

FATAL HUMAN MELIOIDOSIS ACQUIRED IN A SUBTROPICAL AUSTRALIAN CITY

WENDY J. MUNCKHOF, MARK J. MAYO, IAN SCOTT, AND BART J. CURRIE

Department of Infectious Diseases, Infection Control and Sexual Health, and Department of Internal Medicine, Princess Alexandra Hospital and District Health Service, Brisbane, Queensland, Australia; Royal Darwin Hospital Clinical School, Flinders University, and Tropical Medicine and International Health Unit, Menzies School of Health Research, Darwin, Northern Territory, Australia

Abstract. We describe an acute fatal human case of melioidosis acquired in Ipswich, a city at 27.5°S in southern Queensland, south of the area traditionally considered endemic for melioidosis in Australia. Molecular typing revealed that this patient isolate was genetically distinct from 2 other human and 1 bovine isolates of *Burkholderia pseudomallei* from the same region and from 4 tropical northern Australian strains. This finding suggests that if *B. pseudomallei* has been introduced to the region from northern Australia, it was not in recent times, and there has not been a point source of infection. *Burkholderia pseudomallei* is present in temperate southern Queensland, which hitherto has not been well appreciated. Clinicians should consider the diagnosis of acute melioidosis in patients with severe pneumonia or septicemia acquired in subtropical areas such as southern Queensland, particularly after heavy summer rains with flooding.

INTRODUCTION

Melioidosis is a disease of humans and animals caused by *Burkholderia pseudomallei* and is endemic in Southeast Asia, the South Pacific, and tropical Australia. The endemic area for melioidosis has generally been stated to be within 20° of the equator.¹ Human cases are common in tropical northern Australia, with large numbers reported from Darwin (12°S),^{2,3} Cairns (17°S), and Townsville (19°S)^{4–6} (Figure 1). Interestingly, in animals, there have been several well-documented cases of melioidosis in subtropical regions of Australia, particularly after heavy rain. After flooding of the Brisbane River (27°S) in 1974, melioidosis in cattle occurred on a farm where cattle had been imported from north Queensland the previous year.⁷ Between 1981 and 1983, there were 159 cases of melioidosis in piggeries in the subtropical region of the Burnett river at 25.5°S after heavy rainfall and river flooding.⁸ It was suggested that melioidosis in these cases was introduced into the farms by imported carrier animals from tropical Australia, or, alternatively, that the organism had survived in the soil from ancient times, when rainforest covered the area.⁹

Human cases acquired in subtropical regions of Australia are rare. Only 4 human cases of melioidosis acquired in Australia south of latitude 20°S have been reported. A 25-year cluster of animal cases and one human infection in a farmer was reported from southwest Western Australia (31°S).¹⁰ Ribotyping analysis of the animal and human isolates and one isolate from soil in this study showed identical patterns, which were different from isolates from tropical areas of Australia.¹¹ This suggested clonal introduction of *Burkholderia pseudomallei* into this region with environmental contamination, local dissemination, and persistence over the course of 25 years. Second, 3 fatal human cases of melioidosis have been reported from Ipswich (27.5°S), a subtropical city of population 100,000 in southeast Queensland, Australia, 700 km south of the Tropic of Capricorn.¹² All 3 cases (1 in 1974, 2 in 1996) followed high rainfall and local flooding. Medical records for the 1974 human case have been destroyed, and no further clinical details are available.

We report a recent human case of fatal acute melioidosis acquired in Ipswich, and we also report the results of mo-

lecular typing on this isolate, 2 other recent human isolates, and a 1974 bovine isolate from this same subtropical region.

CASE HISTORY

A 51-year-old man presented to Ipswich Hospital in February 1999 with a 6-day history of fever, cough with purulent sputum, breathlessness, right-sided pleuritic chest pain, confusion, and malaise. He had a medical history of chronic obstructive pulmonary disease after years of heavy smoking and an alcohol intake of 60 g per day. His work duties just before the onset of illness included draining excess water that had accumulated in local city parks after a period of hot, humid weather (up to 35°C) with heavy rain and local flooding. He had never traveled overseas; within Australia, he had never traveled north of Brisbane (27°S). He lived in the suburbs of Ipswich, 5 km from the city center. There was no travel to farms or contact with animals.

