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Hydrological connectivity and *Burkholderia pseudomallei* prevalence in wetland environments: investigating rice-farming community's risk of exposure to melioidosis in North-East Thailand

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Abstract In our analysis of 136 water samples from wetland environments (rice paddies, natural wetland sites, man-made water bodies) in rural areas of North-East Thailand, *Burkholderia pseudomallei* was most prevalent in rice paddies (15 of the 30 positive sites). The high prevalence in the water of rice fields is indicative of the inherent vulnerability of farmers in rural agricultural areas in this area of Thailand and likely other locations in the tropics. Nearly all *B. pseudomallei*-positive sites were found within the vicinity of a large wetland associated with the Chi River, in the month of July 2014. Positive samples were found in water ranging in pH from 5.9 to 8.7, salinity ranging from 0.04 to 1.58 ppt, nitrate ranging from 0 to 10.8 ppm, and iron ranging from 0.003 to 1.519 ppm. Of these variables, only iron content was statistically higher in *B. pseudomallei*-positive versus *B. pseudomallei*-negative sites, suggesting that increasing concentrations of iron may encourage the growth of this bacterium, which is responsible for melioidosis. Our results, when combined with data from other published studies, support the notion that *B. pseudomallei* can exist in a wide range of environmental conditions. Thus, we argue that health safety education is a more appropriate means of addressing farmer vulnerability than chemical or physical alterations to fields at large scales. Further, it may be important to investigate melioidosis through transdisciplinary approaches that consider the complex social and ecological contexts in which the disease occurs.

Keywords Microbiological water quality · Waterborne diseases · Disease ecology · Whitemore's disease · Tropical diseases

Introduction

Melioidosis, caused by the bacterium *Burkholderia pseudomallei*, is a potentially fatal infectious disease that affects both humans and animals (Cheng and Currie 2005; Wiersinga et al. 2012). Melioidosis develops after subcutaneous inoculation, inhalation, or ingestion of contaminated particles or aerosols (Barnes and Ketheesan 2005; Inglis and Sagripanti 2006). The disease manifests as septicemia (blood poisoning), acute pneumonia, and abscesses (Wiersinga et al. 2012). The main endemic areas are in Southeast Asia and Northern Australia (Cheng and Currie 2005; Wiersinga et al. 2012). However, sporadic cases are also increasingly being reported in southern India, southern China, Hong Kong, Taiwan, Brunei, Laos, Cambodia, the Americas (particularly Brazil), the Caribbean, Africa, and the

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Middle East (Inglis and Sagripanti 2006; Wiersinga et al. 2006).

Melioidosis is a major public health concern in North-East Thailand, a region known as *Isaan*, due to the high cases of fatalities in humans (Leelarasamee 2000; Limmathurotsakul et al. 2013a). It is the third most frequent cause of death from infectious disease in Isaan, after HIV/AIDS and tuberculosis (Limmathurotsakul et al. 2012). Approximately 2000 cases are diagnosed annually in this poor, rural agricultural region of the country (Limmathurotsakul et al. 2010). Most people come in contact with the bacteria whilst working in the fields. Despite the concern over high prevalence, there is no known vaccine. The disease can be treated with antibiotics, but even with the availability of an effective treatment, the mortality rate in some areas is still greater than 40% (Peacock et al. 2012; White 2003).

High mortality rate is detrimental to the regional economy by reducing the pool of economically productive workers. The economic toll extends to the sphere of the family because of the high medical costs after contracting the disease—an estimated US$14,500 per patient (Bhengsri et al. 2013; Melioidosis.info 2013). The annual income of most Isaan farmers (or outdoor labourers) is less than $5000 per year. Together, the high fatality rate and the economic burden related to morbidity warrant a practical management strategy that reduces the environmental exposure to the bacteria (Baker et al. 2011).

B. pseudomallei is environment-borne, Gramme-negative, and saprophytic (White 2003; Wiersinga et al. 2006; Baker et al. 2011). It is rod-shaped and approximately 0.8 μm in width and 1.5 μm in length (Sprague and Neubauer 2004). It is a facultatively anaerobic bacterium that can adapt and survive in both aerobic and anaerobic environment conditions (Hamad et al. 2011). Soil and various freshwater sources are the primary habitats for *B. pseudomallei* (White 2003; Baker et al. 2013). In hyperendemic Isaan, the recovery of *B. pseudomallei* from soil is estimated to be 20-fold higher than in other regions of Thailand (Smith et al. 1995).

Various settings (including both soil and water) in which *B. pseudomallei* proliferate have been investigated through laboratory microcosm studies that mimic environmental conditions (Tong et al. 1996; Chen et al. 2003; Robertson et al. 2010; Wang-ngarm et al. 2014). Although laboratory studies are informative, the design elements, such as the scale and duration, dependent and independent variables, and the number of variables tested may exclude or distort important features of the natural ecosystem (Carpenter 1996; Fahy and Mckew 2010). Only a handful of studies have examined in situ environmental conditions where *B. pseudomallei* thrives (e.g. Australia (Kaestli et al. 2009; Draper et al. 2010; McRobb et al. 2013), Thailand (Palasathien et al. 2008; Sermswan et al. 2015), Laos (Vongphayloth et al. 2012)).

