An update on plague

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This paper presents an overview of plague, an acute infectious illness caused by Yersinia pestis. The epidemiology, pathogenesis, diagnosis, treatment and prevention of human plague are discussed, accenting recent advances in these areas.

Yersiniosis, or plague, caused by the gram-negative bacillus *Yersinia pestis*, is a zoonotic disease of rodents which is transmitted to humans through the bite of rodent ectoparasites[1]. Dreaded since the third century, the infectiousness and ease of transmission of *Y. pestis* have resulted in at least three major pandemics (worldwide epidemics) which decimated large segments of the population and transformed the society of a medieval age that neither understood the cause nor knew its remedy[2]. The ravages of the Black Death or the Great Pestilence that swept through Europe in the years 1346 to 1352 still echo in contemporary art and literature as in the works of Albert Camus. But it is probably because of the automatic and unconscious associations that the connotations of the word 'plague' trigger in us that we today ascribe the disease a greater malevolence and perniciousness than it perhaps deserves.

Exactly a hundred years ago, in 1894, the French bacteriologist Alexandre Yersin discovered the causative organism[3], but it was only in the 1980s that considerable progress was achieved in understanding the biology of this organism. Although the disease has undergone a precipitous decline since the advent of antibiotic era, it is still present on every continent except Australia, and is endemic in parts of Asia, Africa and the Americas. Table 1 lists the incidence in those countries which have notified the persistent occurrence of human plague during the last ten years or more[4]. In the United States there has been a rising incidence of sporadic human plague in the last three decades, particularly in the southwestern states, with a mortality rate of about 15% (refs. 4, 5). Over the period 1983–1992, 62.5% of the cases (7466) and 81.8% of the deaths (1021) were reported from Africa[4]. In India, suspected plague outbreaks have been reported in 1975 in Maharashtra[6] and in 1985 in Himachal Pradesh[7]. This paper, written in the wake of the recent epidemic in Surat, aims to provide an overview of human plague and review recent developments concerning the pathogenesis of the disease, its diagnosis, treatment, and prevention. Intended for the scientist, not the medical practitioner, the subject is approached from the perspective of the biology of the pathogen, rather than from the standpoint of the response of the host to the disease.

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Epidemiology

The ineradicability of the disease stems from the fact that plague is firmly established as an enzootic (a disease of animals indigenous to a certain locality, analogous to an endemic) in wild rodent populations throughout the world. Over 340 species of mammals are known to be susceptible to the disease and over 30 species of fleas and other ectoparasites are known to transmit the organism[8]. Sylvatic plague, the disease in wild rodents, serves as a reservoir for infection of domestic rats, which along with their ectoparasites live in close association with humans. In urban and semiurban India, *Bandicota bengalensis*[9], the bandicoot, and *Suncus murinus*, the common house shrew[10], along with *Rattus rattus* (domestic rat) or *Rattus norvegicus* (sewer rat) are predominant commensal species and represent a conduit between the two ecosystems. All these rodents harbour the rat flea, *Xenopsylla cheopsis*, the major vector implicated in the transmission of the disease. Following a blood meal from an infected rodent, *Y. pestis* multiplies in the foregut of the rat flea, eventually blocking the proventriculus. During subsequent feeds, the flea regurgitates the organism into the bite wound, thereby infecting a host. When a significant fraction of the rat population is depleted, the rat fleas seek alternate hosts and, may feed on humans. Human outbreaks therefore usually coincide with, or are preceded by 'ratfalls', the consequence of an epizootic (an epidemic in an animal population) in the susceptible rodent population[11]. The dynamics of transfer of the organism from feral ecosystems to the civic environment is not very well understood and may involve several parameters such as vegetation cover, seasonal variations in temperature and humidity[8], etc; for instance, the probable cause of the 1989 plague epidemic in Botswana has been attributed to heavy rains that year which yielded a bumper harvest, leading to

Table 1. Human plague incidence in countries reporting persistent occurrence[a]

	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992
Africa															
Madagascar	25	23	11	44	38	24	39	85	29	23	93	170	226	137	198
Tanzania	nil	nil	49	9	76	569	603	129	360	356	647	31	364	1293	–[b]
Americas															
Bolivia	68	10	26	21	1	21	12	nil	94	2	2	nil	10	nil	nil
Brazil	11	nil	98	59	151	82	37	64	58	43	25	26	18	10	25
USA	12	13	18	13	19	40	31	17	10	12	15	4	2	11	13
Asia															
China	–[b]	8	30	1	0	25	0	6	8	7	6	10	75	29	35
Myanmar	171	73	73	1	165	96	10	35	6	5	8	34	6	100	528
Vietnam	314	306	180	11	116	127	196	137	104	107	196	375	405	94	437
World total[c]	785	661	511	200	753	1067	1356	521	1009	1060	1371	760	1254	1966	1758
	(34)	(33)	(58)	(31)	(48)	(92)	(107)	(58)	(115)	(215)	(153)	(103)	(133)	(133)	(198)

