The Public Health Implications of Melioidosis

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Melioidosis, which is caused by the bacterium *Burkholderia pseudomallei*, is a potentially fatal tropical infection, little known outside its main endemic zone of Southeast Asia and northern Australia. Though it has received more attention in recent years on account of its claimed suitability as a biological weapon agent, the principal threat from melioidosis is a result of naturally occurring events. Occasional case clusters, sporadic cases outside the known endemic zone and infections in unusual demographic groups highlight a changing epidemiology. As melioidosis is the result of an environmental encounter and not person-to-person transmission, subtle changes in its epidemiology indicate a role environmental factors, such as man-made disturbances of soil and surface water. These have implications for travel, occupational and tropical medicine and in particular for risk assessment and prevention. Practical problems with definitive laboratory diagnosis, antibiotic treatment and the current lack of a vaccine underline the need for prevention through exposure avoidance and other environmental health measures. It is likely that the increasing population burden of the tropical zone and extraction of resources from the humid tropics will increase the prevalence of melioidosis. Climate change-driven extreme weather events will both increase the prevalence of infection and gradually extend its main endemic zone.

Key-Words: Public Health, melioidosis, epidemiology.

Public Health Data on Melioidosis

Accurate figures on melioidosis are hard to come by. In many parts of the world, the disease is unknown. Its clinical features are so many, varied and non-specific that there is a danger that an infected patient may go undiagnosed [1]. A further difficulty in reaching an accurate diagnosis of confirmed melioidosis is that it requires an unusual level of diagnostic laboratory expertise. This is lacking even in many developed countries where the infection is not endemic, and in remote parts of countries where the disease is known [2]. A further complicating consideration is its propensity for poorer, rural populations with limited access to health care. Finally, there are very few countries where melioidosis is a notifiable disease. Disease notification to public health authorities ensures consistency of surveillance, but does not guarantee that all cases will necessarily come to the attention of epidemiologists. In Australia, where some states and territories have melioidosis on the list of notifiable diseases, this has led to the development of a standard laboratory case definition of infection [3]. Standardization of public health laboratory methods leads, in turn, to comparability of disease surveillance data between states and cooperative development of diagnostic methods.

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Epidemiology

Clinical Spectrum

Recognizing that laboratory-based surveillance of melioidosis is patchy, inconsistent and poorly targeted with respect to the most vulnerable at-risk populations; it is still possible to draw an outline of the general epidemiology of infection [4]. The infection is a collection of disease states caused by the Gram-negative bacterial species, *Burkholderia pseudomallei* (formerly known as *Pseudomonas pseudomallei*). These conditions include a rapidly fatal Gram-negative septicemia, with or without pneumonia, central nervous system infection, localized abscess formation or soft tissue infection and asymptomatic exposure with seroconversion [5]. These conditions overlap somewhat, since localized infection may spill over to cause systemic infection, and septicemia may result in dissemination to distant tissues in which localized infection occurs. The clinical picture is further complicated by a varied incubation period that varies from 24-48 hours after exposure till septicemia, to late onset disease occurring after an extended period of dormancy. None of the clinical features are specific, pathognomonic indicators of melioidosis, so that the possibility of melioidosis may only be considered when the identity of the infective agent is established by an unsuspecting but alert laboratory scientist. That depends on obtaining a culture of blood, sputum, pus, cerebrospinal or other relevant body fluid. Though reasonably straightforward in laboratories that are familiar with this infection, a first time laboratory encounter with *B. pseudomallei* may not result in a correct identification because some commonly used diagnostic laboratory methods can give incorrect results in up to around 20% of cases [6]. For this reason, we rely on a costly, polyphasic identification process; but we still have trouble confirming this infection in many cases [7].

Geographic Distribution

Authorities recognize that melioidosis is under-diagnosed and may be completely missed in parts of the world where health infrastructure is poorly developed [2]. A clue to the wider distribution of infection comes from occasional reports of international travelers who return to developed countries with melioidosis acquired in other parts of the world [8-10]. The increasing trend towards adventure travel to exotic locations is expected to result in a steady increase in travel-related melioidosis. In Southeast Asia, most cases have been reported from rural locations in people who have frequent contact with soil and water, such as rice farmers [11]. In northern Australia, where there is no rice cultivation, the majority of reported cases have been in indigenous inhabitants of remote communities, particularly those with diabetes, chronic renal disease or alcohol dependency [12]. When these three co-morbidities are taken together, ethnicity ceases to be an independent risk factor, suggesting that underlying disease is the main contributor to epidemiological risk of established *B. pseudomallei* infection. The indigenous population of northern Australia and Asian rice farmers have in common a high level of exposure to soil in a disease-endemic zone. Others who are exposed to moist soil and surface water have also been reported to develop melioidosis [13], suggesting that the principal risk of exposure is determined by environmental conditions.