In areas such as Isaan, rural villagers work in seasonally waterlogged rice fields which, in addition to rainfall, often require additional inputs of irrigation water from natural or man-made water bodies such as lakes, ponds, and reservoirs. The daily interaction with wetland environments places many farmers at risk of melioidosis via direct contact with *B. pseudomallei* in waters where the bacteria is present. Currently, the extent to which local communities are exposed within heterogeneous landscapes with abundant water bodies and the subsequent risk of melioidosis is not understood. Most prior researches on *B. pseudomallei* have investigated the presence of *B. pseudomallei* in potable water supplies where infection can occur mainly via the ingestion of contaminated water (Inglis et al. 2000; Howard and Inglis 2003; Draper et al. 2010; Mayo et al. 2011; McRobb et al. 2013; Limmathurotsakul et al. 2014). Little work to date has examined *B. pseudomallei* in surface water bodies where farmers and labourers are at risk through contact or inhalation (Dance 2000). Even fewer studies have examined the movement of the bacteria within the environment (Kaestli et al. 2009, 2012).

The objective of this research was to investigate the environmental conditions of wetland systems related to the presence and possible mobilisation of *B. pseudomallei* within the associated water bodies. We also endeavoured to understand the vulnerability of farmers and rural labourers who work in the wetland environments to melioidosis. The research was conducted in Khon Kaen Province of Isaan because of the high endemicity of *B. pseudomallei* (Limmathurotsakul et al. 2013b). The study site was also selected because it is an agricultural area juxtaposed with a large wetland system that may serve as a reservoir for the bacteria.

Study area

Khon Kaen is the second largest province of Isaan, with an area of approximately 10,890 km^2 and an estimated

population size of 1,775,000. Isaan is influenced by a seasonal monsoon climate. The dry season (north-east monsoon) occurs between mid-October and March. The wet season occurs between mid-May and October during the south-west monsoon. August and September are the wettest months of the year (Thai Meterological Department 2012).

The wetland system considered in this study spans across the districts of Ban Phai, Chonnabot, and Mancha Khiri (Fig. 1). The 765-km Chi River flows through Khon Kaen Province and is connected with an extensive wetland system. Villagers living along the river experience annual floods during the wet season. The river is important for domestic use, agriculture, and fishing. Several large lakes, including Lawa, Ben, Khao, and Kong Kaeo as well as the Lawa Reservoir, drain into the Chi River and are hydrologically connected to the wetlands, as well as several small lakes, ponds, and streams (Fig. 1). Large man-made reservoirs are also found within these three districts. Rice fields are commonly located near these water bodies that serve as supplies of water resources for irrigation. All these water bodies cluster within what we refer to herein as the '*greater wetland region*'.

Agriculture is the dominant economic activity in Khon Kaen Province. Major crops cultivated in the region are rice, sugarcane, and cassava. Up to 7.9 million of the 9.3 million hectares (almost 85%) of agricultural land in Isaan is devoted to rice farming (Haefele et al. 2006). However, the cultivated area varies year to year and depends primarily on water availability, which is fundamentally governed by the climate (Haefele et al. 2006). Recently, many farmers have turned to irrigation to extend the growing season. As a result, pumps are used to transfer water across the landscape via an elaborate system of irrigation canals and pipes. Thus, the abundance of water in the wetland areas, the water-dependent socio-economic activities of the villages, and the extensive water infrastructure system that connects wetlands together provide a relevant landscape to study environmental exposure of the farmers to *B. pseudomallei* and melioidosis.

Methods and materials

A total of 136 water samples were collected between 1 and 29 July 2014 from a variety of sources, including rice paddies, natural wetland water bodies (e.g. lakes, rivers, streams), and man-made water bodies (e.g. excavated irrigation ponds, canals). Samples were collected both inside and outside the greater wetland region to explore the relationship between the presence of *B. pseudomallei* and environmental conditions. Water samples were collected at the soil-water boundary—the ecotone—where most frequent movement, contact, and

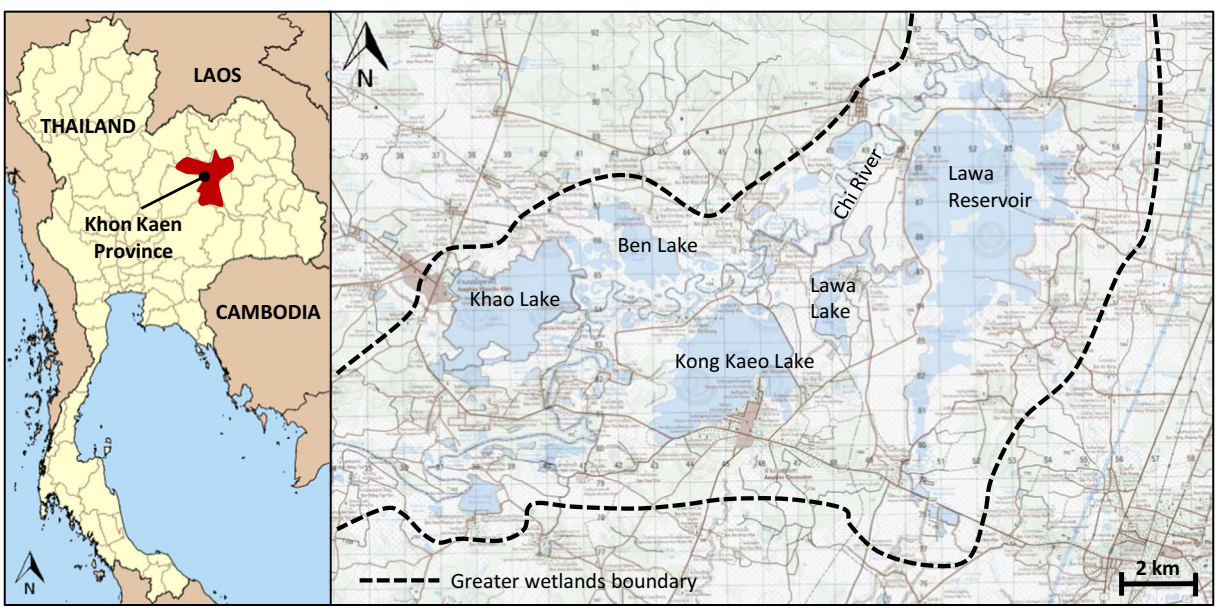

Fig. 1 Location of Khon Kaen Province in Thailand (*left*) and topographic map showing the wetland area at the study site (*right*)