At admission, he appeared acutely ill, with confusion, tachypnea, tachycardia, hypotension, oliguria, and widespread crepitations throughout both lung fields. He was afebrile. Analysis of chest radiographs showed extensive confluent consolidation throughout both lungs. Broad-spectrum antibiotic therapy with intravenously administered ceftriaxone, gentamicin, and erythromycin was commenced. The patient's condition deteriorated despite antibiotics, inotropic support, and oxygenation, and he died 18 hr after admission. Premortem blood and sputum cultures grew *B. pseudomallei*. Antimicrobial susceptibility testing of the isolate by standard National Committee for Clinical Laboratory Standards disk methods showed susceptibility to ceftazidime, ticarcillin-clavulanate, amoxicillin-clavulanate, piperacillin-tazobactam, ciprofloxacin, trimethoprim-sulfamethoxazole, tetracycline, and imipenem and resistance to ceftriaxone and gentamicin.¹³ Serology on the day of admission was tested for both immunoglobulin (Ig) G and IgM to *B. pseudomallei* by hemagglutination assay and was negative, showing no evidence of past infection.

MATERIALS AND METHODS

Isolates. All isolates (Table 1) were stored at –70°C before typing. The frozen isolates were cultured on blood agar

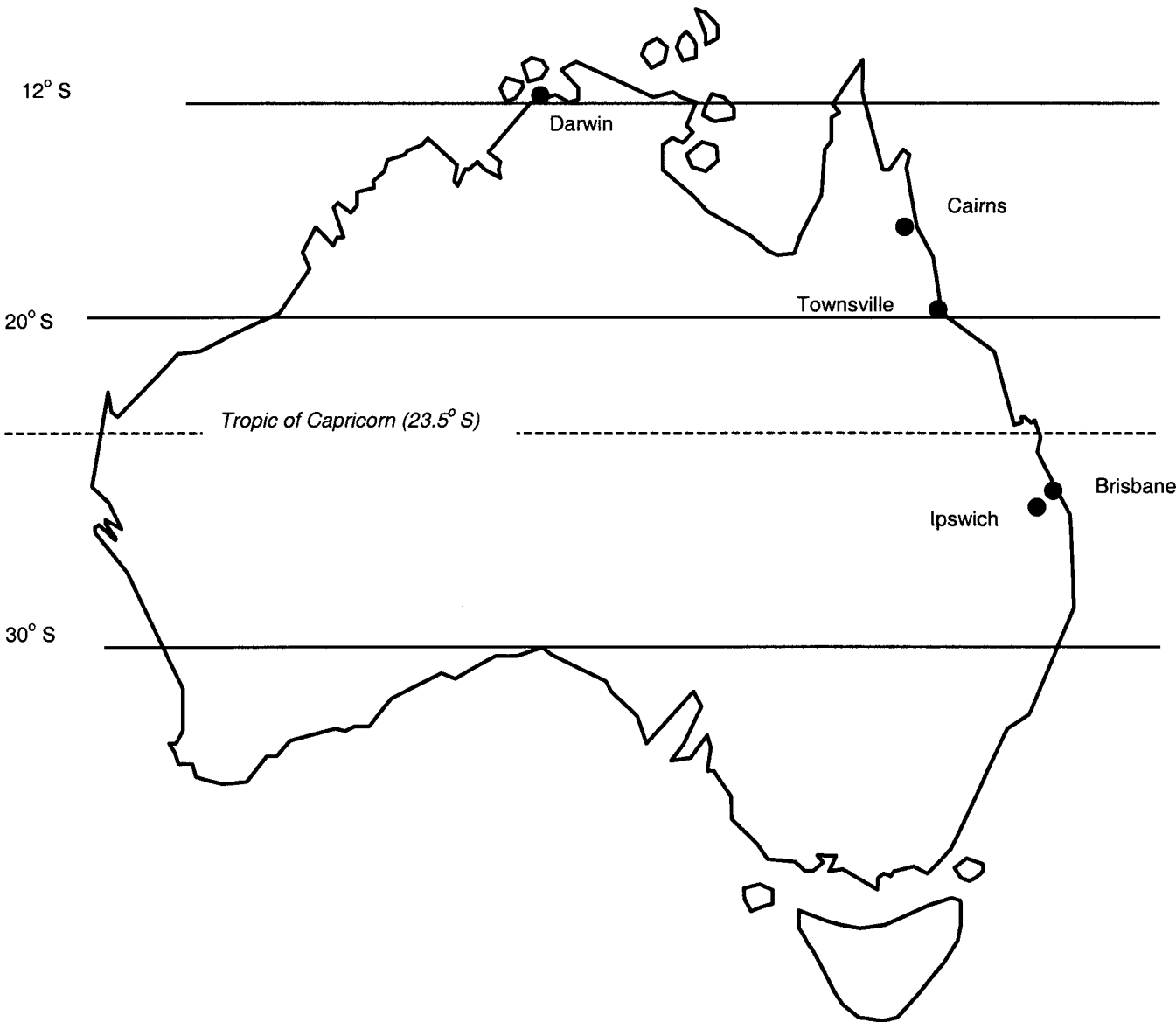


FIGURE 1. Location of human cases of melioidosis in Australia. Large numbers of cases are reported from Darwin, Cairns, and Townsville annually. Cases south of the Tropic of Capricorn are rare.

and the identity of *B. pseudomallei* was confirmed biochemically by API 20NE (BioMerieux Vitek, Baulkham Hills, Sydney, Australia).

Pulsed field gel electrophoresis (PFGE). Block preparation was carried out by a modification of the method of

Haase and others.¹⁴ Pure colonies were grown in Tryptone-soya broth (Oxoid, West Heidelberg, Melbourne, Australia) for 3 days at 37°C. Cells were pelleted, washed twice in normal saline, and adjusted to $240\text{--}300 \times 10^7$ cells/mL in PET IV (10 mM Tris, 1 M NaCl). This suspension was

TABLE 1
Source, location, and year of the *Burkholderia pseudomallei* isolates

Lane	Source of isolate	Location	Year
1	Human blood culture	Ipswich	1996, Patient A
2	Human blood culture	Ipswich	1996, Patient B