[a]From ref. 4
[b]Figures unknown.
[c]Figures in parentheses are the number of deaths.

an increase in rodent population density[12]. Although the primary mode of acquiring the infection is by the bite of an infected rat flea, once established in human populations, further propagation of the disease from person to person can occur by inhaling aerosolized infected lung secretions. The latter route is the principal mode of spread among humans, and is the basis for the highly contagious nature of the disease[1].

Pathogenesis

After the bite of an infected rat flea, *Y. pestis* bacilli multiply at the site of infection and reach the regional lymph nodes via the lymphatics. The organism is highly infectious, and in murine models of plague, a subcutaneous injection of 10 bacilli or less is sufficient to cause disease. A facultative intracellular parasite of the reticuloendothelial system, *Y. pestis* proliferates within macrophages and monocytes in the lymph nodes setting up an acute inflammatory response resulting in the enlarged and inflamed lymph nodes, or 'buboes' in about 2 to 6 days, the hallmark of the bubonic, or primary, form of plague. Hemorrhagic necrosis of the lymph nodes that occur subsequently as a result of the massive proliferation of the bacilli releases these organisms into the systemic circulation, seeding other reticuloendothelial organs (liver, spleen, bone marrow). The lung is commonly infected secondarily and the ensuing pneumonia usually runs a fulminant course. The invasion of the systemic circulation by overwhelming numbers of the organism results in the release of endotoxin, a lipopolysaccharide outer membrane constituent common to all gram-negative bacteria[13]. The 'endotoxin shock' that invariably follows is characterized by disseminated intravascular coagulation and multiple system organ failure, terminating in death[1].

Y. pestis is endowed with several virulence factors which enables it to survive and multiply in the intracellular milieu of macrophages[14] and in the nutrient-deprived, highly acidic environment of necrotic lymphoid tissue. In this respect, *Y. pestis* is unusual among other intracellular pathogens since the phagocytic vesicle containing the organism fuses with hydrolase-laden lysosomal vesicles[14], and the bacillus multiplies in the hostile phagolysosomal environment. Other intracellular organisms such as *Toxoplasma gondii*[15, 16], or *Plasmodium falciparum*[17] proliferate within protective intracellular niches which resist fusion with organelles of the host cell endocytic system; some organisms such as *Listeria monocytogenes*, *Rickettsia prowazekii* and *Trypanosoma cruzi* escape from phagocytic vacuoles and thrive directly in the cytoplasm of the host cell[18], subverting the cell's metabolic resources for its own growth. At least eight virulence factors have been described so far. (i) The presence of a capsular Fraction 1 antigen on the bacterial surface is associated with resistance to phagocytosis[19]. The biosynthesis of capsular material is temperature-dependent and is expressed strongly at 37° C, but not at 26° C (ref. 20); the latter temperature may correspond to that in the rat flea, and it is likely that the elaboration of the protective capsule is an adaptation response to the mammalian host environment. (ii) A 75-kilobase plasmid-mediated[21] temperature- and calcium-dependent response (termed the Lcr^+, Vwa^+, or Cal^+ phenotype) is characterized by the coordinated expression of so-called V and W virulence antigens on the cell surface which confer capsule-independent antiphagocytic properties[22]. An additional cytotoxic factor also appear to be encoded by the Lcr plasmid[23]. Spontaneous mutation to the Lcr^- phenotype results in a 10^6 to 10^7-fold increase in the 50% lethal dose in experimental animals[24]. These antigens