dispersal activities of organisms and species occur (Risser 1995; Chapin et al. 2008). At each sampling location, two sets of water samples were collected: (i) 15 mL was stored in a sterile polypropylene conical centrifuge tube for water physicochemical properties analysis and (ii) 1 L of water was stored in a sterile polypropylene container for microbial analysis. The 15 mL samples were chilled at 4 °C and then sent to the GEOLAB at the Department of Geography at the University of Singapore. Nitrate concentrations were measured using ion chromatography, whilst concentrations of iron were determined using an inductively coupled plasma–mass spectrometer (ICP-OES).

The 1 L samples were stored in the dark at room temperature to prevent exposure to UV light that may change the conditions of the water and affect the survival of bacteria. Bacteria isolation and identification was then performed at the Melioidosis Research Centre in Khon Kaen University. Water samples were filtered through a cascade of three Whatman (USA) filters: (i) 1.2 μm, GF/C, Glass Microfiber Binder Free Filter; (ii) 0.7 μm, GF/F, Glass Microfiber Binder Free Filter; and (iii) 0.45 μm, Nylon Membrane Filter. The cascade facilitated the filtering of 'dirtier' samples (murkier, higher turbidity, contain coarser material) and selectively trapped bacteria onto each membrane. For example, the largest filter removed most sediments in the water samples, but trapped only a limited amount of bacteria.

Filter membranes were plated onto Ashdown's agar (Wuthiekanun et al. 1990) to culture *B. pseudomallei*. The primary contents of the agar (1 L) are Trypticase Soy Broth, 10 g; agar powder, 15 g; glycerol, 40 mL; aqueous crystal violet 0.1% *w/v*, 5 mL; and aqueous neutral red 1% *w/v*, 5 mL. This mixture was autoclaved at 121 °C for 15 min, and gentamicin was added aseptically to a final concentration of 5 mg/L. As the bacteria may be trapped in sediment, the filter papers were first placed upside-down to imprint the sediment onto the agar. The filter papers were then placed upright onto a separate agar dish to allow the bacteria trapped in the pores of the filter membranes to be cultured. All petri dishes were incubated at 37 °C for 4 days.

The *B. pseudomallei* colonies were first identified based on their morphological features. Colonies suspected to be *B. pseudomallei* were tested using latex agglutination test, which has 99.5% sensitivity for detecting *B. pseudomallei* (Amornchai et al. 2007). As the colonies tested in the first latex agglutination test were not pure, those testing positive were then re-streaked onto a new Ashdown agar dish to distil a pure colony. Re-streaked dishes were incubated for another 4 days, and then were tested for *B. pseudomallei* via a second latex agglutination test.

Other on-site water quality variables (pH, temperature, electrical conductivity) were measured during the time of sample collection with handheld probes. Electrical conductivity was converted to salinity by first converting it to specific electrical conductivity, which is standardised to a temperature of 25 °C as such (Radkhe et al. 2006):

$$\mathrm{EC}_{25} = \frac{\mathrm{EC}}{\left[1 + 0.02\left(T - 25^{\circ}\mathrm{C}\right)\right]} \tag{1}$$

where EC_{25} (μS/cm) is the specific electrical conductivity determined from electrical conductivity (EC; μS/cm) and temperature (T; °C). Salinity (S) was determined from the following equation (Wagner et al. 2006):

$$S = 0.0120 + \left(-0.2174^{*}R^{\frac{1}{2}}\right) + \left(25.3283^{*}R\right) + \left(13.7714^{*}R^{\frac{3}{2}}\right) + \left(-6.4788^{*}R^{2}\right) + \left(2.5842^{*}R^{\frac{5}{2}}\right) \tag{2}$$

where R is the ratio between EC_{25} and the standard seawater salinity at 25 °C (53,087 μS/cm) and S has units of practical salinity units (PSU) and is nearly equivalent to parts per thousands (ppt).

The Mann-Whitney non-parametric U test (MW-U) was performed to determine the statistical difference in each water physicochemical parameters (pH, salinity, nitrates, iron, and water temperature) between all *B. pseudomallei*-positive samples and *B. pseudomallei*-negative samples. Nitrate concentrations of some samples ($n = 7$ out of 136 samples) were below the detection limit and, therefore, set to zero because the detection limit for nitrates (0.1 ppm) is already very low (Helsel 2012).

Results

We detected *B. pseudomallei* in 30 out of the 136 (22%) water samples collected (Table 1). Most of the *B. pseudomallei*-positive samples (26 out of 30) were

Table 1 Prevalence of *B. pseudomallei* inside and outside of the greater wetland area

Location	Detected (n = 30)			Not detected (n = 106)		
	Rice field	Natural	Man-made	Rice field	Natural	Man-made
Inside greater wetland area	14	10	2	26	31	12
Outside greater wetland area	1	1	2	2	23	12
Total	15	11	4	28	54	24

collected within the greater wetland area (Fig. 2). Half of the positive samples were from rice paddies (15 out of 30 samples). Natural (n = 11) and man-made water bodies (n = 4) accounted for the other half of the positive samples. Only 4 of the 41 samples collected outside the wetland area were positive for *B. pseudomallei* (Table 1). These sites included one rice paddy, one natural water body site, and two man-made water bodies (Fig. 3).

