

# Chapter 9

## Plague in the 21st Century: Global Public Health Challenges and Goals

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### 9.1 Introduction

*Yersinia pestis*, the Gram-negative bacterial agent of plague, is a zoonotic pathogen that primarily infects wild rodents and is transmitted by fleas. *Y. pestis* is one of the most invasive and virulent bacterial pathogens and has caused devastating pandemics, including the Black Death of 14th century Europe. The last plague pandemic began in Asia in the last half of the 19th century and lingered well into the 20th century, causing tens of millions of deaths as it spread across the world.

Three main forms of plague occur in humans. Bubonic plague, the most common, usually follows transmission by flea bite. Bacteria deposited in the dermal bite site travel through the lymphatic system to the regional draining lymph node. There, bacterial multiplication leads to the swollen, painful lymph node known as a bubo. At this stage, the disease can usually be treated successfully with antibiotics. The infection quickly spreads systemically via the blood stream to produce septicemic plague. If the flea deposits bacteria directly into the dermal vasculature, then primary septicemic plague can sometimes occur in the absence of bubonic plague [1]. In about 5% of humans, hematogenous spread to the lungs results in pneumonic plague. Because it can be spread directly from person-to-person via aerosolized respiratory droplets, this form of the disease presents a public health emergency. The case fatality rate of septicemic and pneumonic plague is high, even with antibiotic treatment.

Plague remains an international public health concern. Periodic plague outbreaks illustrate the ability of *Y. pestis* to suddenly cross over from established enzootic reservoirs and reemerge in human populations [2]. Multidrug resistant strains of *Y. pestis* have been isolated from human plague patients, and *Y. pestis* has recognized potential as an agent of bioterrorism. Plague has been weaponized in the past, and the World Health Organization (WHO) estimated that dissemination of 50 kg of *Y. pestis* in an aerosol cloud over a

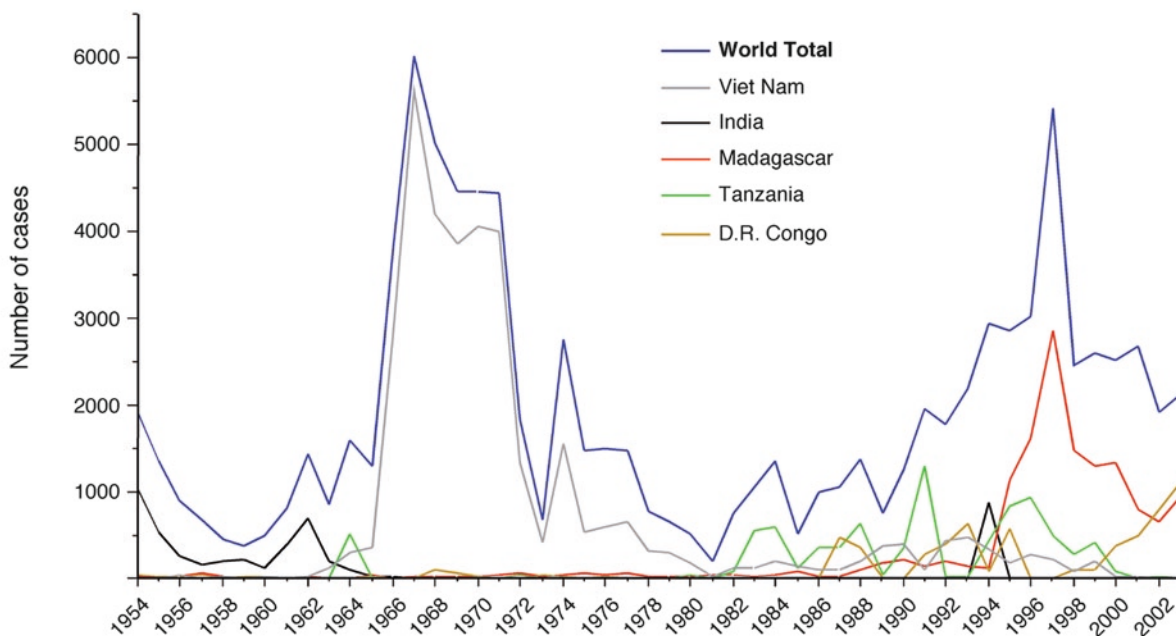
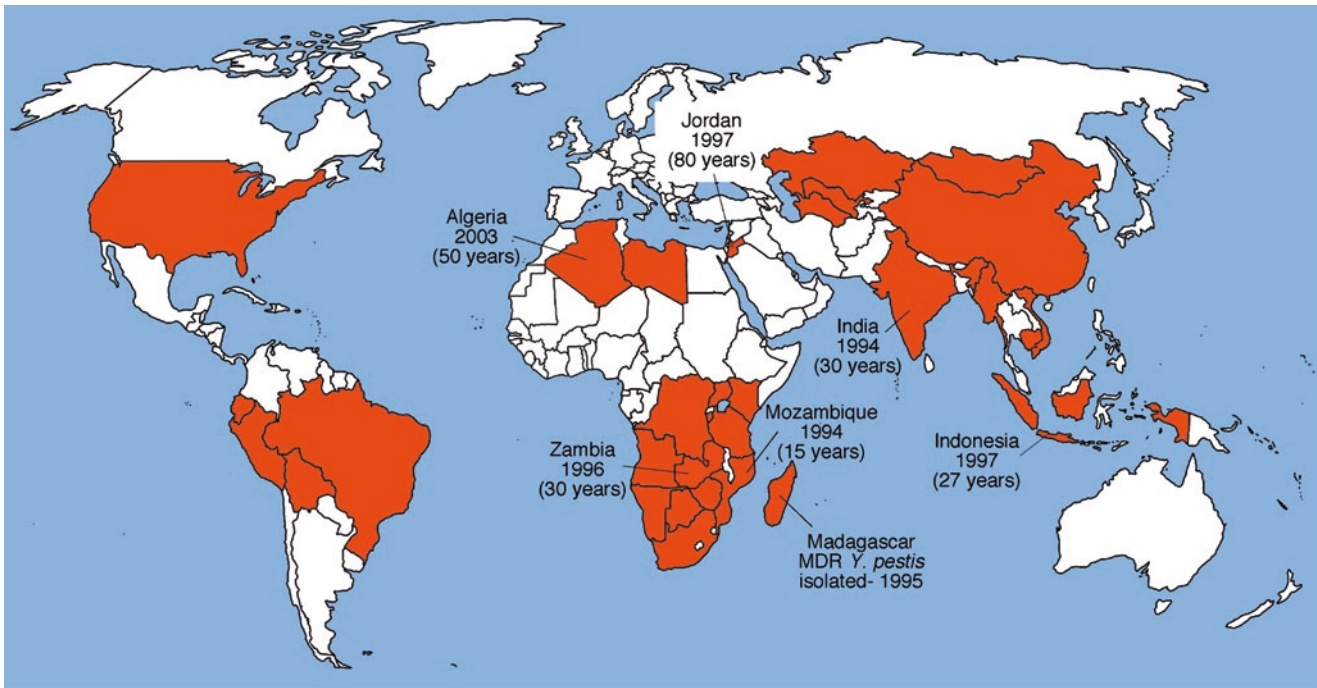
large metropolis could result in 150,000 cases of pneumonic plague [3]. If an antibiotic resistant strain were used, then the fatality rate would be high.

