Association of the Melioidosis Agent *Burkholderia pseudomallei* with Water Parameters in Rural Water Supplies in Northern Australia[▽]

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We analyzed water parameters and the occurrence of the melioidosis agent *Burkholderia pseudomallei* in 47 water bores in Northern Australia. *B. pseudomallei* was associated with soft, acidic bore water of low salinity but high iron levels. This finding aids in identifying water supplies at risk of contamination with this pathogenic bacterium.

Melioidosis is a severe, emerging disease caused by the Gram-negative bacterium *Burkholderia pseudomallei*, a hydrophilic soil saprophyte that is endemic in Southeast Asia and northern Australia (3, 16, 17). Melioidosis is the most common cause of fatal community-acquired bacteremic pneumonia in northern Australia (4).

Melioidosis outbreaks causing fatalities among humans and animals have been attributed to contaminated unchlorinated water supplies in northern Australia (5, 8) (B. J. Currie, personal observation). Rural water bores, of which there are 5,000 around Darwin alone, are mostly unchlorinated due to concerns of bore owners about taste, by-products, and maintenance of chlorination. We have analyzed the association of the occurrence of *B. pseudomallei* with environmental and physicochemical water properties in unchlorinated bore water from rural Darwin in northern Australia. This study is the first report on the habitat of *B. pseudomallei* in water supplies.

Water sampling. Bore water was collected from 47 properties (188 samples) in an area of 22 by 13 km in the 2008 dry season. Twenty-five bores (53%) were fed by carbonate rock aquifers and 22 by aquifers in fractured weathered rock (Groundwater supply prospects and hydrogeology of the Litchfield Shire [map], Northern Territory Department of Lands Planning and Environment, Darwin, Australia) (6). Twenty-six were resampled in the wet season (103 samples). Per bore, 1 liter of water was collected after 1, 30, and 60 min of water pumping to represent water from the bore head, shaft, and aquifer. Water was collected from tanks linked to the bore. Water filtration and *B. pseudomallei* culture were done as previously described (7). Briefly, *B. pseudomallei* was cultured in modified Ashdown's broth and tryptone-soy broth and subcultured onto Ashdown's agar (Oxoid, Australia). Colonies exhibiting *B. pseudomallei* morphology were confirmed by latex agglutination and PCR targeting the type III secretion system

(TTS1) (13). Water samples were tested for pH, temperature, and electroconductivity (Aqua-CP; TPS); total nitrates, total iron, and phosphates (HI3874, HI3834, and HI3833, respectively; Hanna Instruments, Australia); and total hardness (microtest TH 10; Aquaspex, Australia). Water samples were cultured for total coliform counts (Petrifilm coliform count plate; 3 M).

Occurrence of *B. pseudomallei*. In the dry season, 12 of 47 bores (26%) tested positive for *B. pseudomallei*. In the wet season, these 12 were revisited together with 14 bores negative for *B. pseudomallei* and matched for aquifer type and location. Eleven of the 12 initially positive bores were again positive, and 4 of 14 (29%) previously negative bores were newly positive in the wet season.

Multilocus variable-number tandem-repeat analysis (MLVA-4) of *B. pseudomallei* isolates (5a) revealed 33 different MLVA-4 patterns with identical or closely related genotypes also found in *B. pseudomallei* isolates from soil or clinical cases within a 50-km radius. A median of 1.5 different genotypes was found per bore visit (95% confidence interval, 1–2). Isolates with identical or closely related patterns in the dry and wet season were retrieved from seven of the bores, indicating persistent colonization. No geographical clustering of positive bores or of MLVA-4 types was obvious.

No significant variation in bore characteristics was evident between *B. pseudomallei*-positive and -negative bores. These characteristics included bore age, depth, aquifer type, presence of concrete casing/slab, likelihood of water pooling at the bore, and origin of sample (bore head, shaft, aquifer, or tank). Effective bore capping showed reduced prevalence of *B. pseudomallei* (21% versus 44%, $P = 0.21$) (Table 1).