Route of Infection

The precise means of infection has yet to be determined. Some authorities consider it to be mainly inoculation of contaminated material via skin abrasions or incisions [14]. Others cite the high proportion of helicopter crewmembers who developed pulmonary melioidosis during the Viet Nam war as evidence for inhalation exposure [15]. There is recent laboratory animal evidence that explores respiratory exposure, but the relative importance of this route has still to be resolved [16].

Climatic & Seasonal Associations

One of the most interesting aspects of melioidosis epidemiology to emerge in recent years has been an association with extreme weather events. In northern Australia, the acute form of infection is strongly associated with the onset of the summer rainy season [17]. Most septicemic cases occur within a week of the start of the summer rains. In general, cases cluster around severe events, such as the heavy rainfall that occurs when cyclones go through [18]. Though not strictly a weather event, the 2004 Boxing Day tsunami is thought to have caused additional cases of melioidosis around the rim of the Indian Ocean, when many people were forcefully swept through mud and surface water by the tidal wave [19]. Recently, we have documented a possible association between asymptomatic seroconversion and an extreme weather event that occurred in northwestern Australia in 2005 [20]. As the mean annual summer rainfall has more than doubled in these parts of northern Australia over the last two decades, we anticipate an increased melioidosis caseload in years to come if rainfall continues to increase at the current rate.

Transmissibility

Another notable aspect of melioidosis epidemiology is that the infection only rarely transmits from person to person, despite its ability to cause lung infection. The few instances of direct transmission reported have been as a result of intimate contact between the donor and recipient [21,22].

Outbreaks and Case-Clusters

Melioidosis is not an infection of frequent outbreaks. In recognized endemic areas, acute infections occur sporadically, with a seasonal bias. The very few outbreaks that have been documented so far are thus the consequence of unusual interactions between a community and its environment. Only three clear-cut human outbreaks have been attributed to a single source, and the common feature was confirmed or likely contamination of a water source: in the two Australian events, it was the drinking water supply of affected communities [23,24]. In the Brazilian event, the source was an irrigation dam reservoir into which the infected subjects had dived shortly after the dam filled with early rains [25]. Porcine outbreaks occurred in Queensland after heavy rain and river flooding [26]. Detailed laboratory investigation surrounded all these events and in some attributed the source of infection to a specific environmental source [23,24]. It is likely that similar events go unnoticed and uninvestigated in parts of the recognized endemic zone where field investigation tools are lacking. The Brazil outbreak was particularly notable, because it affected a small group of previously healthy children [25]. The explanation for this observation is lacking; it could be due to a particularly high infective dose, an unusually virulent strain of *B. pseudomallei* or a genetic susceptibility. In order to better understand the ecology of melioidosis, field investigation resources need to be made available to the communities where melioidosis case clusters occur.

Environmental Health Investigations

The limited investigational resources available to public health authorities in those places where melioidosis is emerging or already endemic have to be used sparingly. Even in well-resourced countries such as Australia, the remote location of melioidosis-affected communities and at-risk industrial sites places restrictions on what can be assembled of short notice to investigate a suspected outbreak. A short list of alerts or red flags is provided in Table 1 to assist environmental health investigation planning. These are the situations that are most likely to provide environmental threat assessment data useful to public health authorities. Field investigations over a period of more than 10 years in Western Australia and the Northern Territory have concentrated on a small range of sample types most likely to yield a high return on effort (Figure 1,

Table 1. Reasons for increased public health melioidosis alert level and recommended actions targeting unusual disease activity in order to maximize disease control and environmental health measures.

