

Melioidosis: the Tip of the Iceberg?

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INTRODUCTION

Melioidosis is the name given to all infections caused by the bacterium *Pseudomonas pseudomallei*. The term is derived from the Greek name for a variety of conditions resembling glanders and was coined by Stanton and Fletcher in 1921 (143). Although recognized for nearly 80 years, the disease remains poorly understood and continues to receive scant attention in most English language medical texts. In their classic monograph, published over 50 years ago, Stanton and Fletcher predicted that the disease would prove to be far more common than appreciated at the time (144). In the past decade their claim has been vindicated, and melioidosis is now known to be a major cause of human morbidity and mortality in certain areas of the tropics (27, 95). In addition, the disease in livestock has economic and possibly public health implications (159).

Since the comprehensive review of Howe and colleagues, published nearly 20 years ago (68), sporadic reports of the isolation of *P. pseudomallei* have extended the boundaries of likely melioidosis endemicity. Recent reviews have presented incomplete and sometimes conflicting descriptions of the geographical distribution of the disease (78, 95, 137). The purpose of this paper is to review the evidence for the world distribution of *P. pseudomallei* and to summarize methods of

laboratory diagnosis and antibiotic therapy in the hope that this will encourage a greater awareness of the disease among those working in, or managing patients from, known or potentially endemic areas. The clinical and laboratory features of the disease have recently been reviewed extensively elsewhere (37, 78, 95, 137) and therefore will be discussed only briefly here.

HISTORY AND GEOGRAPHICAL DISTRIBUTION

Southeast Asia

Burma (Myanmar). Melioidosis was first encountered in Burma in 1911 by Captain A. Whitmore, a British pathologist, and C. S. Krishnaswami, an assistant surgeon, working in Rangoon General Hospital (172). They reported 38 cases of a "hitherto undescribed pyaemic or septicaemic disease" characterized by widespread caseous lesions and abscesses, notably in the lungs, but also in the liver, spleen, and kidneys, which was particularly prevalent among morphia injectors. From the lesions they were able to isolate a characteristic, motile bacillus ". sufficiently peculiar to distinguish it from all pathogenic bacteria previously known to us" (171). Pure cultures of this organism produced a similar fatal infection when injected into or fed to guinea pigs (171, 172). Further cases were described in Rangoon by Knapp (85) and Krishnaswamy (86) during the next 6 years, and indeed the disease appears to have been very common, being identified in approximately 5% of postmortem reports in Rangoon General Hospital (86). Since 1917, however,

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only five cases of melioidosis probably acquired in Burma have been described (67), although the disease is presumably still endemic in this country.

Malaysia and Singapore. In 1913, *P. pseudomallei* caused an outbreak of a severe, distemperlike illness among laboratory animals in the Institute for Medical Research of the Federated Malay States, although the true nature of the infection was not identified until some years later (144). Stanton and Fletcher later observed a number of cases of human and animal melioidosis arising in the vicinity of Kuala Lumpur (144). Extensive environmental surveys in the 1960s demonstrated the widespread presence of *P. pseudomallei* in mud and surface water in East and West Malaysia and Singapore (48, 146-148, 156). Recent reports of *P. pseudomallei* infections in humans (25, 77, 93, 122, 178) and other animals (31, 51, 109, 136, 138) reveal that clinical melioidosis is still prevalent in both peninsular and East Malaysia, Singapore, and Brunei, and indeed it is being recognized with increasing frequency in Singapore (154).

Indochina (Vietnam, Laos, and Cambodia). Melioidosis in South Vietnam (then Cochin-China) was first diagnosed in 1925 by Pons and Advier, working in the Institut Pasteur, Saigon (123). During the ensuing years of French administration, several further cases were reported in Tonkin (104) and Cochin-China (2, 35) (both now Vietnam), particularly among the European colonizers. The first presumptive evidence of the saprophytic existence of *P. pseudomallei* was obtained by Vaucel in Hanoi (163), and this was later confirmed by Chambon (23) in Saigon.

During the armed conflict in Vietnam, large numbers of western soldiers were intimately exposed to environmental *P. pseudomallei* through contaminated wounds and burns or by inhalation (68). These individuals had access to excellent clinical and laboratory facilities, making possible the laboratory confirmation of a diagnosis of melioidosis. At least 100 cases of melioidosis were recognized among French forces in Indochina from 1948 through 1954 (131), and by 1973, 343 cases had been reported by Moore, as cited by Sanford (133), in American soldiers fighting in Vietnam. Since *P. pseudomallei* may remain latent for many years (100), accounting for the title "time-bomb disease" which has been ascribed to melioidosis (68), cases of melioidosis continue to occur in veterans of the Vietnam war (61, 108). Cases are also seen in immigrants from Indochina (21, 24). These observations suggest that melioidosis is probably highly prevalent among the indigenous population of Indochina, although the disease is seldom diagnosed (3).

