Extreme weather events and environmental contamination are associated with case-clusters of melioidosis in the Northern Territory of Australia

Allen C Cheng,1,2* Susan P Jacups,1,2 Daniel Gal,1 Mark Mayo1 and Bart J Currie1,2

Background Melioidosis, the infection due to the environmental organism Burkholderia pseudomallei, is endemic to northern Australia and South East Asia. It is associated with exposure to mud and pooled surface water, but environmental determinants of this disease are poorly understood. We defined case-clusters in northern Australia, determined their contribution to the observed rate of melioidosis, and explored clinical features and associated environmental factors.

Methods Using geographical information systems data, we examined clustering of melioidosis cases in time and geographical space in the Top End of the Northern Territory of Australia between 1990 and 2002 using a scan statistic. DNA macrorestriction analysis, resolved by pulsed field gel electrophoresis, was performed on isolates from patients.

Results We defined five case-clusters involving 27 patients that occurred within 7–28 days and/or a radius of 100–300 km. Clustered cases were associated with extreme weather events or environmental contamination; no difference in the clinical pattern of disease was noted from other patients not involved in clusters. Isolates from patients linked to environmental contamination were caused by isolates with similar DNA macrorestriction patterns, but isolates from patients linked to severe weather events had more diverse DNA macrorestriction patterns.

Conclusion Case-clusters of melioidosis where isolates exhibit diverse DNA macrorestriction patterns in our region are linked to extreme weather events and outbreaks where isolates are predominantly of the same DNA macrorestriction pattern are linked with contamination of an environmental source.

Keywords Melioidosis, Burkholderia pseudomallei, outbreaks, natural disasters, environmental microbiology

Introduction Melioidosis, the infection due to the saprophytic Gram-negative bacterium Burkholderia pseudomallei, is an infectious disease endemic to South East Asia and northern Australia. It may present as an illness of varying severity and may involve many different sites of the body. As most cases are thought to result from inoculation of the bacterium through the skin, an epidemiological link has been formed between the disease and exposure to mud and/or surface water.1 The spatial and temporal distribution of melioidosis in northern Australia presents unique problems for analysis. The sparse population of the Top End (~150 000 people), encompassing over 500 000 km², is irregularly distributed into three urban areas (Darwin, Katherine, and Gove), and numerous smaller communities of <1000 people (Figure 1). Thus, the main determinant of the location of cases is the location of people, and standard geospatial measures such as autocorrelation must

1 Tropical and Emerging Infectious Diseases Division, Menzies School of Health Research, Charles Darwin University, Australia.
2 Northern Territory Clinical School, Flinders University, Darwin, NT, Australia.
* Corresponding author. Menzies School of Health Research, PO Box 41096, Casuarina NT 0811, Australia. E-mail: allenc@menzies.edu.au
be interpreted with caution. In northern Australia, melioidosis is a seasonal disease with 85% of cases occurring in the tropical wet monsoon season\(^2\) during which localized meteorological events, such as flash flooding, may occur. Unlike other areas with endemic disease, rice farming does not occur in the Northern Territory; thus, exposure to mud and surface water is less dependent on seasonal agricultural practices such as rice paddy flooding and planting.\(^3\)

Previous studies have defined an incubation period of between 1 and 21 days in the 25% of cases where an exposure was defined.\(^4\) However, this may depend on the mode and magnitude of inoculation; for example, the incubation period may be short in situations where exposure to a high bacterial load has been likely, such as near-drownings.\(^5,6\) Additionally, because of the potential for reactivation from a latent focus, cases presenting up to 62 years after exposure have been documented.\(^7,21\)

Temporal-spatial clustering of melioidosis was noted in an epidemiological study in Thailand, but the extent of this phenomenon and the causative factors have not been explored.\(^8\) In this study, we defined cases clustered in time and location in northern Australia, determined their contribution to the observed rate of melioidosis, and explored clinical features and associated environmental factors.

**Methods**

The Top End of the Northern Territory (between latitudes 10°S and 20°S) is regarded as endemic for melioidosis. Since 1989 a database has been maintained containing the demographic and clinical details of all cases of melioidosis in the Northern Territory. We performed an analysis of cases between January 1, 1990 and October 31, 2002.