Reason for alert	Rationale	Action	References
Case cluster	Unusual event, undisclosed cases, Potential secondary prevention	Case finding Environmental health investigation Enhanced laboratory surveillance Isolate genotyping	23, 24, 25
Multiple deaths, septicemic disease	Unusual severity, clustering, potential single source	As in case cluster	12, 23, 24, 24
Pediatric cases	Unusual severity	Case finding, serosurveillance, enhanced laboratory surveillance	25, 42, 43
Epizootic	Potential herald event	Veterinary & serosurveillance	25, 26, 30
Previously unknown location	Extension of endemic area	Enhanced laboratory surveillance	8,9,10
Dry season septicemic cases	Unusual event, specific environmental source	EH investigation, case finding, serosurveillance	27

Figure 1. Burkholderia environmental sample processing flowchart.

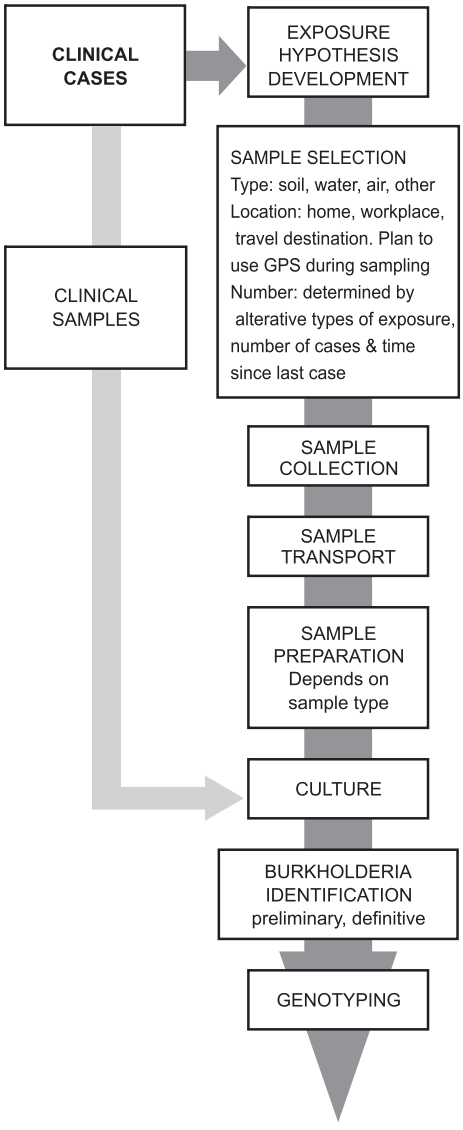

Table 2). It should also be remembered that these methods need prior development and rehearsal by a regional public health reference laboratory in concert with environmental health officers, biologists and public health physicians. Best results will be obtained when all the public health stakeholders are able to prepare, plan, rehearse and deploy as a team effort, as was evidenced by investigations in Western Australia in 1997 and 1998 [23,27], and in Ceará, Brazil in 2004 and 2005 [25]. In both cases, a high degree of interagency cooperation was achieved by exemplary public health leadership and advocacy at an executive level. The aim of a careful field investigation of melioidosis is an understanding of the nature and degree of the environmental hazard, an appreciation of whether it is likely to continue, and if so, what environmental health measures could be taken to reduce the threat of further infection. When field investigations identify a continuing melioidosis threat, they should result in subsequent targeted disease and environmental surveillance [28].

Disease Control Methods

Personal Measures

In the absence of an effective vaccine for melioidosis, the prospect of comprehensive disease control seems a distant reality. However, the predilection of infection for specific high-risk groups in particular locations provides a guide for environmental control methods that can be used with expected benefit. At a personal level, anyone with regular contact with soil or surface water in known endemic locations can be advised about personal precautions to reduce the risk of infection. These include simple personal protective measures such as avoiding skin abrasions, cleaning them thoroughly if they become contaminated with soil or water, and wearing protective equipment such as gloves and suitable clothing for exposure-prone occupations. People with known co-morbidities, such as diabetes or renal disease, need to take special care to apply these measures.

Table 2. Methods for use in environmental health investigation of melioidosis.