3	Bovine lung tissue	Rocklea, Brisbane	1974
4	Human blood culture	Ipswich	1999
5*	Human leg ulcer	Northern Territory	1998
6*	Human hand ulcer	Northern Territory	1998
7*	Human throat culture	Northern Territory	1998

* The 3 Northern Territory isolates were geographically and epidemiologically unrelated.

mixed with an equal volume of 2% low-melting-point agarose (FMC Bioproducts, Rockland, ME) and pipetted into block molds ($0.8 \times 0.4 \times 0.2$ cm). Solidified blocks were incubated in lysis buffer (100 mM ethylenediaminetetraacetic acid [EDTA], 0.5% Brij 58, 0.2% sodium deoxycholate, 0.5% *N*-lauroylsarcosine) containing 20 mg/mL lysozyme (Amersham Pharmacia Biotech, Castle Hill, Sydney, Australia) and 10 μ g/mL RNase (Amersham Pharmacia Biotech) at 37°C overnight. Blocks were then transferred to proteinase K buffer (0.5 M EDTA, 1% *N*-lauroylsarcosine) containing 1 mg/mL proteinase K (Boehringer Mannheim, Indianapolis, IN) and incubated at 55°C overnight. Blocks were washed twice in proteinase K buffer and stored at 4°C until use. Half blocks were dialyzed overnight in low EDTA TE buffer (10 mM Tris, 0.1 mM EDTA). Blocks were preincubated in 200 μ L of digestion buffer for 30 min, then digested with 10 units of *Spe*I (Amersham Pharmacia Biotech) in fresh buffer at 37°C for 4 hr. Digests were run on 1% agarose gels (Promega, Annandale, Sydney, Australia) in 0.5 \times TBE buffer (Tris Base, Boric Acid, 0.5M EDTA) on a Bio-Rad Chef DR-III system (Bio-Rad, Regent Park, Sydney, Australia), with a 10–60-sec pulse ramp at 6 V/cm for 24 hr with yeast chromosome markers as standard (Bio-Rad). Gels were stained with ethidium bromide, visualized with ultraviolet light, and analyzed with the Bio-Rad molecular analyst package.

RESULTS

The isolates that were typed were 1) blood cultures from the patient reported above, 2) blood cultures from both fatal human Ipswich cases acquired in 1996,¹² and 3) a cow that died of melioidosis acquired in Rocklea (27°S), a suburb of Brisbane, after flooding of the Brisbane River in 1974. The typing results for these strains were then compared with isolates from the endemic region in the top end (northern region) of the Northern Territory, Australia.

