CASE REPORT

Two fatal cases of melioidosis on the Thai-Myanmar border

[version 1; peer review: 2 approved, 1 approved with reservations]

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V1 First published: 06 Jan 2014, 3:4
<https://doi.org/10.12688/f1000research.3-4.v1>

Latest published: 31 Mar 2014, 3:4
<https://doi.org/10.12688/f1000research.3-4.v2>

Abstract

Melioidosis is endemic in areas of Southeast Asia, however, there are no published reports from the Thai-Myanmar border. We report the first two cases of fatal melioidosis in this region. This is of great public health importance and highlights the need to increase clinical awareness of melioidosis on the Thai-Myanmar border and to assess the true burden of disease in the area through improved case detection and *Burkholderia pseudomallei* prevalence studies.

Open Peer Review

Approval Status

	1	2	3
version 2 (revision) 31 Mar 2014		 view	 view
version 1 06 Jan 2014	 view	 view	 view

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Any reports and responses or comments on the article can be found at the end of the article.

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Competing interests: No competing interests were disclosed.

Grant information: SMRU, COMRU and MORU are part of the Thailand Major Overseas Program supported by the Wellcome Trust (UK). The blood culture system at SMRU is supported by a grant from the US CDC (Project code 1U50CK000192).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Chu CS, Winearls S, Ling C *et al.* **Two fatal cases of melioidosis on the Thai-Myanmar border [version 1; peer review: 2 approved, 1 approved with reservations]** F1000Research 2014, 3:4 <https://doi.org/10.12688/f1000research.3-4.v1>

First published: 06 Jan 2014, 3:4 <https://doi.org/10.12688/f1000research.3-4.v1>

Presentation

Melioidosis, caused by *Burkholderia pseudomallei*, is endemic in areas of Southeast Asia. Published case reports from the Thai-Myanmar border are absent and the general belief is that melioidosis does not occur in this region. Presented below are two fatal case reports suggesting that melioidosis does in fact occur and may be under-recognized and not reported.

The first case of this series was a 17-year old female who presented with an intermittent fever for two months, more than 10% weight loss and confusion. She had been previously healthy. Her parents were farmers and the family lived in close proximity to the farm in eastern Myanmar along the Thailand border. On physical examination, her temperature was 40.1°C, pulse 130 bpm, respiration 28/min, and blood pressure 80/50 mmHg. In general, she was prostrate with conjunctival pallor. Her Glasgow Coma Score was 10/15. The cardiovascular and pulmonary exams were normal aside from tachycardia and tachypnea. Abdominal examination revealed a 1 cm liver and spleen. There were no other significant findings on clinical exam. Evidence from a random dextrose level test (432 mg/dL) and a urine dipstick was consistent with anorexia. A malaria smear showed *Plasmodium vivax* at a low parasitemia. Because of decreased consciousness, a lumbar puncture was performed and clear cerebrospinal fluid (CSF) was obtained. The CSF cell counts were normal and no organisms were grown after 48 hours of culture. Blood cultures were also sent. The patient was resuscitated with normal saline, given ceftriaxone 1 g intravenous BID and stabilized. Over the following two days, further laboratory results were obtained. A complete blood count (CBC) showed an elevated white blood count (WBC) at $13.5 \times 10^3/\mu\text{L}$ with a neutrophilia, hemoglobin (Hgb) 14.1 g/dL and a normal platelet count. Chest X-ray was abnormal with a left sided consolidation, cavitation on the right side and bilateral hilar lymphadenopathy. Sputum tested for acid fast bacilli was negative. Despite the addition of gentamicin 280 mg intravenous daily and oxygen, the patient deteriorated and died more than 8 weeks after the onset of symptoms. Admission blood cultures grew *Burkholderia pseudomallei* after one day of incubation.