Despite these well-recognized threats, significant gaps in our understanding of many areas of plague biology impede progress in developing effective public health countermeasures. For example, there is currently no vaccine available for plague, but immune correlates of protection against plague have not been well characterized. There is also a recognized need for new diagnostic tests; most plague cases are based on presumptive clinical diagnosis [2].

### 9.2 Ecology and Epidemiology of Plague in the World Today

The highly virulent *Y. pestis* strain responsible for the last pandemic originated in Asia, but it spread across Pacific and Indian Ocean shipping routes to geographic areas where it did not previously exist, including North and South America. An adaptable pathogen, *Y. pestis* quickly established itself in many different species of rodents, among which it circulates using many different flea species as vectors. Thus, plague is more widespread today than it has ever been. It is certain that *Y. pestis* is permanently entrenched in wild rodent reservoir hosts in many parts of the globe, but the geographic extent of the enzootic foci is often poorly defined and subject to rapid expansion and contraction depending on factors such as climate and rodent and flea population dynamics [4].

After the last pandemic waned in the middle of the 20th century, the incidence of human plague declined dramatically. On average, a few thousand cases are reported to the WHO each year, although the actual disease incidence is almost certainly much higher. Beginning in the 1990s, however, outbreaks have occurred in parts of Africa, India, and South America where plague had previously been dormant for many years (Fig. 9.1).



**Fig. 9.1** Epidemiology of plague. Countries reporting plague cases during the last fifty years are depicted in red. In the last ten years, the majority of plague outbreaks have occurred in Africa. Examples of countries in which

plague has re-emerged recently after many years of absence are indicated as well as the first isolation of antibiotic-resistant (MDR) *Y. pestis* strains during the recent plague epidemic in Madagascar. Sources: WHO and CDC

### 9.3 Plague: A Case Study in the Sudden Emergence of New Epidemic Infectious Diseases

Virulent *Y. pestis* strains from around the world constitute a highly uniform clone that diverged from the closely related *Yersinia pseudotuberculosis* only within the last 1,500 to

20,000 years [5, 6]. Despite their recent common ancestry and genetic relatedness, the two sister-species differ radically in parasitic lifestyle. *Y. pseudotuberculosis* is a relatively benign food- and water-borne enteric pathogen. Evolutionarily speaking, therefore, plague is a new disease. The hypervirulence and arthropod-borne transmission of *Y. pestis* evolved quite recently and involved relatively few

genetic changes, making plague an interesting historical case study in the sudden emergence of an epidemic infectious disease. It is sobering to reflect on the fact that only a modest number of minor genetic changes were needed to convert the relatively mild recent ancestor of *Y. pestis* into one of the most feared human pathogens.

