Imported Cutaneous Melioidosis in Traveler, Belgium

To the Editor: In some tropical areas, melioidosis, a disease caused by infection with *Burkholderia pseudomallei*, results in sepsis (1). This disease affects mostly adults with an underlying predisposing condition (2). With the increase in international travel, melioidosis has been identified in patients returning from disease-endemic areas (3). We report a case of a travel-associated cutaneous melioidosis without any systemic involvement.

A 90-year-old woman came to the Hôpital Erasme in Brussels with a nonhealing erythematous and ulcerated cutaneous lesion on the side of her left elbow. The lesion was a papule that gradually increased in size. The patient had diabetes mellitus but was otherwise healthy when she had traveled to Bangladesh 8 weeks earlier. She stayed 3 weeks in a village in the northwestern area of Rangpur District during the rainy season. She reported multiple insect and mosquito bites that evolved into intensely pruritic papules. This led to uncontrolled scratching and repeated washing of bite lesions with untreated well water.

Two weeks after her return, the lesion developed; it increased steadily in size, despite application of topical antimicrobial ointment. Three weeks later, after three visits to a physician, she was admitted to our institution. She did not report any fever, rigors, sweating, malaise, weight loss, or respiratory symptoms. Skin examination showed an irregular $(3.0 \text{ cm} \times 4.0 \text{ cm})$, erythematous, fluctuant, tender, painful plaque (online/Appendix Figure, panel A, available from www.cdc.gov/ EID/content/13/6/946-appG.htm). She did not have palpable regional lymph nodes. Results of a physical examination and laboratory investigations were normal. Five blood cultures at different times failed to isolate any microorganism.

A skin biopsy specimen from the plaque showed an inflammatory granulomatous reaction. Gram staining of biopsy specimens showed scanty lymphocytes, no polymorphonuclear leukocytes, and no microorganisms. Specimens were ground and placed on Columbia agar containing 5% horse blood and Schaedler enrichment broth and incubated aerobically for 3 days and on Schaedler agar containing 5% horse blood and incubated anaerobically for 10 days. After 72 hours, Schaedler broth showed a few colonies of an aerobic gram-negative bacillus that was identified as B. pseudomallei on the basis of typical biochemical characteristics. The strain was mobile at 37°C; grew at 42°C, oxidized but did not ferment glucose; produced cytochrome oxidase, arginine dihydrolase, and gelatinase; and was resistant to 300 IU polymyxin B 300 (DiaTabs; Rosco, Taastrup, Denmark). The isolate had a negative reaction for metabolism of arabinose.

Antimicrobial drug testing showed susceptibility to temocillin, amoxicillin-clavulanic acid (MIC 2 mg/L), piperacillin-tazobactam, ceftazidime, cefepime, meropenem (MIC 0.75 mg/ L), doxycycline, and cotrimoxazole, and resistance to cefazolin, cefoxitin, ampicillin, gentamicin, ciprofloxacine, and amikacin. Results of tests for systemic involvement, as well as sputum and urine cultures, were negative. The patient was discharged and received oral doxycycline, 100 mg twice a day, and amoxicillin/clavulanic acid, 875 mg twice a day, for 32 weeks. The lesion dramatically improved 8 weeks after treatment was started (Appendix Figure, panel B) and had disappeared by 20 weeks after treatment was started (Appendix Figure, panel C). At 24 months after the diagnosis, no relapse had occurred.

Our patient with imported melioidosis had an unusual clinical course. She had never been febrile and had an uncomplicated localized skin infection skin despite her predisposing diabetes. A similar course has been reported in 2 tourists from Finland after the tsunami in Thailand in December 2004 (4), but most imported cases have pulmonary or systemic involvement associated with a severe prognosis (5,6).

The mode of acquisition in our patient herein was probably by an insect bite, contaminated water, or direct contact with wet soil during the rainy season. This mode of acquisition reinforces the hypothesis of a predominant role of percutaneous *B. pseudomallei* infection (7). Although the lesion healed, the patient was advised to have lifelong follow-up because relapses have been observed several years after infection.