The second case was a 45-year-old male who presented with fever and weakness for two days. He also complained of chills, rigors, cough and generalized body aches. He had a history of gastric ulcer and hypertension, neither of which were currently being treated. He was a rice farmer on the eastern border of Myanmar and Thailand and had continued to work until he fell ill. He was treated with ceftriaxone 1 g intravenous daily and metronidazole 500 mg oral TID by a village health worker. At this time a malaria smear was negative and other diagnostic testing was not performed. On the sixth day of illness he had still not improved and was sent to our clinic. At initial consultation, tympanic temperature was 36°C, pulse 76 bpm, respiration 20/min, and blood pressure 100/70 mmHg. His general appearance was weak but he was ambulatory with a Glasgow Coma Score of 15/15. He had icteric sclera and pale conjunctivae. The cardiovascular, pulmonary and abdominal exams were normal. On skin examination, a 3 cm lesion that appeared like a superficial abscess was noted on the right leg. Additional history revealed that this lesion had intermittently appeared and resolved over more than two years.

During his hospitalization, the patient developed congestive heart failure and pulmonary edema that improved with initial management. A random venous dextrose level was 110 mg/dL and field hematocrit 27%. A Urine dipstick result was abnormal with protein 2+ and blood 4+. Urine sediment was normal. Dengue rapid test was NS-1 negative, IgM and IgG positive (Standard Diagnostics, Inc, Kyonggi province, Korea). A scrub typhus rapid test for total antibody was positive (Standard Diagnostics, Inc). Stool test microscopy was negative and the stool sample was noted to be of black color. His CBC showed a Hgb 8.1 g/dL, WBC $26.2 \times 10^3/\mu\text{L}$ with a neutrophilia and normal platelets. Blood culture was negative. Biochemistry was sent to the district hospital, however, the results were not available immediately. His treatment regimen included cloxacillin 1 g intravenous QID, ceftriaxone 1 g intravenous daily, doxycycline 100 mg oral BID and furosemide 40 mg oral BID. Six days after admission the biochemistry results were returned and showed markedly abnormal renal function; blood urea nitrogen (BUN) 112.5 mg% and creatinine 15.4 mg%. He was diagnosed with chronic renal failure exacerbated by dengue and scrub typhus infections. After two weeks of hospitalization, the patient's clinical condition deteriorated and he died despite prolonged broad antibiotic coverage. A second blood culture obtained one day prior to his death grew *Burkholderia pseudomallei* after one day of incubation.

Diagnosis

B. pseudomallei isolates from both cases were obtained using the BacT/Alert® 3D automatic blood culture system (bioMérieux, Marcy L'Etoile, France). The isolate from case one was identified using the API 20NE test kit (bioMérieux) and confirmed with a *B. pseudomallei* latex agglutination test. The *B. pseudomallei* latex test, produced and provided by Mahidol Oxford Tropical Medicine Research Unit (MORU), is based on an anti-exopolysaccharide monoclonal antibody¹. The *B. pseudomallei* isolate from case two was identified using the *B. pseudomallei* latex test and then sent to MORU for confirmation. Susceptibility testing by disc diffusion was performed at SMRU for the first isolate and at MORU for the second (Table 1). Two colony morphologies were found for case 2; one, which was susceptible to meropenem, and one with intermediate susceptibility to meropenem (bioMérieux Etest minimum inhibitory concentration of 8 µg/mL).

Discussion

At the beginning of the 20th century in Rangoon, Burma, Dr. Alfred Whitmore and his assistant Krishnaswami identified a new bacteria causing a "glanders-like illness" in humans. It was originally named *Bacillus pseudomallei*^{2,3} and renamed as *Burkholderia pseudomallei* in 1992⁴. They proceeded to document over a hundred cases and the bacterium was isolated in over 5% of Rangoon's autopsies at that time⁵. The disease, now termed melioidosis, was named by Stanton and Fletcher in 1932⁶ and is characterised by severe sepsis, pneumonia, abscess formation and is associated with a high mortality⁷. It has become an important public health concern across Southeast Asia and northern Australia. In this region, incidence rates vary between 1.1/100000 and 412.7/100000⁸, and Lao PDR has recorded 400 cases between 1999 and 2010^{9,10}. This variability may be explained by the geographical preference of *B. pseudomallei* for specific soils^{11,12}, the locations of the necessary containment level three laboratories for culture diagnosis⁷ and

Table 1. Susceptibility profiles of *B. pseudomallei* isolates from case one (1) and case two (2i and 2ii).