Indonesia. The first human case of melioidosis in Indonesia (formerly Dutch East Indies) was identified in Java in 1929 (40), and wild rats infected with *P. pseudomallei* were later discovered in a rubber plantation near the home of this patient (40). Subsequent reports by Dutch workers confirmed the presence of the disease in both humans and other animals in Indonesia (16, 150). However, as with many other countries within the main endemic area, recent data are scarce (45).

Thailand. The history of melioidosis in Thailand demonstrates the degree to which a highly endemic infection may go unrecognized in the absence of clinical awareness and appropriate laboratory facilities. The earliest evidence for the existence of the melioidosis in Thailand (then Siam) was provided by Gambier (58), who isolated *P. pseudomallei* from a Russian patient normally domiciled in Bangkok. In 1947, two further cases were reported from Siam, in a Dutch prisoner of war and an Indian soldier (118, 120), but it was not until 1955 that the first case was reported in a Thai (30).

In 1962, a serological survey showed that up to 29.1% of healthy Thai volunteers had evidence of exposure to *P. pseudomallei* (112), but by 1966 only three cases of clinical melioidosis had been reported (95). Although the organism was found to be widely distributed in the environment, a SEATO medical research study was unable to detect any clinical cases of the disease (52a, 52b). From 1974 onwards, stimulated by the interest of the Infectious Disease Association of Thailand and facilitated by the improvement of microbiology facilities in rural areas, an increasing number of cases began to be recognized (29, 71, 72, 117, 119, 167). By 1986 the Association, as cited by Leelarasamee and Bovornkitti (95), had collected over 800 case reports. Since laboratory confirmation of the diagnosis is possible only in large regional referral centers, this number may still represent only the "tip of the iceberg."

Within Thailand, clinical melioidosis is most prevalent in the northeast, particularly in Khon Kaen and Ubon Ratchatani provinces (26, 27, 142, 151). *P. pseudomallei* is also present in soil and water in the south of the country (52a, 110), although clinical cases of melioidosis are reported comparatively rarely from this area (1). The disease in Thailand predominantly affects rice farmers and their families, who are thought to contract infection through their daily contact with the soil and water of paddy fields, in which *P. pseudomallei* exists as a saprophyte (52b, 110, 146, 148).

The Philippines. Little is known of the prevalence of melioidosis in the Philippine Islands, although reports of exported cases suggest that the organism must exist there. Possibly the first such case was described in 1948 (66), and several subsequent reports have suggested that infection was probably contracted in the Philippines (55, 73, 74, 94, 126, 169), although in individuals who have travelled extensively within endemic areas it is often impossible to be certain as to the origin of infection.

China, Hong Kong, and Korea. In the past decade, melioidosis in both human and other animals (dolphins and seals) has been reported from Hong Kong (140, 141, 165, 175), and serological evidence indicates that human exposure to *P. pseudomallei* is not uncommon there (139). There is a dearth of information regarding the existence of melioidosis within mainland China. Galimand and Dodin (56) cite research on the disease which has taken place in southern China, and occasional circumstantial evidence has implicated China as the origin of exported cases of melioidosis (106). Certainly other authors have considered China, and also Korea, to be melioidosis-endemic areas (18, 68, 133).

The Indian Subcontinent

Very few cases of melioidosis have been reported from the Indian subcontinent, but sporadic exported cases indicate that the disease is endemic in this region. The first human case was reported by Denny and Nicholls (41) in Sri Lanka (then Ceylon) in 1927. Nicholls (111) subsequently reported a case in a cow, but a more recent serological survey from Sri Lanka demonstrated that few individuals had antibodies against *P. pseudomallei* (162). Several subsequent reports have suggested that infection was acquired in Pakistan or India (70, 99, 107, 145). In the past 2 years, an indigenous case occurring in Bangladesh was reported by Struelens et al. (149), a case probably acquired in India was seen in Switzerland (161), and an imported case from Pakistan was seen in Britain (136a).

Pacific Islands

In 1945, two cases of melioidosis occurring in American soldiers on Guam were described (105). A neonatal case acquired in Hawaii was reported in 1971 (115, 132), although the possibility that the baby was indirectly infected by the father (a Vietnam veteran) via the mother's genital tract cannot be excluded. The recent publication (33) from New Zealand of a case arising in Fiji, where *P. pseudomallei* was reported to be isolated occasionally from wound swabs (K. Singh, personal communication cited in reference 33), is a reminder that melioidosis may be endemic on other Pacific islands.

