y. pestis*. Bubonic plague was historically endemic in the Jordan valley in the 7th century AD. Since 1921, not a single case of plague had been reported in the area. The village of 3,200 inhabitants is located in a desert oasis where most of the population traditiona lly eat raw liver and other raw meat from domestic animals. Two days before the onset of symptoms, 11 of the patients consume meat from the same camel. Among sera collected from 17 local dogs, 3 (17.6%) were found positive for antibodies against *Y. pestis*. 
Table 3. Recommendations for prophylactic antimicrobial therapy for person exposed to \textit{Y. pestis}, by agent and age. From CDC (1996). ^a

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Age</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline\textsuperscript{b}</td>
<td>Adults\textsuperscript{c}</td>
<td>2 g/day in two or four equal doses of 12- or 6-hour intervals for 7 days, by mouth</td>
</tr>
<tr>
<td></td>
<td>Children\textsuperscript{d}</td>
<td>25 - 50 mg/kg/day in two or four equal doses at 12- or 6 hour intervals, respectively, for 7 days, by mouth</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>Adults\textsuperscript{c}</td>
<td>100 - 200 mg/day in two equal doses at 12-hour intervals for 7 days, by mouth</td>
</tr>
<tr>
<td></td>
<td>Children\textsuperscript{d}</td>
<td>2 - 4 mg/kg/day in two equal doses at 12-hour intervals for 7 days, by mouth</td>
</tr>
<tr>
<td>Trimethoprim- \textsuperscript{e}</td>
<td>Adults\textsuperscript{c}</td>
<td>1.6 - 3.2 g/day</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>Adults\textsuperscript{c}</td>
<td>Sulfamethoxazole component in two equal doses at 12-hour intervals for 7 days, by mouth</td>
</tr>
<tr>
<td></td>
<td>Children\textsuperscript{d}</td>
<td>40 mg/day</td>
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<td></td>
<td></td>
<td>Sulfamethoxazole component in two equal doses at 12-hour intervals for 7 days, by mouth</td>
</tr>
</tbody>
</table>

^aInformation is for single exposures.
^bUse of tetracycline during pregnancy should be avoided.
^cPersons ≥ 18 years of age
^dPersons 9 - 17 years of age
^ePersons ≥ 2 months to 18 years of age

Eidsen et al. (1988) reported on the epidemiological features of feline plague from 1977 to 1985 in New Mexico. Among 60 cases of feline plague the most frequent clinical symptoms were lethargy, anorexia, fever, and enlarged lymph nodes or abscesses. A history of hunting rodents was reported in 75% of the cases. Five persons associated with five of the 1977 to 1985 feline cases developed plague.

Plague in Zimbabwe (formerly Rhodesia) was first ported in humans in 1974. Gordon et al. (1979) collected serum from 330 elephants, 391 buffalo, 16 zebras, and 5 sable antelope in the Wankie National Park Zimbabwe. Among the buffalo, 6.6% were positive for \textit{Y. pestis} F1 hemagglutinating antibodies, 0.3% of the elephants were positive, while
none of the zebra or sable antelope were positive. The relatively high percentage of positive buffalo clearly demonstrates the exposure of large wild animals to plague during the course of an epizootic. These reports alter considerably the general concept that rodents and fleas are the primary vectors of plague in humans.

**Molecular taxonomy of Y. pestis**

**Derivation of Y. pestis from Y. pseudotuberculosis**

*Y. pestis* is now considered a subspecies of *Y. pseudotuberculosis* on the basis of DNA/DNA hybridization studies and 16SrDNA sequence analysis (Ibrahim et al., 1993; Bercovier et al., 1980). Recent studies have estimated that *Y. pestis* emerged as a separate clone within *Y. pseudotuberculosis* 1,500 to 20,000 years ago shortly before the first known pandemic (Achtman et al, 1999).

The transformation of *Y. pseudotuberculosis* in the plague bacillus *Y. pestis*, has been accompanied by extensive genetic loss. Gouillot et al. (2008) characterized the chromosomal regions conserved in *Y. pseudotuberculosis* and lost during transformation into *Y. pestis*. Five regions (R1 to R5) and four open reading frames (*orf1* to *orf4*) were found to be conserved in *Y. pseudotuberculosis* and absent from *Y. pestis*. Four loci (*orf2*, *R2*, *R4*, and *R5*) were not required for optimal growth or virulence in *Y. pseudotuberculosis*. In contrast, *orf2*, encoding a putative pseudouridylate synthase involved in RNA stability was necessary for optimal growth at 37 °C in a chemically defined medium. Deletion of R1, a region predicted to encode the methionine salvage pathway, reduced pathogenicity of *Y. pseudotuberculosis* somewhat, suggesting that the availability of free methionine is severely restricted in vivo. R3, a region composed mostly of genes of unknown functions, was necessary for both optimal growth of *Y. pseudotuberculosis* at 37 °C and for mouse virulence. Therefore, despite their absence in *Y. pestis*, five of the nine *Y. pseudotuberculosis*-specific chromosomal loci studied played a role in the survival, growth, or virulence of this species.