Case	Gentamicin	Amoxicillin clavulanic acid	Ceftazidime	Doxycycline	Meropenem	Imipenem	Co-trimoxazole
1	R	S	S	NT	S	NT	S
2i	R	S	S	S	S	S	S
2ii	R	S	S	S	I	S	S

R=resistant, S=sensitive, I=intermediate, NT=not tested

population risk factors that include occupation⁸ or medical conditions such as diabetes, renal failure and immunosuppressed states⁷. Little is known about the current disease burden in Myanmar and along its borders. Indeed the last published case report from within Myanmar was in 1945⁷. A recent serological survey of new Burmese migrants to Thailand revealed that 78% were seropositive for antibodies to *B. pseudomallei*¹³. This high seropositivity rate is supported by Aung *et al* who found that 2% of pus specimens obtained from abscesses from patients examined in Yangon, also known as Rangoon, Myanmar, contained organisms consistent with *B. pseudomallei*¹⁴.

The first case described here was a healthy young female and her only risk factor was environmental exposure. The elevated dextrose on admission was attributed to glycemic dysregulation rather than from new onset diabetes. Concurrent vivax malaria infection may have caused immunosuppression enabling sepsis with *B. pseudomallei*. The second case was a male with environmental exposure as well as undiagnosed chronic renal disease. The first blood culture was negative, however, concurrent infection with dengue and possibly scrub typhus (the rapid test used detects IgG, IgM and IgA as total Ab and cannot differentiate acute or resolved infection) may have caused worsening immunosuppression resulting in *B. pseudomallei* sepsis. Both cases had risk factors and concomitant infections.

Management

Antimicrobial therapeutic options for melioidosis are limited due to the intrinsic resistance of the organism. Current guidelines recommend parenteral ceftazidime 50 mg/kg up to 2 g every 6 hours or meropenem 25 mg/kg up to 1 g every 8 hours for at least 10–14 days (longer in severe or complicated cases) followed by oral co-trimoxazole plus doxycycline (first-line) or co-amoxiclavulanate alone (second-line) for 20 weeks. In these cases, ceftazidime or meropenem was not given due to the low suspicion for *B. pseudomallei* sepsis. New data supports the use of monotherapy with oral

trimethoprim-sulfamethoxazole for the oral phase of treatment¹⁵. This simplifies the treatment regimen and should have a positive impact on prescribing patterns in suspected cases. Melioidosis has not been reported to be endemic in eastern Myanmar along the Thailand border, therefore, health providers may not consider it as a diagnosis in persons with sepsis. The detection of *B. pseudomallei* in this case series highlights the need for further epidemiologic study and case diagnosis in this region.

Consent

Written informed consent to report these cases was obtained from the families of the patients.

Author contributions

CSC, SW, AAP managed the patients, CL, WH, PT, VW performed the laboratory work, all authors contributed to the discussion and writing of the report.

Competing interests

No competing interests were disclosed.

Grant information

SMRU, COMRU and MORU are part of the Thailand Major Overseas Program supported by the Wellcome Trust (UK). The blood culture system at SMRU is supported by a grant from the US CDC (Project code 1U50CK000192).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We would like to acknowledge the contribution of all SMRU staff involved in the management of these two cases including clinic and laboratory staff.

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Current Peer Review Status:

Version 1

Reviewer Report 18 March 2014

<https://doi.org/10.5256/f1000research.3484.r4028>

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Nicholas Feasey

Institute of Translational Medicine, University of Liverpool, Liverpool, UK

The authors have reported two cases melioidosis in an area in which this disease was not believed to occur and make an important point about paucity of diagnostic technology in the region.

Abstract:

- *"We report the first two cases of fatal melioidosis in this region..."* The phrasing of this sentence implies that these are the first actual cases of fatal melioid in the region, rather than the first formally diagnosed cases. It currently implies that Melioid is a new/emerging disease to this region rather than a new diagnosis of a condition which is likely to have been present, but unrecognised.

Presentation:

- *"Presented below are two fatal case reports..."* The case reports were not fatal. "Presented below are two case reports of fatal..." would be better.
- The cases are a little brief on detail and if possible, it would be nice to make the corrections/additions suggested by the other two reviewers. Having said that, the weakness of the case descriptions do not detract from the message regarding the description of Melioid in this setting.