Australia and Papua New Guinea

P. pseudomallei infection arising in Australia was first identified among sheep in Queensland in 1949 (34). A variety of animals have now been affected by the disease in Queensland (80, 81, 88, 90, 91, 114, 153, 158, 160), Western Australia (79, 96), and Papua New Guinea (46). Animal melioidosis in Australia poses economic problems through the death or slaughter of infected animals, and a possible public health risk from drinking contaminated milk has also been proposed (159). Human melioidosis was first recognized in Townsville, Far North Queensland, in 1950 (128), and five more cases were diagnosed there over the ensuing 10 years (128). It is in this region that the majority of human cases have arisen (9, 64, 65), although several have also been reported from the Northern Territory (15, 20, 36, 129, 168), and sporadic cases have occurred in Western Australia (164), the Torres Strait Islands (75), and Papua New Guinea (39, 84, 92, 130). Guard (64) estimated that 20 human cases of melioidosis were diagnosed in Australia each year.

North and South America and the Caribbean

Confusion exists in the literature over the number of cases of melioidosis that have originated in North America. This is largely due to the inadequacy of travel histories (14) or doubts as to the identity of the organisms concerned in individual case reports (17, 102). Certainly, the vast majority of cases seen in the mainland United States have been imported from known endemic areas, most commonly Vietnam (132, 134). The only instance in which *P. pseudomallei* appears unequivocally to have been acquired from soil in North America is the patient reported by Nussbaum et al. (113), although there was no clinical evidence of infection in this case. Laboratory-acquired infections have been reported from Canada (62) and the United States (135), and one possible case of sexual transmission to the wife of a Vietnam veteran with prostatitis (101) has been described, but these represent special situations. The identities of the organisms described by McDowell and Varney (103), Beamer and colleagues (13, 14), and Garry and Koch (59) have subsequently been questioned (17), and the isolate reported by McCormick and colleagues (102) was probably not *P. pseudomallei*.

There is good evidence, however, that melioidosis is endemic in Central and South America. In 1964, Biegeleisen et al. (17) reported a case acquired in Ecuador and cited two cases arising in Panama. Further cases reported from North America have probably been acquired in Panama (103) and Mexico (12), and Larionov (89) cited 18 cases occurring in El Salvador. Although an environmental survey in 1973 failed to yield *P. pseudomallei* from water in the Sao Paulo region

of Brazil (121), French workers have more recently isolated the organism from soil in both Brazil and Peru (57). The 24 indigenous isolates, purported to be *P. pseudomallei*, studied in Chile in 1976 (116) had such atypical antibiograms for the species that their identity must remain in some doubt.

Within the Caribbean, a dramatic epizootic of infection occurred among goats, sheep, and pigs in Aruba and was reported in 1957 (152). The recent description of a human case in Puerto Rico (32) and the isolation of *P. pseudomallei* from soil in Haiti (57) indicate that the Caribbean is yet another endemic area in which the diagnosis of clinical melioidosis may be missed.

Africa

The possibility of the existence of melioidosis in Africa was first raised in 1936 by Girard (60), who isolated a strain of *P. pseudomallei* from the hemorrhagic submaxillary lymph node of a pig in Madagascar after repeated passage of tissue in guinea pigs. Although such lesions were frequently observed in apparently healthy animals at slaughter (60), his observations appear not to have been pursued. In 1960, melioidosis was reported in a goat in Chad (127). Then, in 1972, Ferry isolated *P. pseudomallei* from a number of pigs in an abattoir in Niamey, Niger (52). These animals had been brought from neighboring Upper Volta (Burkina Faso), and Dodin and Ferry (42, 43) were able to isolate the organism from soil samples taken along the route that the pigs had followed. They also demonstrated seropositivity in over 10% of serum samples from villagers in Upper Volta. At about this time, Frazer found serological evidence that melioidosis might exist in Uganda (54), while Mayer, as cited by Larionov (89), also reported the occurrence of human melioidosis in Uganda. Galimand and Dodin (57) later isolated *P. pseudomallei* from soil in the Ivory Coast, Madagascar, and La Réunion (an island off Madagascar), although the presence of the organism in La Réunion was possibly linked to an outbreak among horses in France (44, 57, 106). Further evidence for the existence of melioidosis in Africa was provided by Bremmelgaard et al., who reported a case of human melioidosis possibly acquired in Kenya (19), and by Wall et al. in The Gambia, the latter patient originating from Sierra Leone (166).