Sample	Dry soil	Wet soil	Water	Air	Other
Selection	Potential exposure location 5-10cm below surface, 20-30cm below surface > 50cm deep if loose soil	Moist surface soil Or mud below shallow Surface water	Shallow, permanent or temporary surface water Not flowing May be muddy	Only during extreme weather or other aerosol events	Root soil, water treatment equipment, biofilms IAW exposure hypothesis
Number	Decide according to exposure hypothesis. Fewer samples & thorough laboratory process is better option.				
Quantity	Circa 100gm – 1kg	Circa 25-100g	100-500mL	Circa 10mL filtrate	Variable
Container	clean self-seal plastic bag	Screw-top plastic	Screw-top plastic No preservative Fill to lid	Screw-top 20mL sterile	Screw-top plastic
Transport	To lab within 48hr, with thermal insulation if by air	To lab within 24hr, with thermal insulation if by air	To lab within 24hr, with thermal insulation if by air	To lab within 24hr thermal insulation	To lab within 24hr, with thermal insulation if by air
Preparation	Sieve 100g for AMF[a] 25g in 100mL SDW[b] overnight then 5mL at sample surface	25g in 100mL SDW overnight then 5mL at sample surface	Allow 100mL aliquot to settle overnight, then 6mL sample at surface filter remainder: 0.45µm pore, then 0.22µm pore	If>10mL, split in two, sonicate one portion	According to sample type
Initial culture	1mL onto BPSA[c] and ASA[d] 4mL into 50mL GTSB[e] broth incubate at 37'C x 48hr	1mL onto BPSA and ASA 4mL into 50mL GTSB broth incubate at 37'C x 48hr	1mL onto BPSA and ASA 4mL into 50mL GTSB broth incubate at 37'C x 48hr	1mL onto BPSA, ASA and FBA[f] 4mL into 50mL GTSB broth incubate at 37'C x 48hr	According to sample type
Extra cultures	Digest of AMF contents to BPSA and GTSB	Circa 1mL soil to GTSB	0.22um filter membrane to BPSA	Repeat for sonicate	According to sample type
Prelim ID[g]	GNB[h], oxidase pos, GM R[i]				
Definitive ID	LpxO PCR[j], RecA PCR & sequence, GLC-FAME[k] analysis				
Genotyping	PFGE, VNTR or MLST[l] to refine connection between environmental & clinical isolates				

Abbreviations: a. AMF = arbuscular mycorrhizal fungi; b. SDW = sterile distilled water; c. BPSA = *Burkholderia pseudomallei* selective agar; d. ASA = Ashdown's selective agar; e. GTSN = Gentamicin triptic soya broth; f. FBA = fresh horse blood agar; g. ID = identification; h. GNB = Gram negative bacillus; i. GM R = Gentamicin resistant; j. PCR = polymerase chain reaction; k. GLC-FAME = gas liquid chromatograph-fatty acid methyl ester; l. PFGE, VNTR or MLST = genotyping by pulsed-field gel electrophoresis, variable number tandem repeat or multi locus sequence typing.

Community Measures

At a community level, sporadic infection is difficult to prevent completely, but repetition of true outbreaks may be avoided by careful chlorination or chloramination of the drinking water supply [29]. The occurrence of animal die-off in domestic livestock needs to be thoroughly investigated by veterinary pathologists as a possible sentinel event for subsequent human infection. The porcine melioidosis case cluster in Queensland may thus have acted as an awareness raiser among veterinary and human health authorities, anticipating a whole-health approach to melioidosis control efforts in more recent years [26]. It is possible that a livestock die-off in the Tejuçuoca municipality of Ceará prior to the small melioidosis case cluster of 2004 might have been a melioidosis epizootic [25], but the absence of veterinary analysis of this livestock problem means that this interpretation remains speculative. Sporadic animal cases of melioidosis are well recognised and may have occurred elsewhere in Brazil, from recent anecdotal accounts.

Environmental Management

A fascinating alternative approach is suggested by data obtained from repeated environmental investigations in Western Australia that have consistently returned *B. pseudomallei* negative soil and water cultures from a location that was previously positive and which generated a 25 year long series of caprine infections plus one human infection [30,31]. At this location, culture negativity coincided with the

restoration of native vegetation and removal of chemical fertilizers (urea and superphosphate), both of which are substrates for *B. pseudomallei*. Interestingly, locations in Eastern Malaysia upriver from melioidosis endemic communities have both no evidence of clinical disease or asymptomatic seroconversion, nor do they have *B. pseudomallei* in their organically-cultivated rice fields (personal communication, AKR Hassan). This data is consistent with promotion of environmental risk by modern intensive cultivation methods, remediable by restoration of native flora. Though this interpretation is provisional, it may be worth considering in rural locations that become hyperendemic or which have a persistent association with fatal infection. Specific investigations are currently under way focusing on rice cultivation and rubber plantations, since workers in both types of crop cultivation are at increased risk of melioidosis.