are expressed at 37° C (and not at 26° C) under conditions of very low calcium concentration. Concomitant with the expression of the V and W proteins, the proliferation of *Y. pestis* is inhibited. Since the calcium concentration in the intracellular milieu of the macrophage is known to be very low[25], these events may be part of a regulatory mechanism signalling the release of the organism from sequestered intracellular foci into the systemic circulation. (iii) The production of a surface protease[26] which exhibits plasminogen-activating properties. The deposition of fibrin around bacteria is a common host response and the plasmin-like activity may serve to breach the fibrin barriers, and may therefore play a role in systemic dissemination of the bacteria. Indeed, mutants lacking the plasmid encoding for the protease while capable of producing a local infective focus if injected subcutaneously in mice, require a million-fold higher inoculum to produce systemic disease[26]. (iv) The ability to utilize iron complexones as the sole iron source (Pgm^+ [pigment] phenotype). Virulent phenotypes can utilize hemin and other iron-containing pigments. *In vivo*, a major fraction of iron is complexed to carrier proteins such as transferrin or lactoferrin, and the concentration of free ferrous iron is extremely low. Pgm^- isolates are virulent in mice only if serum transferrin is saturated with exogenous iron[27,28]. (v) The ability for *de novo* biosynthesis of purines since mutants that do not synthesize purines do not multiply within macrophages[14]. (vi) production of a cytoplasmic membrane-associated protein called the plague murine toxin[29]. Although toxic to mice, its role in the pathogenesis of human plague is still unclear. (vii) A 15 kDa protein called the pH 6 antigen, elaborated at 37° C under conditions of low pH[30] (conditions likely to be found in macrophage lysosomes or abscess sites). This protein has been reported to have cytotoxic and proinflammatory actions[31]. (viii) The expression of a bacteriocin (a secreted protein exerting lethal effects on closely related bacteria) called pesticin was thought to be a virulence factor but recent evidence suggests that it is relatively unimportant[26].

Diagnosis

The laboratory diagnosis of plague is relatively straightforward. Aspirates from buboes, if present, usually reveal bacilli with characteristic bipolar 'safety pin' morphology under Giemsa stain. In the bacteremic or pneumonic phases, the organisms can be identified in the blood buffy coat or sputum. Blood or lymph node fluid cultures facilitate unambiguous confirmation of the pathogen by colony morphology, biochemical reactions, and staining with *Y. pestis*-specific fluorescence-labelled antibody. An enzyme-linked immunosorbent assay (ELISA) for detection of *Y. pestis* antigens has been described[32]. Recently, DNA amplification by polymerase chain reaction (PCR) using *Y. pestis*-specific primers has been described[33,34] which may be of value in establishing a rapid, sensitive, and specific diagnosis in early cases of suspected plague. Diagnosis can also be established serologically by demonstrating rising antibody titres, but detectable antibody levels peak only in the second week following the illness. A number of immunological assays including complement fixation, agglutination, passive hemagglutination, and ELISA have been described[35-37]; the ELISA tests appear to be rapid, sensitive, and have a lower false-negative rate[36].

Treatment and prevention

Early therapy with antibiotics reduces the 40–100% mortality rate of bubonic and pneumonic plague to about 5–10%. Streptomycin, tetracycline, and chloramphenicol remain the antibiotics of choice[1] although a variety of beta-lactams, quinolones and aminoglycosides are also efficacious[38]. Drug resistance has not been encountered so far in *Y. pestis* probably because of its isolated ecosystem and it would be prudent to avoid the use of recent-generation antibiotics.

Human-to-human transmission in bubonic or septicemic forms is rare. However, untreated pneumonic plague has been classified as an epidemiologic emergency by the Plague Branch of the Centers for Disease Control, USA[39]. Contracting *Y. pestis* infection can be prevented by antibiotics taken prophylactically (chemoprophylaxis). Tetracycline, sulfisoxazole[40], or trimethoprim-sulfamethoxazole[1] are recommended. A formalin-killed whole bacteria vaccine has been available since the 1960s, and has been administered to over a million US army personnel who went to Vietnam[41], but is recommended only for health-care workers in endemic areas or laboratory workers who handle live cultures of *Y. pestis*. The time-lag in achieving vaccination-induced protection (seroconversion) and the limited duration of adequate antibody level[1] have precluded its use in epidemics[11].

General measures such as rodent control, flea control by the use of pesticides[12], surveillance by public health authorities for the pathogen in commensals, and advising people to avoid contact with rodents are of benefit, but attention must also be paid to the more fundamental problems of sanitation, overcrowding, sewage and waste disposal, the bane of our society.

1. Palmer, D. L., in *Principles of Internal Medicine* (eds. Braunwald, E., Isselbacher, K. J., Petersdorf, R G., Wilson, J. D., Martin, J. B. and Fauci, A. S) McGraw-Hill, New York, 11th edition, vol. 1, pp. 615–617.
2. McEvedy, C., *Sci. Am.*, 1988, **258**, 74–79.
3. Wong, T. W. and Fung, K. P., *Asia Pac. J. Public Health*, 1988, **2**, 144–149.
4. Anonymous, *Bull. World Health Org.*, 1994, **72**, 512–514.