Middle East

Although melioidosis is primarily endemic in the tropics, occasional cases have arisen in subtropical and even temperate climates. In two such reports, that of a horse from Cairo (98) and a human case from Turkey (49), insufficient bacteriological evidence was presented to establish unequivocally the identity of the causative organism.

In 1969, however, unequivocal evidence of melioidosis was obtained when *P. pseudomallei* was isolated from two horses and a mule at the Razi Institute in Iran (11). Naturally occurring melioidosis had previously been identified in sheep and goats in Iran (11), and the organism was subsequently isolated from paddy water near Teheran and further north along the Caspian Sea (125). Although up to 50% of the local population were found to have serological evidence of exposure to *P. pseudomallei* (125), apart from a possibly associated skin rash in rice farmers, serious disease had not been reported at this time (125). In 1977, however, the first human case of pulmonary melioidosis was reported from Iran by Pourtaghva et al. (124).



FIG. 1. World distribution (clinical and environmental isolates) of *P. pseudomallei* and *P. pseudomallei*-like organisms. Shaded area, Main endemic area; hatched areas and asterisks, sporadic isolates.

Europe

The most extraordinary extension of the boundaries of melioidosis took place in France in the mid-1970s. Thanks to the chance visit of a researcher from the Institut Pasteur to a postmortem examination at the Muséum Nationale d'Histoire Naturelle, an epizootic of melioidosis among animals in a Paris zoo was revealed (106). This outbreak subsequently spread to other zoos in Paris and equestrian clubs throughout France and beyond, probably by the transport of infected animals and contaminated manure (44, 106). The infection caused the death or slaughter of an unknown number of animals and at least two fatal human cases. Extensive environmental contamination was documented, resulting in a costly program of disinfection (44, 106) despite which *P. pseudomallei* persisted for years in affected soil (44).

The origin of this outbreak is obscure. Possibly, infected horses from Iran imported the disease (44), or perhaps the index case was a panda donated to France by Mao-Tse-Tung in 1973 (106). "L'affaire du Jardin des plantes" (106) is the only well-documented example of the transmission of melioidosis in a temperate climate and emphasizes the potential dangers of the importation of the disease to a new area. The isolation of *P. pseudomallei* from horses in Spain has also been reported (133), as have several possible cases of human melioidosis in Berlin in 1947 (63, 89), although the evidence for the last observation is uncertain.

Summary

The countries in which *P. pseudomallei* and *P. pseudomallei*-like organisms have been isolated from humans, other animals, or the environment are shown in Fig. 1. Since the boundaries shown on this map are political and do not represent barriers to dissemination of microorganisms, the true distribution is likely to be more extensive.

LABORATORY DIAGNOSIS AND TREATMENT

Isolation

The protean clinical manifestations of melioidosis, a disease which has been nicknamed "the great mimicker" (178), mean that a definitive diagnosis depends on the isolation and identification of *P. pseudomallei* from clinical specimens (95). *P. pseudomallei* will grow on most routine laboratory media (4) and can be isolated from normally sterile sites, such as blood, by using standard techniques (176). In specimens with a mixed normal bacterial flora, *P. pseudomallei* may be overgrown by commensals and consequently overlooked (4, 177). Several media for the selective isolation of *P. pseudomallei* have been developed to overcome this problem (4, 50, 57, 82). During studies in Thailand, use of the medium described by Ashdown (4) significantly increased the frequency of recovery of *P. pseudomallei* from throat, rectum, wounds, and sputum, as compared with the recovery on blood and MacConkey agars, and the isolation frequency from throat, rectal, and wound swabs was further increased by the use of a selective broth preenrichment (177).

Identification

A lack of familiarity with the cultural characteristics of *P. pseudomallei* has often resulted in delays in recognition, identification, diagnosis, and treatment (22, 24, 97, 122, 173). The organism exhibits considerable interstrain and medium-dependent variability in colonial morphology (38), and the wrinkled appearance of older colonies has occasionally resulted in their being erroneously regarded as contaminants (97). *P. pseudomallei* is an oxidase-positive, gram-negative bacillus, which is resistant to aminoglycosides and polymyxins and has a sweet earthy smell (38). Commercial kit systems for bacterial identification such as the API 20NE (38), Microbact 24E (157), and Titertek NF (76) reliably

confirm the identity of *P. pseudomallei*, enabling laboratories in nonendemic areas to diagnose sporadically encountered cases of melioidosis. The results with the API 20E system are more variable (5, 157). In areas of high prevalence, screening systems that use antisera (5, 179) or colonial morphology on Ashdown's medium (38, 177) offer a more practical and economical means of identifying the organism. Unfortunately, commercially produced *P. pseudomallei* antiserum is no longer commercially available.