Of the environmental parameters considered, only iron concentration was statistically different between *B. pseudomallei*-positive and *B. pseudomallei*-negative samples (MW-U; α = 0.01). Sites where *B. pseudomallei* was present had a higher median (0.0932 ppm) and a larger range (0.0032–1.5193 ppm) in iron concentration versus *B. pseudomallei*-negative sites (median = 0.0144 ppm; range = 0.0014–1.2056 ppm). Other variables, including pH, salinity, temperature, and nitrate concentration, were not statistically different between the positive and negative samples. *B. pseudomallei*-positive and *B. pseudomallei*-negative samples had similar median pH values (7.1 versus

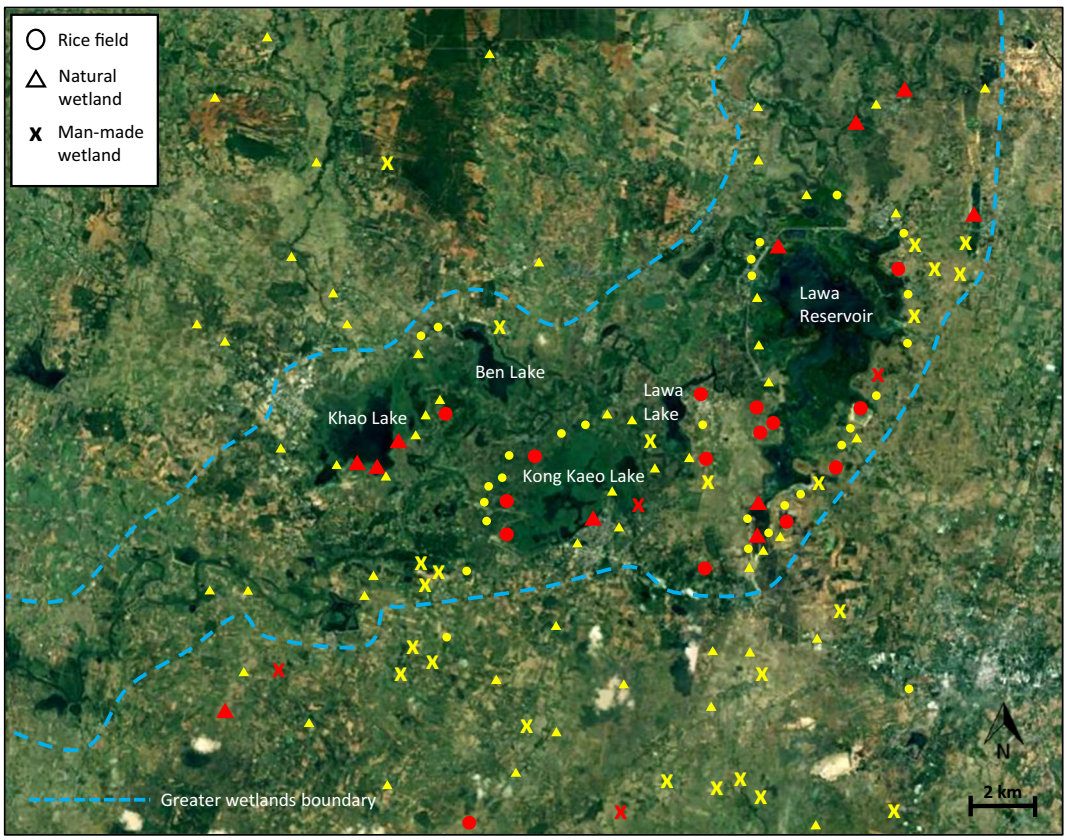

Fig. 2 Map of study area showing the prevalence of *Burkholderia pseudomallei* (Bp) at various sampling sites, i.e. rice fields, natural wetlands, or man-made wetlands (*red* denotes the Bp-positive sites; *yellow* denotes the Bp-negative sites)