#### 9.4 Retracing the Genetic Steps that Led to Flea-borne Transmission and Hypervirulence of *Y. pestis*

Our laboratory participated in an international team effort that reported the complete annotated genome sequence of *Y. pseudotuberculosis* and the comparative genomics of *Y. pseudotuberculosis* and *Y. pestis* [6]. Remarkably, only 32 chromosomal genes occur in *Y. pestis* that do not have identical or highly similar orthologs in *Y. pseudotuberculosis*, as determined from comparative analyses of a panel of isolates of the two species from around the world.

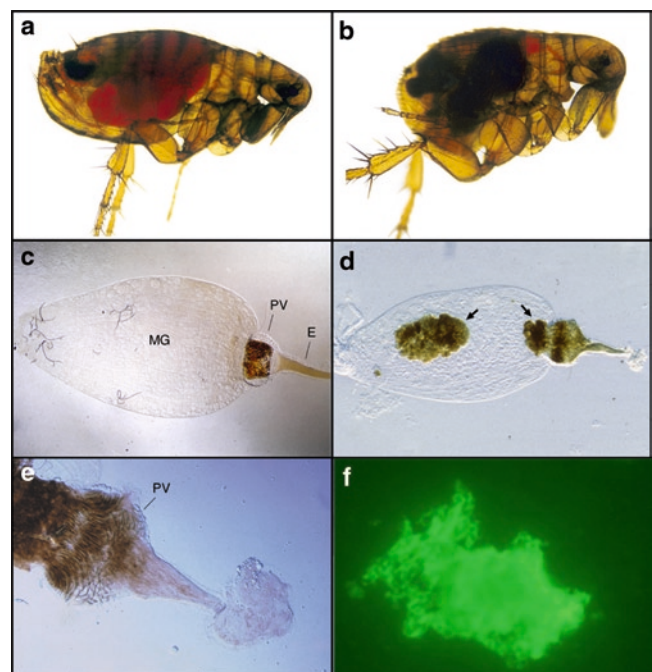
The most obvious genetic difference between the two species is the presence of two *Y. pestis*-unique plasmids that were acquired since the divergence from the ancestral *Y. pseudotuberculosis* strain. Using the flea infection and transmission model systems we developed to investigate the interactions between *Y. pestis* and the rat flea *Xenopsylla cheopis*, the principal vector of human bubonic plague, we reported that both plasmids are essential for flea-borne transmission and virulence of plague. One plasmid encodes a phospholipase D (PLD) that had been originally characterized as murine toxin and presumed to have a role in virulence. We determined that this gene is not required for virulence and identified its true biological function: it is required for *Y. pestis* survival in the flea gut [7].

The other *Y. pestis*-unique plasmid encodes a plasminogen activator (Pla) that is required for dissemination from an intradermal or subcutaneous injection site. Using a *pla*-negative mutant strain, we demonstrated that two distinct pathologies can ensue from a fleabite: bubonic plague, which depends on Pla, and primary septicemic plague, which does not [1]. Our results suggested an evolutionary scenario in which acquisition of the PLD-encoding plasmid was a crucial initial step in the transition to arthropod-borne transmission, and that subsequent acquisition of the Pla-encoding plasmid greatly increased flea-borne transmissibility and epidemic potential.

A major focus of our laboratory has been to understand the genetic and molecular mechanisms that mediate flea-borne transmission. To produce a transmissible infection, *Y. pestis* grows in the flea digestive tract in the form of a bacterial biofilm, a dense multicellular community surrounded by a polysaccharide extracellular matrix (ECM),

and usually attached to a surface [8]. In *X. cheopis* fleas, the *Y. pestis* biofilm often adheres to the hydrophobic, cuticle-coated spines that line the interior surface of the proventriculus, the valve that connects the esophagus to the midgut (Fig. 9.2). Consolidation and continued growth of the biofilm can eventually fill the proventriculus, interfering with its valvular action and blocking the normal flow of blood into the midgut when the flea attempts to feed. Complete or partial blockage of the proventriculus is prerequisite to efficient biological transmission, because it results in regurgitation of bacteria into the flea bite site [9, 10].