The most likely place of exposure to *B. pseudomallei* was regarded as the geographical location in which the patient resided at the time of admission. Latitude and longitude were determined from the Gazetteer of Australia.\(^9\) Population statistics were determined from the mid-interval point, the 1996 census (Australian Bureau of Statistics). We considered patients with first presentations of acute (symptoms of <2 months) cases of culture-confirmed melioidosis. Cyclone tracks, pressures, and wind strength were drawn from the Bureau of Meteorology archive of significant weather events and from an archival web site.\(^10\) Tropical cyclones (TCs) are conventionally classified by maximum wind speed, which may not necessarily correlate with rainfall. Table 1 details TCs in the Top End region.

With permission from affected communities epidemiological investigations were conducted after suspected clusters of disease were identified. Detailed histories were taken from...
individual patients seeking to determine exposure to potential environmental pathogens. Sampling from soil, water, and other possible sources of exposure were performed.

Clinical isolation of *B. pseudomallei* was performed by standard methods. Isolates were stored at −70°C in Todd–Hewitt broth (Oxoid Australia, Melbourne, Australia) with 20% glycerol. DNA macrorestriction analysis was performed using *SpeI* as published elsewhere. In summary, pure colonies were grown overnight in Todd–Hewitt broth. A resuspension was mixed with a 2% low melting point agarose and pipetted into PFGE plug moulds. Plugs were lysed overnight at 50°C in a proteinase K-containing lysis buffer, rinsed with TE buffer, and stored at 4°C. Plugs were digested overnight with *SpeI*. PFGE was performed using the CHEF-III DR system using concatemerized lambda phage DNA as a standard. PFGE gels were stained with ethidium bromide and digitally photographed using a Gel Doc 1000 system (Biorad Laboratories). Strain types were distinguished at the level of ‘closely related’.

The scan statistic determines the probability that incidence of disease within a defined time-space window is the same as the incidence outside the window, adjusted for population and based on the Poisson distribution. We sought clusters defined within a 150 km radius and within 21 days, then used a Monte Carlo simulation with 999 simulations at 0.05 probability level. Clusters were also detected if they occurred within 21 days in all areas. Weather events affect a variable area, and to explore the possibility that incubation may be shorter in case-cluster situations, we performed a sensitivity analysis altering the radius window between 100 and 300 km and the time period between 7 and 28 days. We then examined the clinical characteristics of clustered patients and compared these with all the patients not involved in clusters.

Analysis and statistical calculations for clusters were performed using SaTScan 3.0 (Statistical Research and Applications Branch, National Cancer Institute, Bethesda, MD). Fisher’s exact test compared proportions using Intercooled Stata 7.0 for Windows (STATA Corporation, College Station, TX).

Ethical approval for this study was obtained from the Human Research Ethics Committee of the Department of Health and Community Services and the Menzies School of Health Research. As many remote communities are characterized by small numbers of people with a cultural connection to the area, we have taken specific care not to disclose identifying information for remote Aboriginal communities in this paper pursuant with the conditions of our submission.

### Results

During the period January 1, 1990 and October 31, 2002, there were 274 cases of culture-confirmed melioidosis presenting with acute symptoms. In addition, 50 patients were excluded owing to presentations with reactivated or chronic disease, five owing to residency outside the endemic area, and 27 owing to serological diagnoses only. Thirty communities recorded cases of melioidosis from the 41 census areas (total population of 143,633) defined in this study. The annual rate of acute melioidosis was 14.9 cases per 100,000 population.

Five clusters were identified by the primary analysis parameters (scanning window of 21 days and 150 km radius) involving 22 patients. Sensitivity analysis revealed a further cluster of two patients in cluster 3 using a narrow scan window (7 days, 100 km) and a further three patients in cluster 2 in a wider scan window (28 days, 300 km) giving a total of 27 patients. Table 2 details these clusters.

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**Table 1** Categories of cyclones and a list of cyclones in the Top End region between 1990 and 2002