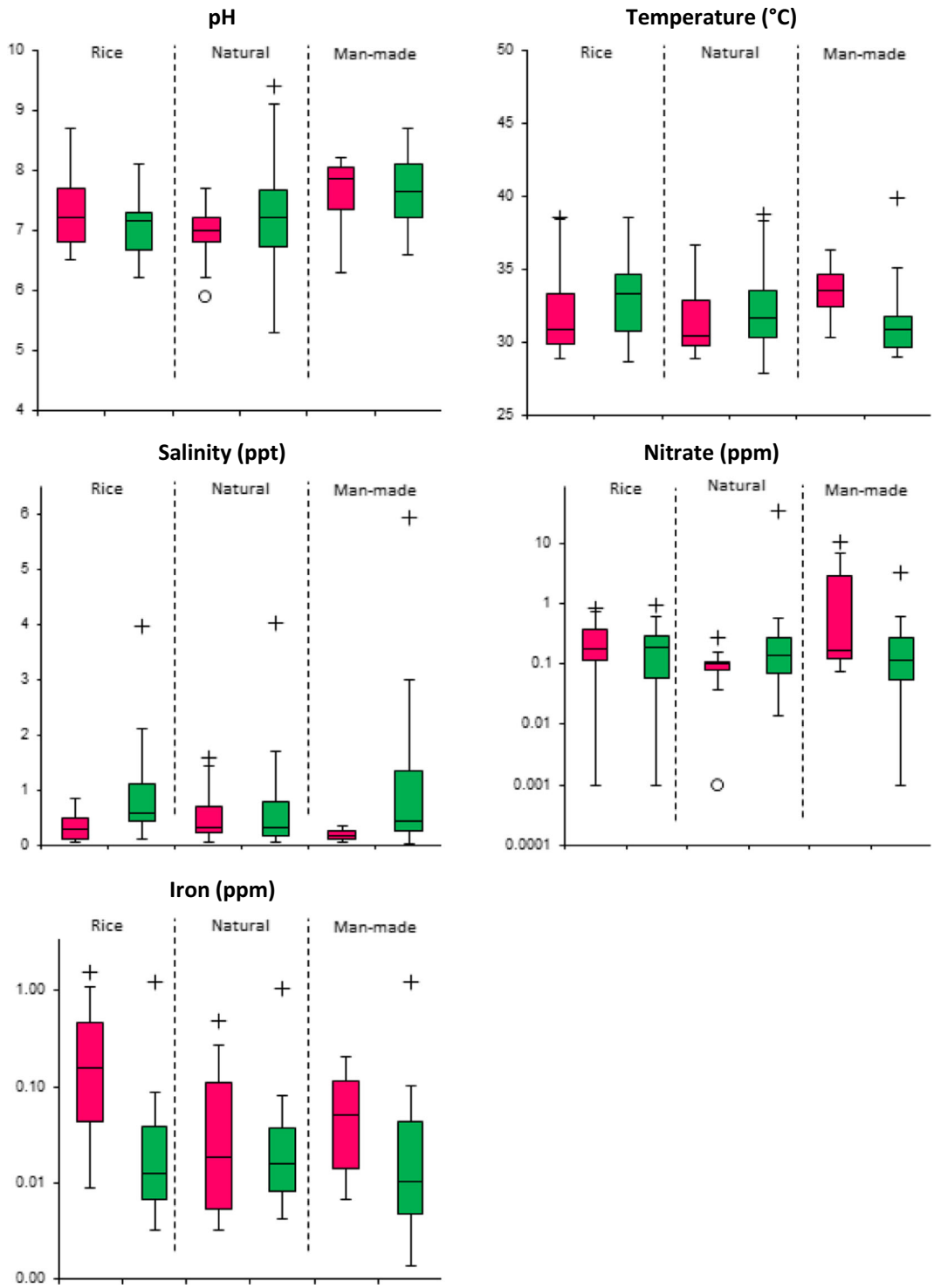

Fig. 3 Box plots comparing pH, temperature, salinity, nitrate, and iron concentrations from rice fields, natural, and man-made wetlands (*red* denotes the samples tested positive for Bp, *green* denotes the samples tested negative for Bp, *cross* denotes the maximum outlier, and *circle* denotes the minimum outlier)

7.2), but the range of the positive samples was narrower (5.9–8.7 versus 5.3–9.4, respectively). The median salinity in *B. pseudomallei*-positive samples (0.2771 ppt) was slightly lower than *B. pseudomallei*-negative samples (0.4346 ppt). The salinity range of positive samples was also much smaller (0.0379–1.5798 ppt) than for negative samples (0.0192–5.9436 ppt). Median nitrate concentrations for *B. pseudomallei*-positive and *B. pseudomallei*-negative samples were again similar (0.1398 versus 0.1434 ppm), but the range of values for the positive sites was smaller (0–10.8055 versus 0–34.7636 ppm). *B. pseudomallei*-positive and *B. pseudomallei*-negative samples appear to have similar median temperature (31.2 versus 31.6). A relatively smaller range of temperature was measured in positive samples (28.9–38.5 °C) versus negative samples (27.9–39.9 °C).

Discussion

Environmental thresholds

pH Prior studies have shown that *B. pseudomallei* are generally detected in a slightly acidic to neutral (pH 5–8) environment (Table 3). Several field surveys in Thailand reported the detection of *B. pseudomallei* in soils with pH ranging from 4.77 to 7.66 (Palasathien et al. 2008; Suebrasri et al. 2013; Sermswan et al. 2015). In neighbouring Laos, Vongphayloth et al. (2012) reported pH values of 5.19 to 8.86 for water samples (mostly from rivers and rice fields) containing *B. pseudomallei*.

Outside the region, Draper et al. (2010) reported that *B. pseudomallei* was found in water supplies with a pH range of 6.5 to 7.1 from bore wells in northern Australia. In another case in rural Darwin (northern Australia), the bacteria was detected in household water supplies with pH of 6.2–6.7 (McRobb et al. 2013). In this study, water samples that tested positive for *B. pseudomallei* had pH values ranging from 5.9 to 8.7 (Table 2).

In laboratory studies, Wang-ngarm et al. (2014) reported the optimum pH range for *B. pseudomallei* growth to be 5 to 7, whilst Chen et al. (2003) proposed a narrower range of 6.5 to 7.5. Finkelstein et al. (1965) reported that the bacteria can exist in pH conditions up to 9. From a microcosm study, Wang-ngarm et al. (2014) suggested that soil pH of greater than 8 can inhibit the growth of *B. pseudomallei* significantly. Robertson et al. (2010) found that *B. pseudomallei* growth was inhibited at pH 3. Earlier reports show that the bacteria can exist in very acidic environments as low as pH 2 (Finkelstein et al. 1965; Strauss et al. 1969). Collectively, these studies suggest that inhibition of *B. pseudomallei* occurs at pH values <4 and >8, with an optimal pH range for growth and survival being 5–7. However, these range estimates do not consider the possibility that other environmental factors (e.g. salinity, temperature) might inhibit *B. pseudomallei* at various levels of pH.