Occupational Risk

Outdoor Occupations

Rural workers engaged in regular exposure to moist soil or surface water are known to be at increased melioidosis risk. These include rice farmers using traditional methods [11]. The risk can be reduced for this group by mechanization or more cheaply by wearing protective footwear. Lowland rice cultivation in flooded paddy fields is probably the highest form of risk for this group. Rubber tree tappers have been shown to be at risk of melioidosis and *B. pseudomallei* can be found in the soil under rubber trees [32], but little work has been done to calculate the relative risk of specific rubber cultivation activities and its possible seasonal variability. More detailed studies would allow specific preventive measures to be recommended. Recent work on the melioidosis risk of mineworkers suggests that at a mine site with identifiable *B. pseudomallei* contamination, a combination of careful occupational health measures, dust suppression and other environmental management measures mitigates infection risk [20]. However, excavation activities on a large industrial site in the humid tropics may result in exposure of some staff to concentrated biological aerosols, particularly during heavy rainfall. Suitable personal protective measures have yet to be designed and evaluated for workers in these tropical industrial environments. Possibly, the most unpredictable melioidosis risks are those to which people engaged in adventure travel may be exposed. This group includes overland trekkers, explorers of the remote tropics, field workers, exploratory mining engineers, eco-tourists, their guides, and military personnel engaged in training expeditions [8-10]. As their specific exposure to previously unidentified *B. pseudomallei* contaminated environments is difficult to anticipate, quantify or locate after the event, only general preventive personal advice can be given prior to departure. Post-travel serosurveillance may be helpful for such groups, or for individuals with unexplained fever. A similar approach may be justified for workers in the power and water supply industries who excavate trenches, install pipes, cables and ducting. We have encountered at least one asymptomatic seroconversion, sub-acute infection or fatal acute melioidosis in each of the above groups.

Laboratory Workers

The occupational risk of melioidosis for diagnostic laboratory workers is often overlooked. In Australia, *B. pseudomallei* is a biological safety level 2 organism and therefore does not appear to require a high level of laboratory biosecurity. But that does not mean that workers can handle it freely on the open bench. Laboratory staff who handle live *B. pseudomallei* cultures may be at risk of infection as a result of laboratory exposure [33,34]. Laboratory-acquired infection has been documented following gross exposure to live cultures [35], but it is possible that asymptomatic seroconversion in laboratory staff may be common but unreported. Uncommon though this is, we recommend that laboratory staff avoid sniffing culture plates to assist identification of *B. pseudomallei* by its characteristic odor. In fact, aerosol-generating procedures with live *B. pseudomallei* cultures should be conducted in a biological safety cabinet [36]. The worker should use latex or similar protective gloves and wear a laboratory gown in accordance with good microbiology laboratory practice. For practical reasons, it may be useful to obtain baseline melioidosis serology prior to commencement of laboratory work with *B. pseudomallei*. This can be repeated periodically, on completion of the work or after potential exposure, according to the intensity of risk. Seroconversion (a more than four-fold increase in titer) can be used as an indication of likely exposure, and is more reliable than a single high titer. The same approach can be used with specific, high-risk occupational groups, subject to their consent and the availability of specialist interpretive advice from the public health reference laboratory running the serological tests.

Biosecurity Aspects

Threat Assessments

The biosecurity threat of melioidosis may have been overemphasized in the wake of the deliberate anthrax dispersal in the USA during late 2001 and has led to some erroneous claims about the potential disease threat [37]. Evidence for deliberate use of *B. pseudomallei* as an agent of terrorism or by a malign foreign power is lacking. We concede that the lack of a vaccine, the high level of intrinsic antibiotic resistance, infection via the respiratory route and the potential fatal outcome of melioidosis might make it appear attractive to those who intend to do harm [38]. However, we argue that the lack of person-to-person spread, the common association of co-morbidity with fatal infection, the requirement for an as-yet unidentified environmental amplifier and the unpredictability of sporadic *B. pseudomallei* infection make it a poor candidate. The listing of *B. pseudomallei* as a category B select agent by the USA [39] may have contributed to the haste with which the initial Ceará outbreak was investigated. It is notable that

Table 3. Melioidosis surveillance methods.