**Biovars (Biotypes)**

Four biovars named *Antiqua*, *Medievalis*, *Orientalis*, and *Microtus* have been distinguished among *Y. pestis* strains collected during the 20th century on the basis of their ability to reduce nitrate and to ferment glycerol and arabinose (Table 2). Molecular analysis of a collection of 72 strains of *Y.
pestis belonging to the first three biotypes collected over a 72-year period established correlations between the ribotypes and the biotypes of strains (Guiyoule et al., 1994). The combination of EcoR1 and EcoRV patterns resulted in 16 ribotypes. In particular, two ribotypes, designated B and O, were found to comprise 65.7% of the strains; ribotype B comprised all of the biotype Orientalis isolates, while ribotype O comprised biotypes Antiqua and Medievalis isolates (Guiyoule et al., 1994). Ribotype B (biotype Orientalis) was found on all five continents, while ribotype O (biotypes Antiqua and Medievalis) was restricted to Central Africa and Central Asia, presumably reflecting the spread of biotypes during each of the three pandemics (Devignat, 1951). Great heterogeneity in rRNA restriction patterns was found among strains isolated from Africa (Guiyoule et al., 1994). This heterogeneity was less pronounced among Asian isolates and was completely absent from the American isolates. Ribotyping has supported the premise that biotype Antiqua, resident in Africa, is descended from the strain that caused the first pandemic arising out of the Central Asian plateau (Devignat, 1951). Later, a second clone (Biovar Medievalis), which lost the ability to reduce nitrate, presumably spread from Central Asia to the Crimea and was responsible for the "Black death" of the second pandemic. Later, a third clone (biovar Orientalis) spread from Asia causing the third pandemic. Isolates linked to the third pandemic are all Orientalis and are currently widespread. Sequence analysis of five housekeeping genes in Y. pestis has lent support to this hypothesis (Achtman et al., 1999). Achtman et al., (1999) analyzed the population genetic structure of Y. pestis (36 strains), Y. pseudotuberculosis (12 strains), and Y. enterocolitica (13 strains). Fragments of 5 housekeeping genes and a gene involved in the synthesis of LPS were sequenced. No sequence diversity was found in any Y. pestis gene, and these alleles were found identical or nearly identical to alleles from Y. pseudotuberculosis. Thus, Y. pestis is a highly uniform clone that evolved from Y. pseudotuberculosis 1,500 to 20,000 years ago, shortly before the first known pandemic of human plague.

The three Y. pestis biovars, Antiqua, Medievalis, and Orientalis are unable to ferment rhamnose. However, in addition to these organisms there is a group of Y. pestis isolates distributed in the former USSR, Mongolia, China, and Morocco that share certain characteristics with the closely related species Y. pseudotuberculosis. These isolates ferment rhamnose, are also dependant on additional nutrients, and are less virulent in guinea pigs but are highly virulent in mice. These strains occasionally cause human or animal plague but have been rarely associated with epizootics of plague. To separate these rhamnose-positive isolates from the main group of Y. pestis strains it has been proposed that they be named Y. pestoides. Such rhamnose-positive
isolates belong phenotypically to the biovar Antiqua. *Y. pestis* G8786, a rhamnose-positive strain was isolated from the high mountainous Caucasian focus. Microarray analysis reflected the remote origin of this isolate and the highest level of divergence from other *Y. pestis* strains (Hinchliffe et al. 2003).

The 137,036-bp plasmid pG8786 from rhamnose-positive *Y. pestis* G8786 is an enlarged form of the pFra virulence-associated plasmid containing genes for synthesis of F1 and phospholipase D. Golubov et al. (2004) sequenced the whole nucleotide sequence of the enlarged pFra plasmid of the rhamnose-positive *Y. pestis* strain G8786 to elucidate its evolutionary origin and its divergence from the pFra plasmids of other *Y. pestis* strains. In addition to the completely conserved genes of the pFra backbone, pG8786 was found to contain two large regions consisting of 4,642 and 32,617-bp, designated regions 1 and 2, respectively. The authors concluded that pG8786 appears to be an ancient form of the pfra group of plasmids that were conserved due to the strict geographical isolation of rhamnose-positive *Y. pestis* strains in the high mountainous Caucasian plague locus.

During the third plague pandemic which started in Hong Kong in 1984, *Y. pestis* colonized new, previously uninfected geographic areas where it has become well established. Giyoule et al. (1997) undertook an extensive study of the genetic stability of *Y. pestis* strains introduced into Madagascar during the third pandemic. The study involved ribotyping 187 strains of *Y. pestis* isolated between 1939 and 1996 from different regions of Madagascar and responsible for mainly human cases of bubonic and pneumonic plague. All *Y. pestis* strains isolated from Madagascar before 1982 were of the classical ribotype B, the ribotype involved in the third pandemic. In 1982, 1983, and 1994, strains of new ribotypes, designated R, Q, and T respectively were isolated on the high plateau region of the island. Restriction profiles revealed that the new variants could also be distinguished by specific genomic and/or plasmid profiles. Strains of ribotypes Q and R were found to be well established in their ecosystem and to have a tendency to spread to new geographic areas and to supplant the original classical strain.

**Y. pestis** as an agent of bioterrorism

**Aerosolized plague**

Since *Y. pestis* does not form spores as *Bacillus anthracis* does, its mechanism of dispersal would be as a liquid aerosol and not as a dry powder. The working Group on Civilian Biodefense (Inglesby et al., 2010) concluded that an aerosolized plague weapon could cause fever, cough, chest pain, and hemoptysis with signs consistent with pneumonia 1 to 6 days after exposure.
Rapid evolution of disease would occur in 2 to 4 days after symptom onset and would lead to septic shock with high mortality without early treatment. Early treatment with streptomycin, gentamycin, tetracycline, or a floroquinone such as ciprofloxacin was advised. Inglesby et al., 2010 have reviewed in detail the efficacy of various antibiotics and those to be avoided. In 1979, the World Health Organization (WHO, 1970) reported that in a worst case scenario, if 50 kg of *Y. pestis* were released as an aerosol over a city of 5 million, pneumonic plague could occur in as many as 150,000 persons, 36,000 of whom would be expected to die. The organism would remain viable as an aerosol for up to 1 hr. for a distance of up to 10 km.

**References**


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History, clinical aspects, epidemiology of plague and characteristics of *Y. pestis*