The chromosomal *hms* genes of *Y. pestis* are responsible for the synthesis of the biofilm ECM, a polymer of  $\beta$ -1,6-*N*-acetyl-D-glucosamine, and therefore are required for the ability of *Y. pestis* to grow as an adherent biofilm *in vitro* and to infect and block the flea proventriculus [8, 11, 12]. The *hms* genes are not required for virulence in the mammal; their biological function is specific to producing a transmissible infection in the flea. Intriguingly, *Y. pseudotuberculosis* never forms a biofilm in infected fleas, despite the fact that it contains identical orthologs to the *Y. pestis* *hms* genes and



**Fig. 9.2** Biofilm life stage of *Y. pestis* in the flea vector. Uninfected (a) and (b) infected, blocked *X. cheopis* fleas immediately after feeding. The presence of fresh blood in the esophagus, but not the midgut, is indicative of proventricular blockage. (c, d) Dissected digestive tracts from an uninfected (c) and a blocked (d) flea. Arrows indicate dense aggregates of *Y. pestis* embedded in an extracellular matrix in the midgut and proventriculus. (e) Micrograph of the anterior end of the digestive tract dissected from a blocked flea showing a viscous *Y. pestis* biofilm extruding from the esophagus. (f) Antibody to poly- $\beta$ -1,6-*N*-acetyl glucosamine binds the ECM of *Y. pestis* biofilm in an immunofluorescence assay. (MG = midgut; PV = proventriculus; E = esophagus)



is able to form *hms*-dependent biofilms *in vitro* [13]. This suggests that regulatory pathways leading to ECM synthesis differ in the two species, and we have shown that loss of gene function in *Y. pestis* (pseudogene formation) has been important in this regard. About 7% of the ~4,000 orthologous gene pairs of the two species are pseudogenes in *Y. pestis* but are intact in *Y. pseudotuberculosis*. One of them, *nghA*, encodes a glycosyl hydrolase that specifically hydrolyzes  $\beta$ -1,6-*N*-acetyl-D-glucosamine and interferes with biofilm formation in the flea [12]. Another, *rcsA*, a negative regulator of the biofilm phenotype, is a functional gene in *Y. pseudotuberculosis* but a pseudogene in *Y. pestis*. Restoring the functional *Y. pseudotuberculosis rcsA* allele in *Y. pestis* essentially eliminated the ability to produce a proventricular-blocking biofilm in fleas [14].

In common with many other bacteria, ECM synthesis in *Y. pestis* is controlled by intracellular levels of cyclic di-GMP, which are determined by competing activities of the *hmsT* diguanylate cyclase and *hmsP* phosphodiesterase gene products [15, 16]. We have recently implicated a second phosphodiesterase gene (*rtn*), whose product is truncated and nonfunctional in *Y. pestis*, in biofilm ECM regulation. As was the case for the *rcsA* pseudogene, replacement of the *Y. pestis rtn* pseudogene with the functional *Y. pseudotuberculosis* allele resulted in a greatly reduced ability to form biofilm in fleas. Because of their transmission-enhancing effects, mutational loss of the ancestral *rcsA* and *rtn* functions would likely have been positively selected during the emergence of *Y. pestis*. In conclusion, we have shown that selective gene loss as well as gene addition via acquisition of the two *Y. pestis*-specific plasmids, played an important role in the evolution of arthropod-borne transmission.

### 9.5 The Sudden Emergence of both Flea-borne Transmission and Hypervirulence in the Genus *Yersinia*: A Case of Mutually Reinforcing Coevolution?

*Y. pestis* is a striking example of a pathogen that has taken the sinister evolutionary path to increased virulence. Modern epidemiological models hypothesize that pathogens evolve a level of virulence that maximizes their transmissibility [17, 18]. We used the flea infection model to quantitatively evaluate two basic components of vector competence – the oral infectious dose ( $ID_{50}$ ) of *Y. pestis* for *X. cheopis* fleas and the transmission efficiency of blocked fleas [19]. The data indicated that the vector competence of fleas is low compared to other parasite-vector systems. The low infectivity (high  $ID_{50}$ ) for fleas mandates that, in order to complete its life cycle, *Y.*

*pestis* must achieve a high density, usually fatal septicemia in peripheral blood. In addition, the low number of bacteria transmitted by fleas mandates that *Y. pestis* must be invasive enough to produce this overwhelming sepsis starting from a small infectious dose that is deposited intradermally by flea bite; the  $LD_{50}$  of *Y. pestis* for mammals is in fact less than 10 bacteria. Thus, the evolutionary change to the flea-borne transmission cycle likely imposed new selective pressures that favored the coevolution of increased virulence. Further epidemiological modeling based on the data indicated that a high flea density per host is required to sustain epizootic plague, supporting the practice of flea control alone as an effective strategy to control plague outbreaks.

