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## A Prospective Study of Melioidosis After Environmental Exposure of Healthy Participants to *Burkholderia pseudomallei* During a Muddy Endurance Challenge

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**Abstract.** In a prospective study of 123 healthy adults competing in a mud-exposing endurance challenge in the melioidosis-endemic tropical north of the Northern Territory of Australia, there were no asymptomatic seroconversions to *Burkholderia pseudomallei* using indirect hemagglutination assay. However, one competitor developed melioidosis attributable to infection acquired during the event.

Melioidosis is endemic in southeast Asia and northern Australia, but the regional and global boundaries for the presence of *Burkholderia pseudomallei* remain uncertain.<sup>1,2</sup> Incidence rates of melioidosis vary greatly by location and can increase substantially after severe weather events, with annual incidence rates as high as 50 per 100,000.<sup>3–5</sup> The environmental parameters and anthropogenic factors that influence the presence and concentration of *B. pseudomallei* in soil and surface water vary by location<sup>6</sup> along with the modes of infection causing melioidosis, with, for instance, the role of ingestion of *B. pseudomallei*-contaminated water recently highlighted.<sup>7</sup> Nevertheless, percutaneous inoculation of *B. pseudomallei* is considered the most common way that melioidosis occurs.

In northern Australia, severe disease is very uncommon in those without identified risk factors for melioidosis.<sup>8</sup> Overall, 20% of cases are healthy with no risk factors, and in this group, isolated skin lesions without sepsis are common, especially in children, where 60% present with primary cutaneous melioidosis.<sup>9,10</sup>

We undertook a prospective study among healthy adults competing in a mud-exposing endurance challenge held in early May of 2013 (late wet season) in the tropical north of the Northern Territory of Australia. The event took place at a recreational site 80 km south of Darwin, an area known to be highly endemic for *B. pseudomallei*. Event participants undertook a 10- or 21-km obstacle course that included wading 1 km through a stream at knee-waist height and crawling through mud beneath electrified wire. Volunteers were asked to provide blood samples for melioidosis serology testing the week before and 3–8 weeks after the event and agree to additional screening in the event of a positive result. Demographic, risk factor, occupational, and recreational details were recorded. Serology was performed using indirect hemagglutination assay (IHA) as previously described,<sup>11,12</sup> with the IHA antigen derived from a combination of three local clinical *B. pseudomallei* strains; a positive titer was defined as being 1:40 or higher, and seroconversion was defined as a twofold rise in titer from the pre-event serology. Approval was obtained from the Human Research Ethics Committee of the Northern Territory Department of Health and the Menzies

School of Health Research (HREC 2013/1990), with written informed consent provided by participants.

We recruited 131 volunteers from among the approximately 2,500 participants, and we obtained paired sera from 123 (94%) participants. Extensive exposure to mud and surface water was universal, and cuts and grazes were common on the lower limbs. Baseline serology was positive for 4 of 123 (3.3%; IHA titers 1:40, 1:40, 1:80, and 1:1,280). Extensive prior recreational exposure to wet-season soil and water was common for all four participants, and two participants reported hazardous alcohol use; however, none had other risk factors for melioidosis. There was no seroconversion in any of the participants screened. The four participants with positive initial serology were further followed up with clinical assessment and a third serology test. All remained well, had normal chest X-rays, and were culture-negative for *B. pseudomallei* from blood and urine cultures and throat and rectal swabs. Post-event and subsequent third serology remained at the same titer for three of four participants, and the other person whose initial IHA was 1:40 had post-event and third IHA titers of 1:20. An additional three volunteers with initial IHA of 1:20 had a third IHA performed after their post-event IHA was 1:40; in two participants, the third IHA was < 1:20, and in one participant, it remained at 1:40.