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
<th>Cyclones in Top End region</th>
<th>Dates</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Strongest gust &lt;125 km/h (Gales; central pressure &gt;985)</td>
<td>TC Laurence</td>
<td>December 10–12, 1990</td>
<td>No landfall (WA border)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC 0858</td>
<td>January 27–28, 1996</td>
<td>Qld border</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC Rachel</td>
<td>January 2–10, 1997</td>
<td>WA border, coast islands, Darwin region</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC Phil</td>
<td>December 26, 1996 to January 3, 1997</td>
<td>Passed over Top End region</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC Steve</td>
<td>February 27 to March 11, 2000</td>
<td>passed inland over Top End region</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC Winsome</td>
<td>February 10–11, 2001</td>
<td>Qld border</td>
</tr>
<tr>
<td>2</td>
<td>Strongest gust 125–169 km/h (Destructive winds; central pressure 985–790 kPa)</td>
<td>TC Mark</td>
<td>January 7–10, 1992</td>
<td>E. Arnhem region</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC Ethyl</td>
<td>March 8–13, 1996</td>
<td>Qld border</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC Sid</td>
<td>December 24–29, 1997</td>
<td>North coast Darwin and E. Arnhem region</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC Les</td>
<td>January 22 to February 1, 1998</td>
<td>Passed over Top End region</td>
</tr>
<tr>
<td>3</td>
<td>Strongest gust 170–224 km/h (Very destructive winds; central pressure 970–945 kPa)</td>
<td>TC Warren</td>
<td>March 4–6, 1995</td>
<td>Qld border</td>
</tr>
<tr>
<td>4</td>
<td>Strongest gust 225–279 km/h (Very destructive winds; central pressure 920–945 kPa)</td>
<td>TC Neville</td>
<td>April 7–13, 1992</td>
<td>Coastal islands, Darwin region</td>
</tr>
<tr>
<td>5</td>
<td>Strongest gust &gt;280 km/h (Very destructive winds; central pressure &lt;920 kPa)</td>
<td>TC Thelma</td>
<td>November 30 to December 11, 1998</td>
<td>Coastal islands, Darwin region</td>
</tr>
</tbody>
</table>

TC: Tropical cyclone; Qld: Queensland; WA: West Australia.
Clusters associated with weather events

During the study period, 13 cyclones were recorded in the Top End area of the Northern Territory (Table 1). Of these, two were linked with the identified clusters. The first TC Thelma developed in the Timor Sea on December 6, 1998 and progressed into a severe TC of Category 5 (estimated central pressure 925 hPa, maximum estimated gusts of up to 320 km/h). Destructive winds and extreme rainfall were experienced in the Darwin regional and adjacent coastal communities on December 10, 1998. Areas of Darwin recorded their highest rainfall totals for over 25 years, with one station in the Darwin region recording 1022 mm of rain in 24 h. TC Thelma moved away from the coast to the west and weakened the following day. This extreme weather event was linked to Clusters 1 and 2. Patients included in Cluster 1 were from one coastal community in the Darwin region. Cluster 2 incorporated all six patients from Cluster 1 and a further six patients from the Darwin region.

The second TC in the time period, TC Les, formed in the Gulf of Carpentaria to the east of the Top End in mid-January, 2000. Although not associated with strong winds (Category 2), it was associated with heavy rainfall (300–400 mm), especially in the catchment area of the Katherine and Upper Roper rivers. For 48 h prior to January 27, 2000, this was the highest total recorded in the region and caused extensive flooding of the Daly and Katherine rivers. At the town of Katherine, the water level was above the flood level of 16 m from January 26 to January 31, 2000 and peaked at 20.4 m on January 27, 2000, the highest

Table 2 Outbreaks identified and probable explanatory events

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Location</th>
<th>Cases/population</th>
<th>Relative risk (P-value)</th>
<th>Period and area of scan window</th>
<th>Source</th>
<th>Weather event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Coastal communities A</td>
<td>6 cases/1734 people</td>
<td>425 (0.001)</td>
<td>December 8–27, 1998; 38 km radius</td>
<td>–</td>
<td>TC Thelma</td>
</tr>
<tr>
<td>2</td>
<td>All areas</td>
<td>16 cases/143 633 people</td>
<td>9.8 (0.001)</td>
<td>December 8, 1998 to January 9, 1999</td>
<td>–</td>
<td>TC Thelma</td>
</tr>
<tr>
<td>3</td>
<td>Katherine</td>
<td>6 cases/7046 people</td>
<td>97 (0.001)</td>
<td>February 3–20, 1998</td>
<td>Extensive flooding following TC Les</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Coastal community B</td>
<td>3 cases/2059 people</td>
<td>95 (0.003)</td>
<td>March 4–8, 1994; Single community</td>
<td>Contamination of drinking water supply</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Katherine district community C</td>
<td>2 cases/273 people</td>
<td>9000 (0.002)</td>
<td>January 23–24, 2001; Single community</td>
<td>Contaminated detergent</td>
<td></td>
</tr>
</tbody>
</table>

Incorporates all patients in Cluster 1.