### 9.6 Functional Genomics Approaches to the Discovery of *Y. pestis* Transmission, Infectivity, and Virulence Mechanisms

A number of important virulence factors of *Y. pestis* have been discovered and an important basic pathogenesis strategy recognized – the ability to retard and attenuate the mammalian innate immune response [20, 21]. Thus, *Y. pestis* combines the dangerous attributes of stealth (the ability to prevent immune detection and stimulation) and aggression (rapid invasiveness) to produce rapidly fatal systemic sepsis. The molecular mechanisms by which it does so are incompletely known, however, and often based on *in vitro* evidence and inferences from studies of the enteropathogenic *Yersinia* species or attenuated *Y. pestis* strains. Thus, the molecular mechanisms underlying specific impairments of host defense remain to be determined or validated *in vivo*. To this end, we have developed, characterized, and used mouse and rat models of bubonic plague [22, 23]. The rat model had not been employed for many years, but disease progression and gross pathology in the rat more closely resembles human bubonic plague. In addition, the microbial pathogenesis of *Y. pestis* is seldom studied in the context of the natural flea-borne route of transmission. Nearly all studies have employed needle-injection of cultured bacteria. While this artificial transmission model is valuable, it ignores specific aspects of the natural transmission route, such as the unique biofilm phenotype assumed by *Y. pestis* in the flea and the effect of flea saliva. In fact, the true role and significance of some *Y. pestis* virulence factors were only revealed in studies employing transmission by flea bite [1, 24].

One way to discover new transmission and virulence factors is to analyze gene expression response patterns of the host-parasite interaction. Such microarray-based functional genomics strategies reveal genes that are specifically upregulated during infection, implicating them as being involved in the disease process. Furthermore, where and when a gene is

expressed provides important clues about what the gene does [25]. In collaboration with the Genomics Unit of NIAID's Research Technologies Branch, we have characterized the *in vivo* transcriptome, or gene expression profile, of *Y. pestis* in infected fleas and in infected lymph nodes (buboes) of rats, as well as the transcriptome of rat lymph node cells during bubonic plague. A comparison of normalized gene expression levels from the two data sets provides insight into the biology of the flea-mammal life cycle and supports the hypothesis that *Y. pestis* differentially expresses distinct subsets of genes to establish a productive infection in its two disparate hosts. About 24% of *Y. pestis* genes showed significantly higher relative expression levels in the bubo than in the flea; 15% were higher in the flea than in the bubo; and 61% were not differentially expressed in the two hosts.

### 9.7 Virulence Determinants Discovered by Analysis of *Y. pestis* Gene Expression in the Bubo

The microarray profile of *Y. pestis* gene expression in the rat bubo compared to the profiles of exponential and stationary phase *Y. pestis* cultured *in vitro* at 37° and 21°C revealed an adaptive response of *Y. pestis* to iron limitation and nitric oxide (NO)-derived reactive nitrogen species (RNS) in the extracellular environment of the bubo [26]. Expression of 10 of the 14 proven or potential iron or heme transport systems, including three siderophore-based systems, five ABC iron transporters, a heme transport system, and an iron permease, was upregulated >5-fold in the bubo. Several genes that have been implicated in the bacterial protective response to RNS were also upregulated *in vivo* compared to *in vitro*. In particular, expression of *hmp*, which encodes a flavoglobin that is induced by NO and confers resistance to RNS, increased 10 to 20-fold in the bubo. NO-derived antimicrobial activity is generated by macrophages, neutrophils, and other host cells by the inducible NO synthase (iNOS) in response to bacterial infection [27]. In subsequent pathogenesis studies, we showed that large numbers of iNOS-expressing neutrophils are recruited to the infected bubo and are in close association with extracellular masses of *Y. pestis*, serum NO levels are elevated in rats with bubonic plague, and that mutation of the *Y. pestis hmp* gene resulted in decreased virulence.

Current models of plague pathogenesis stress the ability of *Y. pestis* to inhibit phagocytosis and the inflammatory response, thereby avoiding exposure to innate immune effector molecules. Our results show that *Y. pestis* encounters and responds to NO-derived reactive nitrogen stress in the bubo, where it is extracellular. Although the importance of NO-derived stress in innate defense against intracellular pathogens is well-established, evidence for a role against

extracellular bacteria such as *Y. pestis* is lacking. In addition to its antibacterial role in innate immunity, NO has known concentration-dependent immunosuppressive and other regulatory effects on the developing adaptive immune response; and high NO levels contribute to septic shock, the ultimate cause of plague mortality [27]. Thus, the elevated systemic NO levels we detected during plague disclose a new potential factor in disease pathogenesis and immunomodulation.