Salinity Suebrasri et al. (2013) detected the presence of the bacteria in soil samples from Khon Kaen, Thailand, containing pore water with the salinity of 0.02–0.42 ppt. In northern Australia, *B. pseudomallei*-positive environmental samples had salinity of 0.01–0.11 ppt (measured as EC of 0.01–0.25 mS/cm) as reported by Draper et al. (2010). Meanwhile, household drinking water that contained *B. pseudomallei* in another study from northern Australian had a salinity of approximately 0.02 ppt (EC of about 0.02–0.03 mS/cm). All of our *B. pseudomallei*-positive samples had salinity of under 1.58 ppt (Table 2).

Laboratory experiments by Wang-ngarm et al. (2014) showed that high salinity inhibited the growth of *B. pseudomallei.* Their soil microcosm showed that soil pore water with 2.5% NaCl (25 ppt) greatly inhibited the growth of *B. pseudomallei*. Salinity of 0 to 0.5% NaCl (0–5 ppt) appeared to be optimal for survival and growth according to their study.

Temperature In our study, *B. pseudomallei* was present in water samples with temperatures ranging from 29.0 to 33.5 °C (Table 2). Other studies showed survival in a relatively greater temperature range. In Laos, surface and sub-surface water sources with temperatures of 29.9–39.5 °C tested positive for *B. pseudomallei* (Vongphayloth et al. 2012).

In the laboratory, Tong et al. (1996) showed that optimum survival temperatures were between 24 and 32 °C. Experiments by Chen et al. (2003) revealed a higher optimum range of 37–42 °C. In the latter study, inhibition appeared to occur below 4 °C. Meanwhile, microcosm studies by Robertson et al. (2010) showed that inhibition occurred at 2 °C.

Considering all the evidence, *B. pseudomallei* appears to be able to exist in temperatures as low as 4 °C and as high as 42 °C. The optimal range appears wide and may very well lie at the upper end from 24 to 42 °C determined by Tong et al. (1996) and Chen et al. (2003).

Table 2 Summary of water quality parameters with and without the presence of *B. pseudomallei*

Water quality variable	Detected (n = 30)				Not detected (n = 106)				p value
	Mean	Median	Minimum	Maximum	Mean	Median	Minimum	Maximum	
pH	7.2	7.1	5.9	8.7	7.3	7.2	5.3	9.4	0.6672
Temperature (°C)	32.2	31.2	28.9	38.5	32.1	31.6	27.9	39.9	0.8650
Salinity (ppt)	0.3987	0.2771	0.0379	1.5798	0.8426	0.4346	0.0192	5.9436	0.0203
Nitrate (ppm)	0.5410	0.1398	0	10.8055	0.6217	0.1434	0	34.7636	0.8337
Iron (ppm)	0.2244	0.0932	0.0032	1.5193	0.1017	0.0144	0.0014	1.2056	0.0041

Nitrate In anaerobic environments such as waterlogged rice fields, *B. pseudomallei* will require an alternative respiratory pathway to grow. Dance (2000) proposed that the proliferation of *B. pseudomallei* can occur through the reduction of nitrate from fertilisers. Nitrate respiration by *B. pseudomallei* has also been observed by Wongwanich et al. (1996). Because the nitrate concentrations were not statistically different at sites where *B. pseudomallei* were absent/present, the results from our study could neither support nor refute the hypothesis by Dance (2000). The median values of nitrate concentration in *B. pseudomallei*-positive and *B. pseudomallei*-negative samples collected from rice fields were very similar at approximately 0.17 and 0.19 ppm, respectively (Fig. 3). Ultimately, we cannot delineate a meaningful threshold for nitrate from this study.

Iron From this study, sites that were positive with *B. pseudomallei* had significantly higher (MW-U; $\alpha = 0.01$) iron concentrations than sites where the bacteria was not detected (median = 0.0932 versus 0.0144 ppm, respectively; Table 2). Whilst some studies (Baker et al. 2015; Hantrakun et al. 2016) reported otherwise, our finding supports researches showing enhanced *B. pseudomallei* in iron-rich medium (Yang et al. 1991; Kaestli et al. 2007; Draper et al. 2010). In addition, clinical conditions causing the overloading of iron, such as thalassemia, are also associated with an increased incidence of melioidosis (Cheng and Currie 2005; Draper et al. 2010).

In water, from this study, *B. pseudomallei* was detected in samples containing iron concentrations of 0.0032–1.5193 ppm (Table 2). In soil, the bacteria have been shown to be able to thrive in very wide range of concentrations from 1 to almost 300 ppm (Draper et al. 2010; Suebrasri et al. 2013; Wang-ngarm et al. 2014; Table 3). Given the very large range differences of iron concentrations in water versus soil, we cannot establish a reliable threshold for inhibition of *B. pseudomallei*.

Water content The main determinant of *B. pseudomallei* presence at our site was simply location inside (versus outside) the greater wetland area (Fig. 2), supporting the supposition that *B. pseudomallei* requires a threshold level of moisture to survive (cf. Inglis and Sagripanti 2006). Yip et al. (2015) also shared similar observations—in their study, they associated the incidences of melioidosis to exceptionally heavy rainfall which resulted in widespread flooding in a normally parched region in Central Australia.