Figure 2 Incident cases of melioidosis in relation to TC Thelma, December 1998 (Clusters 1 and 2)

Figure 3 Incident cases of melioidosis in relation to Katherine floods in January, 2000 (Cluster 3)
recorded level. Inundation of the central business district led to the evacuation of the area and was associated with severe disruption to communications and extensive property damage in Katherine. This event was linked to the six cases in Cluster 3 (Figure 3). This compares with the corresponding 28 day period in the previous year, where no cases of melioidosis presented from the Katherine region.

Clusters associated with environmental contamination
Two clusters were associated with environmental contamination. Cluster 4 was part of a longer outbreak that involved nine patients over 28 months that has previously been reported.15 Finally, the two patients in Cluster 5, from a small community of 273, were exposed at a garage to a common detergent container from which \textit{B. pseudomallei} was recovered.16

Of the 27 patients involved in clusters, the mortality was 11\%, the proportion with severe sepsis was 15\%, pneumonia was seen in 52\%, and genitourinary disease was seen in 15\%. The proportion of patients with a history of diabetes was 30\%. These proportions were not statistically different from patients not involved in case-clusters (mortality 16.2\%, severe sepsis 25\%, pneumonia 50\%, genitourinary disease 13\%, diabetes 38\%, Fisher’s exact: all $P > 0.05$).

Pulsed field gel electrophoresis
PFGE was performed from six patients in Cluster 3 and all six patients in Cluster 1, linked to severe weather events. Two of six isolates from patients in Cluster 1 were identical. Two isolates from patients in Cluster 3 were closely related but others were unrelated (Figure 4). In contrast, isolates from patients in Clusters 4 and 5, linked to environmental contamination, have demonstrated similarities on DNA macrorestriction analysis.15,16

Discussion
This analysis using a geographical information system seeks to detect unusual clusters of cases. The background rate of ‘usual’ disease is defined and simulated using the Poisson distribution for the geographic area during a time period ‘window’. Observed cases within each window are compared with expected cases, and a relative risk with its associated probability calculated. Where it has been suggested that a large window between 10\% and 50\% of the population of interest is defined, we have chosen a much smaller geographic area to reflect the relatively localized influence of meteorological conditions and the broad study area. Similarly, the temporal window we used for the primary analysis, 21 days, covers the incubation period of acute melioidosis and sought to detect relatively short-lived meteorological phenomena. Sensitivity analysis allowed for variations in the incubation period potentially associated with high or low inoculum and non-point source case-clusters of up to a week in duration, occurring over a wider area.

In this study, we have defined five such unusual clusters during a 12 year period involving 10\% of the cases of acute melioidosis seen during this time. We have observed a link between extreme weather events and three of the clusters of melioidosis identified by this method. For the residents of the Top End, TC Thelma and the Katherine floods undoubtedly represent the most catastrophic weather events during the study period; both caused extensive property damage and required evacuation of inhabitants from the worst-affected areas. A recent review of all Australian cases also noted an association between a cluster of five cases from an isolated Queensland community and flooding following a TC in January, 2002.17 Although TCs are an annual occurrence in the region, apart from TC Tracy in 1974, which destroyed Darwin city, most have not been associated with widespread destruction to communities in recent decades.
The relative contribution of each possible mode of acquisition of melioidosis is yet to be defined, whether inhalation, inoculation, or ingestion. It has been thought that the majority of cases arise from inoculation or contamination of minor wounds, based on a common exposure history of pooled surface water and mud, the clinical features of the illness suggesting haematogenous dissemination, and in some patients, a clear history of an inoculating injury. More recent work has noted an association between the severity of illness and incidence of pneumonia in relation to higher rainfall, suggesting that inhalation may be more important than had been previously recognized. Although ingestion has been postulated as a mode of acquisition, the lack of localization to the gastrointestinal tract, as seen in goats, points away from this occurring commonly; it should be noted that case clusters of disease related to contaminated drinking water may not necessarily represent acquisition via ingestion. In this study, significant differences between the pattern of disease during case-clusters and those at other times have not been found, although, an association between monsoonal rainfall and severity of illness and the incidence of pneumonia has been previously noted, suggesting a possible shift from inoculation to inhalation during periods of heavy wind and rain.18