The PFGE patterns of DNA from the human and bovine isolates of *B. pseudomallei* showed that the isolates were all distinct from each other (Figure 2, lanes 1–4) and from isolates from the top end of the Northern Territory (Figure 2, lane 5–7).

DISCUSSION

This study demonstrates that *Burkholderia pseudomallei* isolates from 3 fatal human cases of melioidosis acquired presumptively in subtropical Australia are genetically distinct from each other and are also not genetically identical to isolates obtained from tropical northern Australia. These results are different than typing results from the only other known human and animal cluster of melioidosis previously reported from nonendemic Australia. All isolates from the previous cluster, which occurred in southwest Western Australia (31°S), were genetically identical, although, as with the Ipswich cases, the cluster was genetically distinct from tropical northern Australia isolates.¹¹ It was suggested that this cluster was due to a clonal introduction of *B. pseudomallei* from an animal imported from northern Western Australia, with subsequent persistence and dissemination of the same strain over the course of 25 years.¹⁰

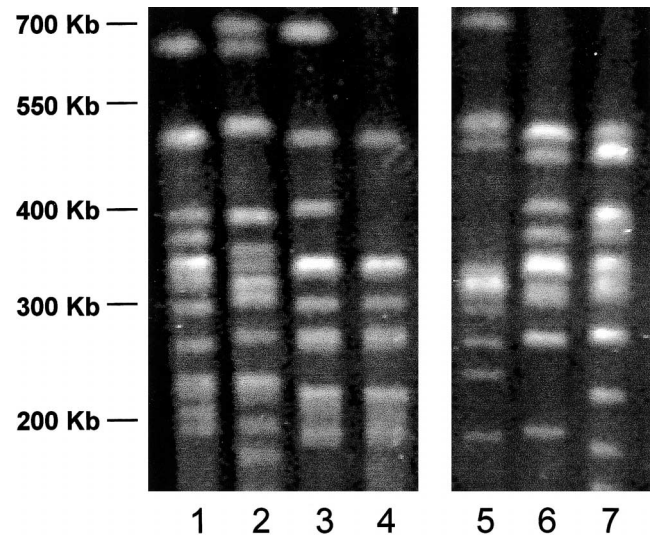


FIGURE 2. Pulsed field gel electrophoresis of *Spe*I-digested chromosomal DNA of *Burkholderia pseudomallei* isolates. Procedures are described in Materials and Methods. **Lane 1**, human blood culture, Ipswich, Patient A, 1996. **Lane 2**, human blood culture, Ipswich, Patient B, 1996. **Lane 3**, bovine lung tissue, Rocklea, Brisbane, 1974. **Lane 4**, human blood culture, Ipswich, 1999. Lanes **5–7**, human cultures from the top end of the Northern Territory, 1998. The 3 Northern Territory isolates were geographically and epidemiologically unrelated.

Our results clearly show that the 4 southern Queensland isolates are genetically distinct and not clonal. The patient reported here lived in the suburbs of Ipswich 5 km north of the city center, whereas the two 1996 human cases lived within 5 km of each other, 65 km north of Ipswich in a rural area near a recreation and irrigation reservoir.¹² The 1974 fatal case of bovine melioidosis was that of a cow from a farm in Rocklea (27.5°S), a suburb of Brisbane that experienced heavy flooding in 1974 (Bell M and Thomas R, unpublished data).

Our cases show that the geographic boundaries of endemicity of melioidosis in Australia remain unclear. Geographically distinct cases in southern Queensland after periods of heavy rain and high humidity have been found in different studies.^{7,8,12} The diagnosis of melioidosis in patients from southern Queensland with severe pneumonia or septicemia should be considered, particularly after heavy summer rains with flooding. Patients with chronic pulmonary diseases, diabetes, heavy alcohol intake, steroid use, renal impairment, or malignancy are at increased risk of both acquiring and dying from melioidosis.¹⁵ If the diagnosis is likely or possible, appropriate antimicrobial therapy to cover *B. pseudomallei* should be considered, including administration of ceftazidime or a carbapenem (imipenem or meropenem), while awaiting results of sputum and blood cultures.