We analyzed the association between the occurrence of *B. pseudomallei* and water parameters (Table 1 and Fig. 1). A significant association was found between the occurrence of *B. pseudomallei* and more acidic water. These results support data showing the preference of *B. pseudomallei* for more acidic soil (2, 10, 14).

Water hardness, i.e., calcium carbonate levels, and salinity and pH showed a significant positive correlation with each other (Spearman's correlation, $P < 0.001$), attributed to the

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TABLE 1. Summary of water parameters[a]

Variable	Dry season			Wet season			Multivariable analysis [OR (95% CI); P value][b]
	Median value (95% CI) or %		P value[c]	Median value (95% CI) or %		P value[c]	
	Positive	Negative		Positive	Negative		
Total iron (mg/liter)	2 (1–3)	1 (1–1)	<0.001	4 (2–5)	1 (1–1)	0.002	1.34 (1.12–2.62); 0.002
pH	6.8 (6.5–7.1)	7.3 (7.2–7.4)	<0.001	6.3 (6.1–6.6)	7.4 (7.3–7.6)	<0.001	<0.01 (<0.01–0.36); 0.027[d]
Salinity (mS/cm)	0.07 (0.02–0.25)	0.25 (0.17–0.27)	0.037	0.02 (0.01–0.05)	0.25 (0.09–0.28)	<0.001	
Hardness (mg/liter)	100 (40–170)	180 (150–200)	0.066	25 (10–80)	170 (80–180)	<0.001	
Phosphate (mg/liter)	2 (1–3)	3 (2–3)	0.114	6 (4–6)	3 (3–6)	0.069	
Turbidity (NTU)	14.0 (6.0–22.0)	3.1 (2.6–4.0)	<0.001	15.0 (10.0–26.0)	6.4 (5.0–8.0)	0.001	
Coliforms (CFU/ml)	100 (20–220)	12 (5–25)	0.033	120 (21–220)	9 (2–24)	0.004	
Effective bore capping							
Yes (38 bores)	21		0.205[e]	29		0.438[e]	0.38 (0.14–1.06); 0.067
No (9 bores)	44			44			

[a] Summary statistics of the occurrence of *B. pseudomallei* in water bores and bore parameters. Positive, bores that were positive for *B. pseudomallei*; Negative, bores that were negative for *B. pseudomallei*. Using Stata/IC 10.0 (StataCorp LP, College Station, TX), 95% confidence intervals (CI) are percentile bootstrap estimates. Nitrate levels are not reported as most were below test detection levels of 10 mg/liter.

[b] Odds ratios (OR) for *B. pseudomallei*-positive bores were calculated using multivariable logistic regression clustered by bore and allowing standard errors for intragroup correlation and including season. The model was specified correctly as tested by a linktest.

[c] Mann-Whitney test unless otherwise noted.

[d] Odds ratio for interaction of pH and salinity.

[e] Fisher's exact test.

buffering capacity of carbonates and salts. *B. pseudomallei* was significantly associated with low hardness, i.e., soft water and low salinity. This correlates with *in vitro* research showing *B. pseudomallei* counts dropping rapidly in salt concentrations above 2.5% (9) or seawater (15). Soft water can be corrosive and compromise bore piping, potentially facilitating the introduction of *B. pseudomallei* into bores and creating a rough inner bore surface, promoting biofilms (1).

Coliform counts were significantly higher in *B. pseudomallei*-positive bores, suggesting the presence of nutrients for microbial growth. There was also a significant association between increased turbidity and *B. pseudomallei*. Higher turbidity likely reflects failure of the bore casing in the subterranean environment or backflow of surface water into the bore. Contamination of the bore with soil may well explain the origins of *B. pseudomallei* and other microbes in these bores, but this requires formal study. More organic matter in these bores would be favorable to the saprophytic *B. pseudomallei*, and decomposition of organic matter would contribute to acidification of the water.