Imported melioidosis is no longer a rare disease. With the increase in international travel and adventure tourism to disease-endemic regions, melioidosis is more likely to develop among travelers, even in those with short-term exposure. Recent reports suggest that melioidosis is probably widespread but poorly recognized throughout Bangladesh (5). Clinicians who treat patients returning from disease-endemic tropical areas, including the Indian subcontinent, should consider the disease in the differential diagnosis of febrile illnesses and isolated skin ulcers. Diagnosis is based on isolation of B. pseudomallei from blood, sputum, or biopsy specimens from lesions. Microbiologists should also be aware of the characteristics of the agent, and cultures should be handled under laboratory biosafety level 3 containment. Moreover, B. pseudomallei is a potential bioterrorism agent (8). Assessment of geographic and seasonal exposure is needed for identifiving this polymorphic exotic disease. Furthermore, travel advertisements to disease-endemic countries should include prophylactic measures to avoid contact with wet soils and contaminated water.

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Coronaviruses in Children, Greece

To the Editor: Two recently detected human coronaviruses (HCoVs), NL63 and HKU1, increased the number of coronaviruses known to infect humans to 5 (1-3). HCoV-229E and HCoV-NL63 belong to antigenic group 1, HCoV-OC43 and HCoV-HKU1 belong to antigenic group 2, and severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV) is most closely related to group 2 coronaviruses. In 2005, an optimized pancoronavirus reverse transcription-PCR assay was used to explore the incidence of HCoV-NL63 infection in children in Belgium who had a diagnosis of respiratory tract infection (4). We report the results of an epidemiologic study that used a universal coronavirus RT-PCR assay to detect coronaviruses among children in Greece with acute respiratory tract infections.

We tested throat swab specimens obtained from children hospitalized in Greece during June 2003 through May 2004 (200 children 2 months to 14 years of age, mean 4.09 years) and during December 2005 through March 2006 (44 children 1.6–8.5 years of age, mean 5.05). Specimens were obtained the first day of each child's hospitalization, and all specimens were included in the study, regardless whether other respiratory microorganisms were detected.

The 25- μ L reaction contained 200 μ M dNTPs, 0.2 μ M primer PC2S2 (equimolar mixture of 5'-TTATGGGTTGGGATTATC-3' and 5'-TGATGGGATGGGACTATC-3'), 0.8 μ M primer PC2As1 (5'-TCAT-CAGAAAGAATCATCA-3'), 1 μ L of enzyme mix from the QIAGEN One-Step RT-PCR Kit (QIAGEN GmbH, Hilden, Germany), and 5 μ L of RNA. The initial 30-min reverse transcription step at 48°C was followed by 10 cycles of 20 sec at 94°C, 30 sec at 62°C with a decrease of 1°C per cycle, 40 sec at 72°C; 40 cycles of 20 sec at 94°C, 30 sec at 52°C, 40 sec at 72°C; and a final extension step at 72°C for 10 min. To determine the sensitivity after optimization, we tested quantified RNA in vitro transcripts that included the natural primer binding sites of the respective coronavirus genomes. Sensitivities for SARS-CoV, HCoV-OC43, HCoV-229E, and HCoV-NL63 were 61.0, 800.0, 8.2, and 82.3 nominal RNA copies per assay, respectively. A separate test was not done for HCoV-HKU1 because it had the same primer binding sites as HCoV-OC43. A phylogenetic tree based on a 400-bp genome fragment of the polymerase gene was constructed (online Appendix Figure, available from www.cdc. gov/EID/13/6/947-appG.htm).

Of 200 samples collected in 2003–2004, 5 (2.5%) were positive for coronaviruses (2 each for HCoV-NL63 and HCoV-229E and 1 for HCoV-OC43), and of 44 samples collected in 2005–2006, 2 (4.5%) were positive for coronaviruses (1 for HCoV-229E and 1 for HCoV-OC43) (GenBank accession nos. EF103180–EF103184, EF394298, and EF394299). CoV-HKU1 was not detected.

The amplified genome region is one of the most conserved regions of the coronavirus genome. However, sequences for HCoV-NL63 strains isolated in Greece are genetically closer to the sequence for a strain (AY567487) isolated in Amsterdam in 2003 (1) than to a strain (AY518894) from a specimen collected in Rotterdam in 1988 (2) (0.6% vs. 1.1% nucleotide divergence). Sequences for HCoV-229E and HCoV-OC43 strains isolated in Greece differ from sequences for strains isolated elsewhere by 0.5%–1.7%.

The HCoV-NL63–positive specimens in our study were obtained from a 9- and a 14-month-old child during winter 2003–2004; no cases were identified during 2005–2006. Specimens positive for HCoV-229E and HCoV-OC43 were detected during both study