Investigations of soil moisture thresholds for *B. pseudomallei* survival are limited and most are laboratory-based. Chen et al. (2003) noted the inhibition of growth at 10% in their laboratory experiments. Another microcosm study by Tong et al. (1996) showed that the survival of *B. pseudomallei* in soils with 20% moisture is 439 days. Without water, the survival was only 30 days. In the environment, the bacteria could be detected in soils with only 1.37% water content (Sermswan et al. 2015), casting doubt on the notion of a lower threshold in laboratory studies. Palasathien et al. (2008) also found the bacteria in soils with moisture ranging as low as 9%. Comparison of the evidence supports the idea that a lower threshold of soil moisture cannot be determined.

Hydrological connectivity

The higher presence of *B. pseudomallei* inside the greater wetland area versus outside highlights the role of hydrological connectivity, or the linkage of various water bodies, in facilitating the spread of the bacteria across the landscape (Chaturongkasumrit et al. 2005). Hydrological connectivity is controlled naturally by local-

Table 3 Summary of environmental conditions observed for the survival of *Burkholderia pseudomallei* (Bp) as reported in various controlled (laboratory) and uncontrolled (field) settings

Location	Sample type	pH	Temperature (°C)	Salinity[a] (ppt)	Nitrate (ppm)	Iron (ppm)	Moisture (%)	Reference
Setting: laboratory (microcosm study)								
South China	–	[5–8]	[24–32]	–	–	–	[≥40]	Tong et al. (1996)
North Australia	–	(4–7)	(20–40)	–	–	–	–	Robertson et al. (2010)
Kaohsiung, Taiwan	–	(4–8) [6.5–7.5]	(4–45) [37–42]	–	–	–	(15–20)	Chen et al. (2003)
NE Thailand	–	(4–8) [5–7]	–	(0–10)[b] [0–5][b]	–	(50–150)	–	Wang-ngarm et al. (2014)
Setting: field								
Saravane Province, Laos	Water	(5.19–8.86)	(29.9–39.5)	–	–	–	–	Vongphayloth et al. (2012)
North Australia	Soil	{5.5}	–	–	–	–	–	Kaestli et al. (2009)
North Australia	Water	(6.5–7.1)	–	(0.01–0.11)	–	(1–5)	–	Draper et al. (2010)
North Australia	Water	(6.2–6.7)	–	(0.018–0.021)	–	–	–	McRobb et al. (2013)
NE Thailand	Soil	(5.1–5.9) {5.6}	–	–	{38.5}	–	(9–18) {14.92}	Palasathien et al. (2008)
NE Thailand	Soil	(4.77–7.66) {5.85}	–	–	–	–	(1.37–36.86) {13.08}	Sermswan et al. (2015)
NE Thailand	Soil	(5.05–7.53)	–	(0.02–0.42)	–	(6.17–288.48)	(8.26–40.82)	Suebrasri et al. (2013)
NE Thailand	Water	(5.9–8.7) {7.2/7.1}	(28.9–38.5) {32.2/31.2}	(0.0379–1.5798) {0.3987/0.2771}	(0–10.8055) {0.5410/0.1398}	(0.0032–1.5193) {0.2244/0.0932}	–	This study

Note that (#–#) is the observed range for Bp survival, {#} is the observed mean/median for Bp survival, and [#–#] is the observed optimal range for Bp growth

[a] Converted from mS/cm or dS/m

[b] Converted from % NaCl

scale flooding that occurs in North-East Thailand annually during the wet monsoon seasons. Seasonal flood waters (re)connect isolated wetland areas, for example rice paddy fields, within the greater wetland area.

Flood waters likely aid in dispersing *B. pseudomallei* by transferring it from ideal habitats to sites where the bacteria may survive but is potentially inhibited during the lengthy drought period. These ideal habitats, or 'reservoirs', may be wetland areas or other sites where soil-water conditions favour *B. pseudomallei* survival. In contrast, flooding is less in areas further away from the greater wetland area, potentially reducing the movement of the bacteria. Hydrological connectivity via seasonal flooding may greatly explain the general trend of fewer positive sample points located outside the greater wetland area.

Hydrological connectivity is also affected anthropogenically by irrigation networks that are prominent in the study area. Villagers now transfer water to rice fields from ponds, canals, and larger natural water bodies including rivers and lakes, especially during the dry season (Polthanee and Promkhambut 2014). These systems likely affect the distribution of *B. pseudomallei.* Kaestli et al. (2009, 2012) hypothesised that irrigation systems might spread *B. pseudomallei* within gardens during the dry season. At our study site, because irrigation systems are difficult to build and maintain at locations away from abundant water sources (Mekpruksawong et al. 2012), distance may be a factor-limiting dispersal of *B. pseudomallei* away from the greater wetland area. It may also be simply related to a lower ground water table (not examined in this study).

Risk and rice

Amongst the different types of wetlands sampled, *B. pseudomallei* was most frequently detected in the rice paddies (Table 1). This finding is in general agreement with other studies showing that *B. pseudomallei* flourish in rice fields (Suputtamongkol et al. 1994; Dance 2000; Kao et al. 2003). In general, the neutral to slightly acidic, warm, and low-saline conditions that generally occur in rice wetland environments should be favourable for bacterial growth. Several authors have noted that *B. pseudomallei* prefer stagnant and/or slow-flowing water, as well as moist soil conditions (Kanai and Kondo 1994; Suputtamongkol et al. 1994; Kaestli et al. 2009; Wang-ngarm et al. 2014)—conditions that all pertain to rice field environments across several months at our study area.

