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 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 molecular typing on this isolate, 2 other recent human isolates, and a 1974 bovine isolate from this same subtropical region.
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. Pure colonies were grown in Tryptone soy broth (Oxoid, West Heidelberg, Melbourne, Australia) for 3 days at 37°C. Cells were pelleted, washed twice in normal saline, and adjusted to 240–300 × 10<sup>7</sup> cells/mL in PET IV (10 mM Tris, 1 M NaCl). This suspension was

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

* 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 × 0.4 × 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 μg/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 pre-incubated in 200 μL of digestion buffer for 30 min, then digested with 10 units of SpeI (Amersham Pharmacia Biotech) in fresh buffer 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 pre-incubated in 200 μL of digestion buffer for 30 min, then digested with 10 units of SpeI (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 × 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 (27.5°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

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.3,5,12 The diagnosis of melioidosis in patients from southern Queensland with severe pneumonia or septicaemia 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 cefazidime 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.

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