The occurrence of *B. pseudomallei* was strongly associated with high iron levels. This finding supports previous research showing enhanced *B. pseudomallei* growth in iron-rich medium (18) and red-colored soil (indicating oxidized iron) (11). Clinical conditions causing iron overload, such as thalassemia, are associated with increased melioidosis rates (3), and *B. pseudomallei* is able to produce siderophores under limited iron supply (18). It is of interest that the acidic pH associated with *B. pseudomallei*-positives bores increases the bioavailability of iron by reduction of precipitated Fe^{3+} to soluble Fe^{2+}, especially under more anaerobic conditions, such as if water is pumped up from aquifers (12).

A comparison of the dry and wet season data showed that the water parameters in *B. pseudomallei*-positive bores were even more favorable for *B. pseudomallei* in the wet season. Heavy rainfall might explain the reduced salinity in the wet

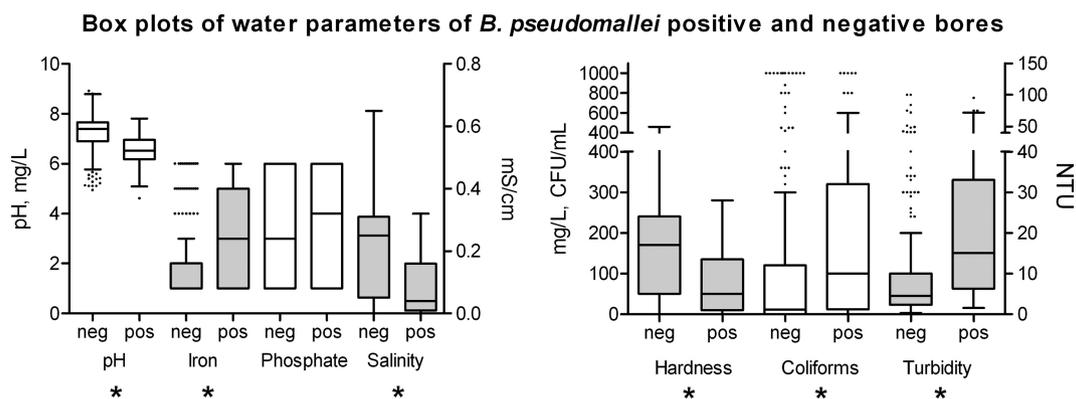### Box plots of water parameters of *B. pseudomallei* positive and negative bores

FIG. 1. Box-and-whisker plots of water parameters in *B. pseudomallei*-negative (neg) and -positive (pos) bores in both the dry and wet season. The box spans the interquartile range of the data, and the median is marked with a vertical line. An asterisk indicates a significant difference between *B. pseudomallei*-negative and -positive bores with a *P* value of <0.01 (Mann-Whitney U test). The unit of salinity (conductivity) is mS/cm (mS/cm × 640 = ppm total salts); the unit of turbidity is the nephelometric turbidity unit (NTU).

season, with higher iron levels due to water influx from shallow aquifers in an iron-rich lateritic layer which are only active in the wet season.

Clustered multivariable analysis showed that the most significant predictors for the presence of *B. pseudomallei* in water were high iron levels and the interaction of low pH with low salinity (Table 1).

Conclusion. We have found a close association between the presence of *B. pseudomallei* in bore water and water parameters such as low pH, low salinity, and high iron levels. This indicates that the occurrence of *B. pseudomallei* in bores is not only the result of an initial contamination event but also depends on water conditions favorable for *B. pseudomallei*. The strong association of *B. pseudomallei* with an abiotic fingerprint aids in the identification of water bores at high risk of *B. pseudomallei* contamination. Future studies will examine whether changing the levels of abiotic parameters, such as through pH correction filters, creates an environment unfavorable for the growth of *B. pseudomallei* and, thus, could be used as a preventive measure against the persistence of *B. pseudomallei* in unchlorinated water supplies.

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