In Isaan, farmers typically maintain 5 to 10 cm of water in their fields through irrigation inputs and rainfall (Bouman et al. 2007). Maintaining the water level potentially moderates the temperature, pH, and salinity. In general, low salinity is expected to be found to be associated with rice paddies because concentrations exceeding 2 ppt can reduce rice production and growth (Summer and Miller 1996; Bouman et al. 2007; Clermont-Dauphin et al. 2010). Whilst farmers tend to avoid planting on saline soils, we found salinity concentrations as high as 3.9652 ppt (EC_{25} = 7220 μS/cm) in rice fields (Fig. 3). However, we did not find the bacteria in waters with salinity higher than 1.5798 ppt (EC_{25} = 3040 μS/cm). The detection rate of *B. pseudomallei* in rice paddy samples was much higher than in the samples of natural and man-made water bodies, suggesting that the water conditions in the latter two may be less favourable to *B. pseudomallei* survival.

The high presence of *B. pseudomallei* in rice paddy environments aligns with the widely recognised exposure and the corresponding health risk to farmers, who comprise about 80% of the population in Isaan (Haefele et al. 2006; Wiersinga et al. 2006). The rice growing season coincides with the monsoon and annual localised 'flooding' that is integral for rice production (Kao et al. 2003; Wiersinga et al. 2006; Bouman et al. 2007). Numerous activities related to the rice growing season occur every year from July to November in Isaan (Kawasaki and Herath 2011). The nature of rice farming can result in cuts and abrasions during planting and harvesting, particularly to the feet and hands. Exposed wounds provide a point of entry for *B. pseudomallei*. Another possible route of transmission is through inhalation of water vapour from the flooded surface water in the rice paddy fields. These potential routes of transmission increase the farmer risk of acquiring melioidosis (Chaturongkasumrit et al. 2005).

It is tempting to use the data from the growing number of studies to suggest ways to manipulate the environmental conditions to reduce farmer risk to infection. For example, results suggest that increasing pH or salinity to levels that inhibit bacterial growth, but still allow for farming, are potentially useful small-scale (field) strategies (Kaestli et al. 2009; Wang-ngarm et al. 2014). However, they may not be appropriate at landscape scales as they may produce adverse effects to surrounding wetland environments (Haefele et al. 2006).

A more feasible strategy is to promote the awareness of high-risk groups, such as farmers, to the risk of melioidosis. Currently, 74% of the Thai population have not heard of melioidosis; 19% had heard of the disease but had no further knowledge (Chansrichavala et al. 2015). Awareness and education programs could begin by encouraging farmers to avoid cuts and abrasions by wearing boots and gloves in high-risk areas. Educational information should include explanations of the various infection routes of the bacteria and the symptoms of melioidosis.

At a higher level, the interaction of good hygiene and disease ecology should be addressed because of the potential coupled human-bacteria ecological cycle that may allow for the persistence of *B. pseudomallei* in rural environments (Dance 2000). In this cycle, humans and other animals play a role in the transmission of *B. pseudomallei* through direct urination or defecation (or passing other body fluids) in the wetland environments. Both are still common in rural farming areas where sanitation practises are basic at best. This transfer mechanism represents another form of connectivity, whereby the bacteria can move great distances to otherwise 'clean' (uncontaminated) environments.

Limitations and suggestions

Herein, we recognise that *B. pseudomallei* may have been present at some (negative) sites, but we failed to detect it in the limited volume of water sampled or because the bacteria entered a non-culturable state under unfavourable or stressed conditions (Wang-ngarm et al. 2014; Inglis and Sagripanti 2007). To address this, we suggest a more sensitive, molecular method as described by Knappik et al. (2015). Due to resource limitations, we were also unable to replicate our samples. Further, the bacteria may have passed through the use of filter with the finest pore size (0.45 μm). Future researches can consider the use filter membranes with finer pore size, i.e. 0.2 μm. An improvement to our method would be testing for the bacteria concentrations (colony-forming unit), which could provide quantitative information about the bacteria population within a collected sample, and perhaps its relationship with water quality parameters. Whilst we focused on water, determining environmental variables for surrounding soil conditions would have been informative and allowed comparison with other studies. We also recognise that sampling across seasons (e.g. dry versus wet) may have produced different results.

Conclusion

This research contributes to the current epidemiological knowledge of melioidosis and its etiological agent, *B. pseudomallei*, by emphasising how surface water environments provide suitable habitat for the survival and mobility of the bacteria, and correspondingly, melioidosis as well. Whilst the rice field environment is a preferential habitat for *B. pseudomallei*, the bacterium was also detected in other natural and man-made water bodies. Most positive sites were located within the greater wetland area of our study site, highlighting the role of water in regulating favourable conditions in which the bacteria can move across the landscape. Hydrological connectivity between the various types of water bodies in the wetland system is affected both naturally by seasonal floods and anthropogenically by large-scale irrigation systems that support local agriculture. These results underpin the inherent risk to rice farmers who work unprotected in the fields and who do not understand the risks of infection. Given the identified role of water as the medium of growth, as well as the agent of transport, control programs should also emphasise on reducing vulnerability through education and awareness programs. Finally, we further believe that transdisciplinary approaches of investigation are needed to address the complex social and ecological aspects of the disease. These approaches are arguably urgently needed because this community-acquired infectious disease may be substantially more prevalent than currently believed (Limmathurotsakul et al. 2016).

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