Serodiagnosis

The most widely available serodiagnostic test for melioidosis is the indirect hemagglutination test, using a crude autoclaved culture filtrate as antigen (7). While useful as an indicator of exposure to *P. pseudomallei*, this test lacks diagnostic specificity in areas of high melioidosis prevalence (27) and is also of unreliable sensitivity (10). Tests to detect specific immunoglobulin M antibodies to *P. pseudomallei*, either by indirect immunofluorescence (6, 83) or ELISA (enzyme-linked immunosorbent assay) (10, 87) have shown better correlation with disease activity. Assays for the detection of *P. pseudomallei* antigens (174) or exotoxin (69) offer the possibility of rapid diagnostic tests but await validation in clinical use.

Treatment

Until recently, antibiotic therapy for melioidosis had not been studied in prospective trials. Empirical regimens of combinations of antibiotics had often been used (133, 170), probably because of the disappointing results of single-agent therapy, since no evidence of in vitro synergy between the components had been obtained (47). Several new beta-lactam agents are active and bactericidal against *P. pseudomallei* (8, 28), and one of these, ceftazidime, has recently been compared in an open randomized trial with the combination of chloramphenicol, doxycycline, and trimethoprim-sulfamethoxazole for the treatment of acute severe melioidosis (170). Ceftazidime treatment (120 mg/kg/day) was associated with a 50% (95% confidence interval, 19 to 81%) lower overall mortality (170). The optimum duration of therapy and most effective oral antibiotics remain to be determined. Important adjuncts to antibiotic treatment include resuscitation and intensive care and the surgical drainage of abscesses (133).

DISCUSSION

Since the review by Howe et al. (68), knowledge of the worldwide epidemiology of melioidosis has advanced in three ways. First, further evidence has accrued that melioidosis is endemic in Africa (19, 42, 43, 52, 54, 57, 89, 166), the Indian subcontinent (136a, 149, 161), Iran (124, 125) and Central and South America (12, 57, 89). Second, sporadic reports have indicated the presence of *P. pseudomallei* in regions geographically separated from known areas of melioidosis endemicity: Hong Kong (139–141, 165, 175), Hawaii (115, 132), Fiji (33), Haiti (57), Puerto Rico (32), France (44, 106), and North America (113). Whether these are areas in which *P. pseudomallei* has always been present or areas into which it has recently been introduced (as was considered by Fournier [53] to be the case in Australia and Aruba) is uncertain, with the exception of France, where the subsequently documented disappearance of the organism from the environment (44) suggests a recent introduction. Third, in

one area of known melioidosis endemicity, northeast Thailand, the importance of the disease as a cause of morbidity and mortality in the indigenous population has been demonstrated bacteriologically (26, 27, 95, 142, 151). The true worldwide importance of melioidosis as a public health problem, however, remains unknown.

The factors that contribute to a high local incidence of clinical melioidosis are poorly understood. Within Thailand, although environmental *P. pseudomallei* has been found more frequently in the south than in the northeast part of the country (52a), the incidence of clinical disease is paradoxically higher in the northeast (95). Possible influences on the true incidence of infections include the nature of the environment (chemical composition of surface water and soil, climate), the virulence of local strains of *P. pseudomallei*, the nature of contact with environmental *P. pseudomallei* (e.g., through rice farming), and the nature of the exposed population (e.g., the prevalence of underlying diseases). The perceived incidence of clinical melioidosis is influenced greatly by the level of health care available to the exposed population and the familiarity of clinical and laboratory staff with the disease and its causative organism, as evidenced by the history of melioidosis in Thailand. Until these aspects are better understood, it is impossible to predict whether the high incidence of melioidosis in northeast Thailand can be extrapolated to areas from which the disease has been reported only sporadically. However, since it is the rural poor within endemic areas who are most likely to come into contact with *P. pseudomallei* in soil and water, but who are least likely to have access to sophisticated laboratory facilities, it is probable that active clinical melioidosis is underdiagnosed in many countries. Furthermore, seroprevalence data (6, 10, 42, 54, 83, 112, 139) indicate that many more individuals may have latent melioidosis (7) and be at risk of subsequent relapse (132, 134). It is possible that the spread of the human immunodeficiency viruses through melioidosis endemic areas may unmask large numbers of such cases (155).

There has been a recent resurgence of interest in melioidosis, and yet we remain ignorant of many aspects of the disease and its causative organism. Further epidemiological studies as well as an increased level of awareness of the condition are urgently needed to help define the size and extent of the problems posed by this elusive disease, to develop rational strategies for prevention, and to allow improved therapeutic regimens to be applied.

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