### 9.8 The Infective Phenotype of *Y. pestis* Characterized by Analysis of Gene Expression in the Flea Vector

After transmission, many known virulence factors of *Y. pestis* are induced that confer resistance to innate immunity. However, these factors are not produced in the low-temperature environment of the flea, suggesting that *Y. pestis* is vulnerable to the initial encounter with innate immune cells at the flea bite site. Therefore, in a complementary project we compared the *Y. pestis in vivo* transcriptome in infective fleas to *in vitro* transcriptomes in temperature-matched biofilm and planktonic cultures, and to the previously characterized *in vivo* gene expression profile in the rat bubo. The results characterized a specific phenotype *Y. pestis* in the flea vector and implicated previously unrecognized genes involved in biofilm formation. Notably, several genes with known or predicted roles in resistance to innate immunity and pathogenicity in the mammal were upregulated in the flea; and *Y. pestis* from infected fleas had increased resistance to phagocytosis. The results indicate that cycling through the flea vector pre-adapts *Y. pestis* to face the mammalian innate immune response that it encounters immediately after transmission, and that the vector-specific phenotype of *Y. pestis* as it exits the flea and enters the mammal enhances infectivity [32].

## 9.9 Wanted: 21st Century Public Health Countermeasures Against Plague

### 9.9.1 Epidemiology and Surveillance

Until recently, all human cases of plague as well as cholera and yellow fever were required by international law to be reported to WHO. This somewhat outdated emphasis on only three diseases was eliminated in the latest version of the International Health Regulations of the WHO, which came into effect in June 2007. Now, notification to WHO is mandated only for an unexpected event presenting a risk of international spread, for example a SARS or avian influenza virus outbreak.

Although any unusual occurrence of plague would still be reportable under the new regulations, one fallout will likely be less complete data on the worldwide prevalence and incidence of human plague. However, because of poor diagnostic facilities in many countries where plague occurs, plague almost certainly has been chronically underreported.

Plague cannot be eradicated; it is widespread and firmly entrenched in many wild rodent reservoirs. As a zoonosis, the risk of plague in human populations is closely linked to the dynamics of plague in its natural reservoirs [4]. Surveillance to monitor the geographical extent of natural foci and to detect plague epizootics in wild rodents is well recognized as being an important public health measure. Surveillance typically entails serological testing of urban and wild rodents and testing their fleas for the presence of *Y. pestis*, but is rarely done. Consequently, the threat that plague poses at any given time to human populations in most parts of the world remains poorly understood.

### 9.9.2 Diagnostics, Treatment, and Prophylaxis

Globally, laboratory diagnosis is problematic because plague tends to occur in remote areas and in countries with limited public health resources and infrastructure. Diagnosis of human plague is most often based on the following: (i) compatible clinical and epidemiological features and microscopic observation of Gram-negative coccobacilli in a direct smear from a clinical sample (suspected case); (ii) detection of *Y. pestis* F1 capsular antigen in a clinical sample or anti-F1 antibody in a single serum sample (presumptive case); and (iii) *Y. pestis* culture and identification or a fourfold increase in anti-F1 antibody titer in paired sera (confirmed case). Most human plague that is reported to WHO are suspected or presumptive cases. Simple and rapid diagnostic tests for F1 antigen detection have been developed and are being assessed [2]. Such tests would provide much-needed new tools if their reliability, sensitivity, and specificity can be verified in field conditions. The use of PCR, real-time PCR, and other molecular biology tools for diagnosing plague from clinical specimens is also being assessed for use in reference laboratories. At their most refined, these methods can genotype different *Y. pestis* isolates with exquisite sensitivity, which is of great value in epidemiological and forensic (bioterrorism-related) investigations [28].

Our *in vivo* genomics studies have identified outer surface protein genes of *Y. pestis* that are highly expressed in the flea, indicating that their protein products are prominent antigens initially exposed to the mammalian immune system when the bacteria exit the flea and enter the mammal. We are investigating them as candidate markers for new efficacious vaccines, diagnostics, and therapeutic drug targets. Diagnostic

markers specific for flea-borne versus primary pneumonic (aerosolized) plague transmission would be valuable in epidemiologic investigation. For example, if a case of pneumonic plague occurred in Los Angeles, it could either be primary pneumonic plague, a highly unusual event that might indicate a deliberate, nefarious act of bioterrorism; or secondary pneumonic plague that developed rapidly from flea-borne plague, more easily explainable because enzootic plague foci have been reported in suburban parks of that city.

If it is diagnosed early enough, then plague can be successfully treated with several widely available antibiotics [3]. The public health response to a plague outbreak in most instances consists of isolation and antibiotic treatment of cases, prophylactic treatment of contacts, and local application of insecticide to kill fleas. Because of the explosive nature of plague outbreaks in certain conditions, rapid response is of the essence, making reliable and readily available diagnostic tests all the more critical. Although pneumonic plague has a reputation as being highly contagious via respiratory droplets, the simple precaution of wearing a mask, even an improvised one consisting of layers of cotton gauze, effectively prevents aerosol transmission [29, 30].