Melioidosis surveillance	Sensitivity	Specificity	Comment
Interest driven	High	Variable	Commonest, dependent on interested individuals, patchy application
Disease notifications	Low	High	Uncommon
Laboratory-based: culture	Low	High	Common, since often a laboratory – based diagnosis
Laboratory-based: serology	High	Low	Commoner methods can be non-specific
Laboratory-based: combined reference methods	High	High	But often limited to low prevalence locations, away from high endemicity areas
Veterinary	Low	Low	May give early warning of increased human risk
Biosecurity	High	High	Results may be restricted to government services

Table 4. Check list for shipping samples to melioidosis reference laboratories (NB details should be checked before shipment as specific requirements may change with increased regulation of biological material transfer and will differ according to the specific circumstances of international transfer of biological material).

Sample	Clinical sample	Bacterial isolate	Extracted DNA	Serum
Initial letter of request from sending laboratory	+	+	+	+
Letter of understanding from reference laboratory	+	+	+	+
Import requirements from receiving laboratory	+	+	+	+
Import license from receiving laboratory		+	+	
Material transfer agreement		+	+	
Written confirmation of absence of viable bacteria in shipped sample			+	+
Shipment temperature	1-4°C	1-4°C	-20°C (dried ice)	1-4°C
Copies of all documents to courier company	+	+	+	+
Follow-up correspondence from sending laboratory confirming courier tracking number	+	+	+	+

further occurrences of melioidosis in northeastern Brazil did not attract the same level of international attention.

Laboratory Networking

One of the consequences of *B. pseudomallei*'s listing as a potential bioweapons agent has been an increased difficulty transporting live cultures of this species between public health laboratories. Clearly, the previous *ad hoc* approach was unsatisfactory, and the current approach is a work in progress, making it doubly difficult to cooperate in public health capacity-building across international boundaries. At present, the best way forward is careful recording of bacterial isolate transfers, defined material transfer agreements, rigorous adherence to import licensing, incorporation in registered culture collections and controlled access to archived, imported strains (Table 3). While some may find these requirements unduly restrictive, it will place more pressure on the international network of public health laboratories to use standard molecular identification and genotyping methods that can be applied to *B. pseudomallei* DNA, reducing reliance on trans-border shipment of live bacterial isolates.

Emerging Public Health Issues

We can anticipate emergence of melioidosis in a growing number of locations where the infection was previously unrecognized due to steadily-improving diagnostic capability. Filling diagnostic capability gaps will also go some way towards covering gaps in the geographic and demographic distribution of melioidosis. Targeted surveillance, where it is possible, may throw more light on high-risk activities and specific forms of occupational exposure, but until systematic surveillance based on disease notification is more widespread [4,28,40,41], there will be uncertainty over the incidence of sporadic infection and the true frequency of point-source case

clusters. In many temperate countries where melioidosis is experienced only as an imported disease, there is no strong pressure for development of an effective human vaccine. Cooperation between countries in the melioidosis endemic zone, whether relatively developed or developing, requires a more single-minded advocacy of an effective vaccine. At present, melioidosis still qualifies as a neglected tropical disease. There is another reason to collaborate in international surveillance of human melioidosis, since septicemic disease often occurs as a result of extreme weather events, some of which can be attributed to climate change. A long-term regional change in melioidosis epidemiology could serve as an indicator of effective climate change, or of direct encounters with a contaminated environment through environmental disturbance. The infection may thus provide us with an index of both direct local and indirect regional anthropogenic environmental stress.

Conclusions

Melioidosis is a complex bacterial infection that includes a cluster of overlapping disease entities, resulting from exposure to a contaminated environment. While incomplete, knowledge of the epidemiology, biology and ecology of melioidosis can be applied to improving disease surveillance, outbreak identification and environmental control. The absence of a vaccine and difficulty with both diagnosis and treatment place a heavy reliance on environmental health resources, which are often scarce in melioidosis-endemic locations. Targeted disease surveillance and selective environmental investigation represent the most effective use of those scarce public health resources, and will establish a knowledge base for introduction of a melioidosis vaccine when one eventually becomes available. International cooperation is needed for public health laboratory capacity-building and effective advocacy for vaccine development for this neglected tropical disease.

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