Diagnosis:

- This is a challenging condition to diagnose and it is important to have a little more detail here – reassuring readers that this really was *B. pseudomallei* is the most important part of this paper. The authors should have described the colonial morphology of the pathogen as *B. pseudomallei* has a characteristic appearance (although I would discourage them from re-culturing it simply to answer this point unless there is a safe laboratory environment to do so). It would also have been useful to record the API20NE score (both the 7 digit number and the percentage ID). What does SMRU stand for? Lastly, what confirmatory test did MORU perform?

Management:

- It is a little unusual that management follows the discussion. In particular, the last 2

sentences are conclusions and they should be separated and labelled as such.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response ( ) 27 Mar 2014

Francois Nosten, Mahidol University, Mae Sot, Thailand

- *"We report the first two cases of fatal melioidosis in this region..." The phrasing of this sentence implies that these are the first actual cases of fatal meliod in the region, rather than the first formally diagnosed cases. It currently implies that Meliod is a new/emerging disease to this region rather than a new diagnosis of a condition which is likely to have been present, but unrecognised."*

It is true that there are multiple interpretations of this phrase. This report did clarify, and further stated that melioidosis is endemic in areas of Southeast Asia and points out that there are no published reports from the Thai-Myanmar border. Prior to these two cases it was thought not to occur in the region. The report also highlights the need to assess the true burden of disease in the area, which we feel will help shed light on whether this is a new, emerging or unrecognised infection in the area.

- *"Presented below are two fatal case reports..." The case reports were not fatal. "Presented below are two case reports of fatal..." would be better."*

We agree that this should be corrected.

- *"The cases are a little brief on detail and if possible, it would be nice to make the corrections/additions suggested by the other two reviewers. Having said that, the weakness of the case descriptions do not detract from the message regarding the description of Meliod in this setting."*

Details to the cases have been added to the updated version of the article.

- *"This is a challenging condition to diagnose and it is important to have a little more detail here – reassuring readers that this really was B. pseudomallei is the most important part of this paper. The authors should have described the colonial morphology of the pathogen as B. pseudomallei has a characteristic appearance (although I would discourage them from re-culturing it simply to answer this point unless there is a safe laboratory environment to do so). It would also have been useful to record the API20NE score (both the 7 digit number and the percentage ID). What does SMRU stand for? Lastly, what confirmatory test did MORU perform?"*

We agree with the reviewer that it would be useful to add more detail to this section and have made changes to the diagnosis section of the manuscript.

- *"It is a little unusual that management follows the discussion. In particular, the last 2 sentences are conclusions and they should be separated and labelled as such."*

The initial manuscript followed an example from the journal. We agree with this comment, and will amend the manuscript to follow a more traditional layout.

Competing Interests: No competing interests

Reviewer Report 17 February 2014

<https://doi.org/10.5256/f1000research.3484.r3595>

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Blandine Rammaert

Service des Maladies Infectieuses et Tropicales, Necker Sick Children's Hospital, Paris, France

F. Nosten and colleagues report two cases of fatal melioidosis in the Myanmar border and remind the international community that this disease was discovered in Myanmar. Melioidosis is probably a forgotten public health issue in Myanmar and this article has the advantage of emphasizing it. However a description of the Myanmar environment, healthcare system and available antibiotics could help to better understand the general background around these two cases.

I have a few comments on the cases:

The first case illustrates a chronic pulmonary melioidosis with a fatal septic choc due to *B. pseudomallei*.

- *"lived in close proximity to the farm"* - Could you please reiterate the context of the Myanmar border; are there irrigated rice fields in the area? Is the patient in close contact with wet soil or any kind of water sources?
- *"Abdominal examination revealed a 1 cm liver and spleen"* - This statement is not really clear. Do you mean that there are an hepatomegaly and a splenomegaly?
- *"died more than 8 weeks after the onset of symptoms"* - It would be more helpful to have the time from hospitalization.

The second case is about a fatal disseminated melioidosis from cutaneous inoculation. What is less clear is the global clinical presentation. The patient had initial pulmonary oedema. Maybe it would be helpful to discuss pulmonary melioidosis and to have the result of the chest X-ray (if it could have been done in this setting).

- *"stool sample was noted to be of black color"* - This statement doesn't bring out any new

evidence.