### 9.9.3 The Need for a Third-Generation Plague Vaccine

Two types of plague vaccines have been used in the past [30]. Beginning in 1914, live attenuated *Y. pestis* vaccine strains were administered to millions of people in some Asian countries and Madagascar and are still being used in parts of the former Soviet Union. These vaccines protected against both bubonic and pneumonic plague, but were associated with severe side effects and would never be approved today. A different whole-cell vaccine, consisting of formalin-killed, virulent *Y. pestis* in saline (the USP plague vaccine) was used from about 1950 to 1995. This formulation reportedly provided short-term protection against bubonic plague only, and is no longer available.

Two new subunit vaccines, both based on the surface-exposed F1 and V antigens of *Y. pestis*, were developed at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) and the United Kingdom's defense department. Mouse and non-human primate trials have been completed for the USAMRIID vaccine, and both are now in human trials. Protection against bubonic plague was good in all animal models but mixed results were obtained for efficacy against pneumonic plague—good protection against aerosol challenge occurred in cynomolgus macaques, but not in African green monkeys [30]. The mechanisms of vaccine protection and predictive correlates of protection are not well understood. For example, humoral immunity is important, but antibody titers were not predictive of protection against

aerosol challenge in non-human primates. Another potential shortcoming of these vaccines is the fact that the F1 capsular protein is not required for *Y. pestis* virulence; therefore, vaccines might be unprotected against naturally occurring or deliberately engineered F1-negative strains. In addition, antigenic variants of the *Y. pestis* V antigen occur, and V antigen has been shown to have immunosuppressive effects, an undesired property of a vaccine component (30). Thus, there is a need for further research on new vaccine candidates, but only limited information is available about the mammalian protective immune response to plague and the corresponding *Y. pestis* targets. Optimal delivery routes, vehicles, and adjuvants also remain to be established [30, 31].

Our microarray analyses of the *Y. pestis* *in vivo* transcriptome identified eight outer membrane protein genes that were highly expressed during bubonic plague. In collaboration with Dr. Susan Buchanan, a membrane protein biochemist at NIH, NIDDK, we are evaluating these proteins as potential vaccine candidates. In addition, X-ray crystallographic structural analyses of the highly expressed essential virulence factors required for iron acquisition and transport may point to new chemotherapeutic strategies with novel modes of action.

## 9.10 Summary

Plague is exceptional for its extreme virulence and potential for rapid epidemic spread in the right conditions. These capabilities of *Y. pestis* evolved relatively recently, making plague a sobering example of how a new epidemic can suddenly appear on the basis of just a few genetic changes. Far from being an historical curiosity, plague in the 21st century remains a public health threat that is incompletely understood. Plague still periodically reemerges, often after decades of quiescence, for enigmatic reasons. The occurrence of antibiotic resistant *Y. pestis* isolates and the documented potential of *Y. pestis* as an agent of bioterrorism increase the urgency for new medical countermeasures. Paramount among them is an effective plague vaccine, a goal that should be achievable. Successful development of these new tools, however, depends on further research to clearly define the molecular mechanisms of *Y. pestis* transmission, infection, and immunity.