Acknowledgments: We thank Brian Parker and Joseph Kan of the Microbiology Laboratory at Ipswich Hospital and Maree Brennan, formerly of the Menzies School of Health Research, for isolate culture and identification; Dr. David Drummond of Queensland Medical Laboratories for forwarding cultures to Darwin for typing; Dr. Robert Norton of Townsville Hospital for serological testing; and Morton Bell of Darling Downs Public Health Unit and Rod Thomas of the Animal Research Institute at Yeerongpilly, Brisbane, for historical veterinary advice and for the 1974 bovine isolate of *B. pseudomallei*.

Financial support: This work was partly funded by a grant from the National Health and Medical Research Council of Australia.

Author's addresses: Wendy J. Munckhof, Department of Infectious Diseases, Infection Control and Sexual Health, Princess Alexandra Hospital, Ipswich Road, Woolloongabba, Brisbane, Queensland, Australia, 4102. Mark J. Mayo and Bart J. Currie, Royal Darwin Hospital Clinical School and Tropical Medicine and International Health Unit, Menzies School of Health Research, P.O. Box 41096, Casuarina, Northern Territory, 0811, Australia. Ian Scott, Department of Internal Medicine, Princess Alexandra Hospital, Ipswich Road, Woolloongabba, Brisbane, Queensland, Australia, 4102.

REFERENCES

1. Howe C, Sampath A, Spotniz M, 1971. The *pseudomallei* group: a review. *J Infect Dis* 24: 598.
2. Currie BJ, Fisher DA, Howard DM, Burrow JNC, Selvanayagam S, Snelling PL, Anstey NM, Mayo MJ, 2000. The epidemiology of melioidosis in Australia and Papua New Guinea. *Acta Trop* 74: 121–127.
3. Merianos A, Patel M, Lane JM, Noonan CN, Sharrock D, Mock PA, Currie B, 1993. The 1990–1991 outbreak of melioidosis in the Northern Territory of Australia: epidemiology and environmental studies. *Southeast Asian J Trop Med Public Health* 24: 425–435.
4. Ashdown LR, Guard RW, 1984. The prevalence of human melioidosis in Northern Queensland. *Am J Trop Med Hyg* 33: 474–478.
5. Guard RW, 1987. Melioidosis in Australia. A review. *Aust Fam Physician* 16: 119–123.
6. Guard RW, Khafagi FA, Brigden MC, Ashdown LR, 1984. Melioidosis in Far North Queensland. A clinical and epidemiological review of twenty cases. *Am J Trop Med Hyg* 33: 467–473.
7. Ketterer PJ, Donald B, Rogers RJ, 1975. Bovine melioidosis in south-eastern Queensland. *Aust Vet J* 51: 395–398.
8. Ketterer PJ, Webster WR, Shield J, Arthur RJ, Blackall PJ, Thomas AD, 1986. Melioidosis in intensive piggeries in south-eastern Queensland. *Aust Vet J* 63: 146–149.
9. Currie BJ, 1993. Medicine in tropical Australia. *Med J Aust* 158: 612–615.
10. Golledge CL, Chin WS, Tribe AE, Condon RJ, Ashdown LR, 1992. A case of human melioidosis originating in south-west Western Australia. *Med J Aust* 157: 332–334.
11. Currie B, Smith-Vaughan H, Golledge C, Buller N, Sriprakash KS, Kemp DJ, 1994. *Pseudomonas pseudomallei* isolates collected over 25 years from a non-tropical endemic focus show clonality on the basis of ribotyping. *Epidemiol Infect* 113: 307–312.
12. Scott IA, Bell AM, Staines DR, 1997. Fatal human melioidosis in south-eastern Queensland. *Med J Aust* 166: 197–199.
13. National Committee for Clinical Laboratory Standards, 2000. Performance standards for antimicrobial disk susceptibility tests: approved standard. Seventh edition. National Committee for Clinical Laboratory Standards document M2-A7, vol. 20, no. 1. Villanova, PA: National Committee for Clinical Laboratory Standards.
14. Haase A, Smith-Vaughan H, Melder A, Wood Y, Janmaat A, Gilfedder J, Kemp D, Currie B, 1995. Subdivision of *Burkholderia pseudomallei* ribotypes into multiple types by random polymorphic DNA analysis provides new insights into epidemiology. *J Clin Microbiol* 33: 1687–1690.
15. Sanford JP, 1995. *Pseudomonas* species (including melioidosis and glanders). Mandell GL, Bennett JE, Dolin R, eds. *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases*. Fourth edition. New York: Churchill Livingstone, 2003–2009.