- *"A second blood culture obtained one day prior to his death grew Burkholderia pseudomallei after one day of incubation."* - Does that mean that you got the result after his death? How long did it take to get the result?
- Do the authors think that earlier result of blood cultures could have had a positive impact on the outcome of their two patients? It could be interesting to discuss it.
- Do the authors have any comments on the Ashdown media, which is a cheap, specific and easy-to-make medium. Could it be implemented in the Myanmar border to help diagnosing cases?
- Is ceftazidime available in Myanmar?

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 24 Feb 2014

Cindy Chu, Shoklo Malaria Research Unit, Thailand

Thank you for reviewing this case series. Our reply to your comments are as follows:

Response to comments on the first case:

1. Land use along the Thailand Myanmar border is primarily agricultural. Crops that are grown are wide ranging, but most importantly, rice farming is common. All rice farms have some form of irrigation. As is custom, this patient and her family lived in a wooden home situated beside the fields of the farm where they worked and would certainly have been exposed to wet soil and natural water sources on a daily basis.
2. "Abdominal examination revealed a 1cm liver and spleen" would mean that both the liver and spleen were enlarged and both measured 1cm.
3. The patient's symptoms had started approximately 8 weeks before admission. She was admitted 3 days prior to her death and the case report describes the events over those 3 days.

Response to comments on the second case:

1. At our clinics, situated in a resource-limited setting, we are not able to test stool for occult blood, therefore we rely on the visual examination to determine if there is gastrointestinal bleeding. In this particular case, the presence of melanic stool would indicate either the severity of his sepsis or an underlying gastrointestinal disorder contributing to his poor clinical condition.

2. The second blood culture result grew *B. pseudomallei* within 24 hours. Results were called immediately to the clinical doctor, however, the patient had already expired the day prior.
3. Gram stain, blood culture results, and sensitivities when available are called immediately to the clinical doctor. Usually, microbiological identification is available within 24 hours of blood culture positivity. If the first blood culture had been positive then the course of events may have been different. We agree that that early recognition and confirmation of *B. pseudomallei* by blood culture would save lives.
4. Ashdown media would be a suitable selective media for testing a variety of samples for *B. pseudomallei*, particularly from sites with normal flora and if incubated for 48 hrs. However, it must be noted that when handling *B. pseudomallei* cultures appropriate biosafety level three precautions must be taken, which may be a limiting factor in some areas of Myanmar.
5. Ceftazidime is available in hospitals and pharmacies in Myanmar, however, the fee for service medical system currently present in Myanmar may limit patient access to medical care and treatment.

Competing Interests: No competing interests

Reviewer Report 22 January 2014

<https://doi.org/10.5256/f1000research.3484.r2977>

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Kavitha Saravu

Department of Medicine, Kasturba Medical College, Manipal, India

This article certainly raises awareness about the presence of melioidosis in Thai Myanmar border and the expanding map of melioidosis globally. The two cases also reiterate the importance of timely diagnosis and appropriate chemotherapy for successful outcome. This report also underscores the fact that there may be many undiagnosed cases in the region, because of low index of suspicion by the clinicians and lack of facility for diagnosis.

However, a few points could have been included as follows:

1. Evidence from a random dextrose level test (432 mg/dL) and a urine dipstick was consistent with anorexia. This statement is not clear. It would have been better if a glycated hemoglobin value was available to rule out diabetes. Additionally, further values of plasma

glucose would have been helpful. Also, the finding of the urinary dipstick is not mentioned.

2. It is not clear if the patient was treated for vivax malaria.

3. The course and events of the patient's hospitalization of 8 weeks has not been mentioned.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reader Comment 28 Jan 2014

Cindy Chu, Shoklo Malaria Research Unit, Thailand

Thank you for your useful comments. Our reply to your points are as follows:

1. We agree that it would have been better had diabetes been ruled out. A blood sample for glycated hemoglobin was not sent to the sub-district hospitals, and was not available at our hospital. In this case, repeated random dextrose levels remained elevated with the highest measure being 520 mg/dL. The urine dipstick results were WBC 1+, ketones 3+, glucose 1+. Serum biochemistry for K⁺ was 3.14 mmol/L and for Na⁺ was 129.5 mmol/L, not consistent with dehydration.
2. Oral chloroquine 500 mg daily was given for 2 days. The patient expired before the final dose could be given.
3. The patient's symptoms had started approximately 8 weeks before admission. She was admitted 3 days prior to her death and the case report describes the events over those 3 days.

Competing Interests: No competing interests

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