## References

- Sebbane F, Jarrett C O, Gardner D & et al (2006). Role of the *Yersinia pestis* plasminogen activator in the incidence of distinct septicemic and bubonic forms of flea-borne plague, *Proc Natl Acad Sci USA*, **103**, 5526–5530
- World Health Organization (2008). Interregional meeting on prevention and control of plague, April 2006, Antananarivo, Madagascar: WHO, 2008
- Inglesby T V, Dennis D T, Henderson D A & et al (2000). Plague as a biological weapon, *JAMA*, **283**, 2281–2290
- Stenseth N C, Atshabar B B, Begon M & et al (2008). Plague: past, present, and future. *PLoS Med*, **5**, e3
- Achtman M, Morelli G, Zhu P & et al (2004). Microevolution and history of the plague bacillus, *Yersinia pestis*, *Proc Natl Acad Sci USA*, **101**, 17837–17842
- Chain P S G, Carniel E, Larimer F W & et al (2004). Insights into the evolution of *Yersinia pestis* through whole-genome comparison with *Yersinia pseudotuberculosis*, *Proc Natl Acad Sci USA*, **101**, 13826–13831
- Hinnebusch B J, Rudolph A E, Cherepanov P & et al (2002). Role of the *Yersinia murine* toxin in the survival of *Yersinia pestis* in the midgut of the flea vector, *Science*, **296**, 733–735
- Jarrett C O, Deak E, Isherwood K E & et al (2004). Transmission of *Yersinia pestis* from an infectious biofilm in the flea vector, *J Infect Dis*, **190**, 783–792
- Bacot A W & Martin C J (1914). Observations on the mechanism on the transmission of plague by fleas, *J Hyg Plague Suppl* **3**, 13, 423–439
- Bacot A W (1915). Further notes on the mechanism of the transmission of plague by fleas, *J Hyg Plague Suppl* **4**, **14**, 774–776
- Hinnebusch B J, Perry R D & Schwan T G (1996). Role of the *Yersinia pestis* hemin storage (*hms*) locus in the transmission of plague by fleas, *Science*, **273**, 367–370
- Erickson D L, Jarrett C O, Callison J A & et al (2008). Loss of a biofilm-inhibiting glycosyl hydrolase during the emergence of *Yersinia pestis*, *J Bacteriol*, **190**, 8163–8170
- Erickson D L, Jarrett C O, Wren B W & et al (2006). Serotype differences and lack of biofilm formation characterize *Yersinia pseudotuberculosis* infection of the *Xenopsylla cheopis* flea vector of *Yersinia pestis*, *J Bacteriol*, **188**, 1113–1119
- Sun Y C, Hinnebusch B J & Darby C (2008). Experimental evidence for negative selection in the evolution of a *Yersinia pestis* pseudogene, *Proc Natl Acad Sci USA*, **105**, 8097–8101
- Kirillina O, Fetherston J D, Bobrov A G & et al (2004). HmsP, a putative phosphodiesterase, and HmsT, a putative diguanylate cyclase, control Hms-dependent biofilm formation in *Yersinia pestis*, *Mol Microbiol*, **54**, 75–88
- Bobrov A G, Kirillina O & Perry R D (2005). The phosphodiesterase activity of the HmsP EAL domain is required for negative regulation of biofilm formation in *Yersinia pestis*. *FEMS Microbiol Lett*, **247**, 123–130
- Anderson R M & May R M (1982). *Population biology of infectious diseases*, Springer Verlag, New York
- Lenski R E & May R M (1994). The evolution of virulence in parasites and pathogens: reconciliation between two competing hypotheses, *J Theor Biol*, **169**, 253–265
- Lorange E A, Race B L, Sebbane F & et al (2005). Poor vector competence of fleas and the evolution of hypervirulence in *Yersinia pestis*, *J Infect Dis*, **191**, 1907–1912
- Perry R D & Fetherston J D (1997). *Yersinia pestis*—etiologic agent of plague, *Clin Microbiol Rev*, **10**, 35–66
- Heesemann J, Sing A & Trulzsch K (2006). *Yersinia's* stratagem: targeting innate and adaptive immune defense, *Curr Opin Microbiol*, **9**, 55–61
- Sebbane F, Gardner D, Long D & et al (2005). Kinetics of disease progression and host response in a rat model of bubonic plague, *Am J Pathol*, **166**, 1427–1439
- Lemaitre N, Sebbane F, Long D & et al (2006). *Yersinia pestis* YopJ suppresses tumor necrosis factor alpha induction and contributes to apoptosis of immune cells in the lymph node but is not required for virulence in a rat model of bubonic plague, *Infect Immun*, **74**, 5126–5131
- Sebbane F, Jarrett C, Gardner D & et al (2009). The *Yersinia pestis* *cafMIAI* fimbrial capsule operon promotes transmission by flea bite in a mouse model of bubonic plague, *Infect Immun*, **77**: 1222–1229



25. Manger I D & Relman D A (2000). How the host 'sees' pathogens: global gene expression responses to infection, *Curr Opin Immunol*, **12**, 215–218
26. Sebbane F, Lemaitre N, Sturdevant D E & et al (2006). Adaptive response of *Yersinia pestis* to extracellular effectors of innate immunity during bubonic plague, *Proc Natl Acad Sci USA*, **103**, 11766–11771
27. Bogdan C (2001). Nitric oxide and the immune response, *Nat Immunol*, **2**, 907–916
28. Girard J M, Wagner D M, Vogler A J & et al (2004). Differential plague-transmission dynamics determine *Yersinia pestis* population genetic structure on local, regional, and global scales, *Proc Natl Acad Sci USA*, **101**, 8408–8413
29. Erickson D L & Hinnebusch B J (2006). Pneumonic plague. In: *Microorganisms and Bioterrorism* (Ed: B. Anderson, H. Friedman & M. Bendinelli) Springer, New York
30. Smiley S T (2008). Current challenges in the development of vaccines for pneumonic plague, *Expert Rev Vaccines*, **7**, 209–921
31. Yang X, Hinnebusch B J, Trunkle T & et al (2007). Oral vaccination with salmonella simultaneously expressing *Yersinia pestis* F1 and V antigens protects against bubonic and pneumonic plague, *J Immunol*, **178**, 1059–1067
32. Vadyvaloo V, Jarrett C, Sturdevant D E, Sebbane F, Hinnebusch B J (2010). Transit through the flea vector induces a pretransmission innate immunity resistance phenotype in *Yersinia pestis*. *PLoS Pathogens* 6: e1000783