One case of cutaneous melioidosis occurred in the context of this study. A healthy young competitor with no known risk factors reported onset of fevers, myalgia, and lethargy 12 days post-event. A left subinguinal abscess was detected 19 days post-event. *B. pseudomallei* was subsequently cultured from pus swabs. There was no evidence of dissemination, and the individual was successfully treated with standard therapy.<sup>1</sup> Cutaneous inoculation was considered likely given multiple lower leg abrasions sustained during the event. Interestingly, this individual's IHA serology from both before the event and on four occasions over 5 months post-event showed non-specific reactivity, and sera sent to Townsville Hospital for *B. pseudomallei* enzyme immunoassay (EIA) tested in parallel showed initial negative immunoglobulin G (IgG) and post-event equivocal IgG.<sup>13</sup>

In the 18 months since the event, there have been no other competitors diagnosed with melioidosis. This is consistent with previous data from the Darwin prospective melioidosis study<sup>8</sup> showing that melioidosis is surprisingly uncommon in healthy sports persons in melioidosis-endemic regions, despite the widespread presence of *B. pseudomallei* in the soils with which they have frequent sporting contact.<sup>14</sup>

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This prospective study of seroconversion identified no case of asymptomatic infection among 123 people with likely exposure to *B. pseudomallei* during the endurance event. At the same time, there was a single confirmed case of melioidosis attributed to the event. The baseline melioidosis seropositivity of 3.3% in this study is consistent with the 3% from the same region of northern Australia previously seen in healthy adults with extensive environmental exposure to *B. pseudomallei*.<sup>11</sup> This contrasts sharply with the background seropositivity rate in northeast Thailand, which has been documented to exceed 50%, with most seroconversion occurring between 6 months and 4 years of age.<sup>15</sup> This is notable because the incidence rate of confirmed melioidosis in the Darwin region<sup>5</sup> is actually higher than that documented from northeast Thailand, notwithstanding that surveillance for melioidosis may be more comprehensive in Darwin.<sup>3</sup> Furthermore, modeling of serology data in northeast Thailand suggested that only 1:4,600 antibody-producing exposures results in clinical infection in that region.<sup>16</sup> This dramatic disparity between the Thai and Australian data on seropositivity and progression to melioidosis highlights a major gap in our understanding of the epidemiology of infection with and disease from *B. pseudomallei*. Regional variations in exposure are likely to be critical (for instance, the possibility that early childhood ingestion of *B. pseudomallei* from unchlorinated contaminated water is commonly occurring in northeast Thailand).

A limitation of this study is the recognized insensitivity of IHA as a marker of infection with *B. pseudomallei*.<sup>2,12,13</sup> Nevertheless, it was the assay used in the noted prior studies from both Thailand<sup>15,16</sup> and Australia<sup>11</sup> and remains the mainstay of serology in endemic regions.<sup>2</sup> Although IHA was found to be positive at the time of admission in only 56% of cases of melioidosis in Australia, subsequent seroconversion occurred in the majority of those initially seronegative.<sup>12</sup> Although it is possible that, by using IHA, we have underestimated the sub-clinical infection rate from this prospective study of intense exposure to *B. pseudomallei*, we are confident that we have not missed any resulting clinical cases of melioidosis.

Our previous serology study of healthy adults in the Darwin region, which is highly endemic for melioidosis, showed that, among the small number found to have high IHA titers, some described prior clinical illness that may well have represented primary melioidosis that resolved spontaneously and without melioidosis-specific antibiotics.<sup>11</sup> What remains unclear is how many asymptomatic people with positive melioidosis serology, presumably reflecting prior infection with *B. pseudomallei*, have not cleared their infection and have bacteria persisting in undetermined latent foci and what proportion of these will subsequently have activation of infection, resulting in clinical disease (i.e., melioidosis).

Although the prospective design of this study and the extreme nature of the exposure event are informative, the small sample size is another limitation. In addition, extrapolation to other locations and other exposure scenarios requires caution. For instance, the 2012–2013 monsoon was late in arriving in northern Australia, and rainfall preceding the event was substantially less than in recent years, potentially reducing the risk to competitors. The low attack rate in this study is also in contrast to a report from Malaysia, where 10 cases of melioidosis (7 fatal cases) occurred amongst 153 people involved in a river search-and-rescue operation. However, that cohort was generally older, and all melioidosis cases were diabetic.<sup>17</sup>

In conclusion, opportunistic serosurveillance of competitors in an endurance event with heavy mud exposure in a location highly endemic for melioidosis showed no evidence of resulting subclinical infection with *B. pseudomallei*. However, a single competitor developed melioidosis attributable to infection acquired during the event. The strong associations between rainfall, soil and surface water exposure, and melioidosis case numbers caution against extrapolating the serology data from this single study when planning location and month of future similar sporting events in tropical locations where melioidosis is endemic.

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