5. Anonymous, *J. Am. Med. Assoc.*, 1980, **244**, 1764.
6. Renapurkar, D M., *Indian J. Public Health*, 1981, **25**, 74–75
7. Saxena, V. K , *J. Commun Dis*, 1985, **17**, 344–346.
8. Christie, A B , *Ecol Dis*, 1982, **1**, 111–115
9. Renapurkar, D M , *J. Hyg Epidemiol Microbiol Immunol*, 1988, **32**, 407–413.
10. Renapurkar, D M , *J. Hyg Epidemiol. Microbiol Immunol*, 1989, **33**, 45–49.
11. Butler, T., *Trans R Soc. Trop. Med Hyg.*, 1989, **83**, 458–460.
12. Kumaresan, J A , Grova, J. B , Mmatli, P K. and Maganu, E D., *Trop Doct.*, 1991, **21**, 142–146
13. Westphal, O , *Transact. College Int. Aller.*, 1975, **49**, 1–43.
14. Straley, S C. and Harmon, P. A , *Infect Immun*, 1984, **45**, 655–659.
15 Jones, T. C. and Hirsch, J G., *J. Exp Med*, 1972, **136**, 1173
16 Sibley, L. D , Weidner, E and Krahenbuhl, J L , *Nature*, 1985, **315**, 416–419
17 Desai, S A , Krogstad, D J. and McCleskey, E. W , *Nature*, 1993, **362**, 643–646.
18 Portnoy, D A and Jones, S., *Ann NY Acad Sci*, 1994, **730**, 15–25.
19 Janssen, W A , Lawton, W. D , Fukui, G. M and Surgalla, M J , *J. Infect. Dis*, 1963, **113**, 139–143
20. Fox, E. N and Higuchi, K , *J. Bacteriol*, 1958, **75**, 209–216.
21. Ferber, D. M and Brubaker, R. R , *Infect Immun*, 1981, **31**, 838–841.
22. Burrows, T W. and Bacon, G A , *Br J Exp. Pathol.*, 1956, **37**, 481–491
23 Goguen, J D , Walker, W. S , Hatch, T P. and Yother, J , *Infect Immun.*, 1986, **51**, 788–794.
24. Burrows, T. W , *Ergeb. Mikrobiol. Immunitaetforsch*, 1956, **37**, 59–113.
25 Pollack, C , Straley, S. C and Klempner, M. S , *Nature*, 1986, **322**, 834–836
26 Sodeinde, O. A , Subrahmanyam, Y V B K , Stark, K., Quan, T., Bao., Y. and Goguen, J. D , *Science*, 1992, **258**, 1004–1007.
27 Jackson, S. and Burrows, T W., *Br J Exp. Pathol*, 1956, **37**, 577–583
28 Carniel, E., Mercereau-Puijalon, O and Bonnefoy, S , *Infect Immun*, 1989, **57**, 1211–1217
29. Montie, T. C , *Pharmacol. Ther*, 1981, **12**, 491–499
30 Lindler, L. E , Klempner, M S. and Straley, S. C., *Infect. Immun*, 1990, **58**, 2569–2577
31. Bichowski-Slmonicki, L and Ben-Efraim, S., *J Bacteriol.*, 1963, **86**, 101–111
32. Williams, J. E , Gentry, M K., Braden, C. A , Leister, F. and Yolken, R H , *Bull WHO*, 1984, **62**, 463–466.
33 Hinnebusch, J and Schwan, T. G , *J. Clin Microbiol*, 1993, **31**, 1511–1514.
34. Grimont, P A , Lefevre, M. and Carniel, E., *J Clin. Microbiol.*, 1994, **32**, 634–641
35 Williams, J E , Arntzen, L , Robinson, D M., Caganaugh, D C. and Isaacson, M , *Bull WHO*, 1982, **60**, 777–781
36. Shepherd, A J , Leman, P. A., Hummitzsch, D. E. and Swanepoel, R., *Trans R. Soc Trop. Med Hyg.*, 1984, **78**, 771–773.
37. Montenegro, S. M , De Almeida, A. M. and Carvalho Jr , L. B , *Mem. Inst Oswaldo Cruz*, 1993, **88**, 119–123
38 Bonacosi, S. P , Scavizzi, M R , Guiyoule, A , Amouroux, J. H and Carniel, E , *Antimicrob. Agents Chemother.*, 1994, **38**, 481–486.
39 White, M E , Gordon, D , Poland, J. D. and Barnes, A. M , *Infect. Control*, 1980, **1**, 324–329.
40 Wilson, W R , *Med Clin. North Am*, 1983, **67**, 99–112.
41. Butler, T , in *Plague and other Yersinia infections*, Plenum, New York, 1983, pp. 198–207