Other associations also resulting in clusters of melioidosis cases were noted in relation to environmental contamination, of which two were detected in this study. It was determined that one of these clusters was part of a larger outbreak of nine cases occurring over a 28 month period. This outbreak has previously been reported and was found to be linked, both epidemiologically and by molecular typing, to contamination of an unchlorinated drinking water supply.15 Contaminated water supplies have been implicated in two case-clusters elsewhere in Australia.19,20 A second outbreak in a small community was linked epidemiologically to contaminated detergent used in a garage. In this outbreak multiple strain types were isolated from the detergent but none was found to match the two clinical isolates, which had identical DNA macrorestriction patterns.16 We believe that the observation that these outbreaks have not reoccurred since the removal of the putative source vindicates our approach to the investigation of these outbreaks. We acknowledge that it is possible that these case-clusters may have been self-limited owing to natural changes in the bacterial concentration in the environmental source unrelated to our control measures, but it is unlikely that the fall in incident cases relates to changes in the susceptibility of the population, a significant proportion of which have risk factors for melioidosis such as diabetes.

The molecular similarities of isolates from patients involved in outbreaks linked to environmental contamination reflect the acquisition of a strain established in a particular environmental niche from which exposure occurs. However, the isolates from melioidosis patients affected by extreme weather events generally exhibited molecular diversity, suggesting that widespread weather events that favour the acquisition of _B. pseudomallei_ are responsible for these case-clusters rather than exposure to a particular strain.

There are several limitations to our study. We did not consider host parameters, where risk factors such as diabetes, excessive alcohol intake, and renal disease are known to be important determinants of disease.2,8 We note that census data from 1990 and 2002 have only revealed small changes to the total population and do not believe that this would affect our results significantly. Changes to the risk factor profile of this population are not likely to affect the incidence of melioidosis over the short time windows in this analysis.

Ascertainment of all cases linked to weather events is not possible owing to the variation in incubation period for melioidosis, which can be up to 62 years after exposure.21 Previous work suggests an incubation period of up to 21 days in the majority of cases,4 with shorter incubation associated with situations of exposure to high inocula. Subsequent to the cases identified in Cluster 3, additional melioidosis cases in Katherine presented in the 4th, 8th, 9th, and 20th weeks after the floods. These four cases were not included in the cluster as they were outside the primary and secondary analyses windows (21 day and 7–28 days, respectively) used in this study.

In addition, meteorological phenomena are complex events; it is difficult to define their location not only in terms of time and space but also in terms of the resulting rainfall, wind speed, and hydrology. Because of this complexity and the paucity of such data from remote communities, we chose to analyse our data using disease incidence, but the exact meteorological determinants of disease and their probable interaction with soil types are yet to be defined. Sampling studies have suggested that _B. pseudomallei_ is associated with clay layers in soil; this remains to be confirmed by GIS studies.

We conclude that geospatial clusters of cases of melioidosis where clinical isolates exhibit molecular diversity are associated with extreme wind and rainfall conditions. In contrast, outbreaks where clinical isolates have similar DNA macrorestriction patterns are associated with environmental contamination. This work also suggests that environmental sampling at the time of extreme weather events might be expected to have a higher yield, and that clusters of melioidosis not related to such events should prompt a search for an environmental focus. This work also suggests that public awareness and prevention campaigns might help prevent such case-clusters if commenced during such weather events, although outbreaks only contribute a minority of cases to the total public health burden of melioidosis. Ongoing work is aimed at elucidating factors responsible for the background rate of disease such as water contamination, soil type, and patterns of rainfall as well as factors determining the different manifestations of melioidosis.

Acknowledgements
We are grateful to Michael Foley from the Bureau of Meteorology for data on extreme weather events, and Chris Devonport and Phillippe Puig for early advice regarding the utility of GIS in disease epidemiology. We acknowledge the contribution of the clinical microbiology laboratories, particular Dr Gary Lum and staff at the Royal Darwin Hospital, for their expert isolation and identification of _B. pseudomallei_. Financial support: A.C.C. is supported by a training grant from the Australian National Health and Medical Research Council and the study is supported by a project grant from the Australian National Health and Medical Research Council.

Conflict of Interest
The authors have declared no conflicts of Interest.
Case-clusters of melioidosis can be identified by a scan statistic.

Case-clusters where patient isolates exhibit diversity based on DNA macrorestriction analysis are linked to extreme weather events such as flooding and TCs.

Case-clusters where patient isolates are genetically similar based on DNA macrorestriction analysis are linked to environmental foci.

References