Digging for melioidosis

New insights into the epidemiology and pathophysiology

Birnie, E.

Link to publication

Creative Commons License (see https://creativecommons.org/use-remix/cc-licenses):
Other

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (http://dare.uva.nl)

Download date: 25 Jan 2020
CHAPTER 6
DIFFERENCES IN INFLAMMATION PATTERNS INDUCED BY AFRICAN AND ASIAN BURKHOLDERIA PSEUDOMALLEI ISOLATES IN MICE
CHAPTER 6
DIFFERENCES IN INFLAMMATION PATTERNS INDUCED BY AFRICAN AND ASIAN BURKHOLDERIA PSEUDOMALLEI ISOLATES IN MICE

Emma Birnie*, Tassili A.F. Weehuizen*, Bart Ferwerda, Joris J.T.H. Roelofs, Alex F. de Vos, Martin P. Grobusch, and W. Joost Wiersinga


*These authors contributed equally
ABSTRACT

Burkholderia pseudomallei is the causative agent of melioidosis, an emerging tropical disease of high mortality. Sub-Saharan Africa represents potential melioidosis "hotspots"; however, to date, only a few cases have been reported. Herein, we compared the inflammatory patterns induced by a B. pseudomallei strain recently isolated from a fatal Gabonese case with the Thai reference strain B. pseudomallei 1026b and B. thailandensis E264. Ex vivo, no differences were observed in terms of cellular responsiveness between strains. However, when compared with the B. pseudomallei 1026b strain, the Gabonese isolate was significantly less virulent in terms of bacterial dissemination, inflammatory response, and organ damage in mice. Genomic comparison between strains showed differences in regions containing a fimbriae/adhesion virulence protein. In addition to a lack of microbiology facilities, differences in virulence of Burkholderia strains might contribute to the diverse global clinical occurrence of melioidosis.
The Tier 1 biothreat agent *Burkholderia pseudomallei* is an environmental Gram-negative bacillus and the causative agent of melioidosis, a tropical infectious disease classically characterized by pneumonia and abscess formation throughout the body.\textsuperscript{1-3} The *Burkholderia* genus contains over 40 species, of which *B. pseudomallei* and *B. mallei* are considered the most pathogenic.\textsuperscript{1,4} *B. thailandensis* is closely related to *B. pseudomallei* but rarely causes disease.\textsuperscript{3} *B. pseudomallei* governs an impressive arsenal of virulence factors, including the capsular polysaccharide, lipopolysaccharide, type-III secretion systems, flagella, fimbriae, adhesion virulence proteins, and the toxin named BPSL1549 *Burkholderia* Lethal Factor-1.\textsuperscript{6,7} However, the relative impact of each on clinical outcome in human infection remains largely unknown. One could hypothesize that potential differences in the virulence of *B. pseudomallei* strains explain in part differences in clinical occurrence of melioidosis around the globe.

Melioidosis is endemic in Northern Australia and Southeast Asia with annual incidence rates of up to 50 patients per 100,000 people\textsuperscript{1,8}. There are about 165,000 human melioidosis cases per year worldwide, of which 89,000 people succumb to their disease.\textsuperscript{9} It is estimated that melioidosis is widespread in sub-Saharan Africa as well, with 24,000 cases of melioidosis occurring each year associated with a predicted mortality rate of 62.5%\textsuperscript{9}. However, less than 20 cases of melioidosis from Africa have been reported. In addition to severe underreporting as well as a lack of diagnostic facilities,\textsuperscript{10,11} differences in the virulence of *Burkholderia* strains could explain this disparity.

Therefore, in the present study, we determined the differences in patterns of virulence of a recently isolated *B. pseudomallei* strain from a fatal Gabonese case by comparing it to the virulence of the well-typed virulent Thai isolate 1026b and the nonpathogenic *B. thailandensis* E264 by investigating the host response to these *Burkholderia* strains in mice ex vivo and in vivo.\textsuperscript{4,12,13} In addition, we compared whole genome sequences on the presence of potential virulence factors of both strains.

Male wild-type C57Bl/6 mice (Charles River, Leiden, the Netherlands) between 8-10 weeks of age were used. *B. pseudomallei* strain 1026b has been isolated from a blood culture of a septic 29-year-old Thai female rice farmer presenting with bacteremia with soft tissue, skin, joint, and splenic involvement.\textsuperscript{13} The Gabonese *B. pseudomallei* isolate was isolated from a blood culture of a 68-year-old diabetic septic female with soft tissue and joint involvement.\textsuperscript{12} *B. thailandensis* E264 is an environmental isolate from Northeast Thailand.\textsuperscript{5}
Murine whole blood, peritoneal macrophages and alveolar macrophages were isolated as described\textsuperscript{4,14,15} and stimulated overnight with medium, *Escherichia coli* LPS or *Burkholderia* isolates. Pneumonia was induced by intranasal inoculation with $7.5 \times 10^2$ colony forming units (CFU) of *B. pseudomallei* 1026b, the Gabonese *B. pseudomallei* isolate, or *B. thailandensis* E264. Sample harvesting, processing, and determination of bacterial growth, assays, and pathology were done as described.\textsuperscript{4,14,15} Whole-genome sequencing was performed using the MiSeq platform (Illumina, San Diego, CA, USA) as described.\textsuperscript{11,12} Genome alignments and annotation of *B. pseudomallei* 1026b strain (NC\_017831.1 and NC\_017832.1) were obtained from GeneBank.\textsuperscript{16} For full methods see Supplemental material.

To investigate the differences in inflammation induced by infection with the Gabonese *B. pseudomallei* isolate and *B. pseudomallei* 1026b, we first stimulated different murine cell types considered important for the regulation of inflammation in melioidosis. In terms of pro-inflammatory cytokine release, we observed no differences between the Gabonese isolate when compared with *B. pseudomallei* 1026b in whole blood (Figure 1A), peritoneal macrophages (Figure 1B), or alveolar macrophages (Figure 1C). Interleukin (IL)-6 levels were comparable to tumor necrosis factor (TNF)-\(\alpha\) (data not shown).

In vivo, however, mice infected with $7.5 \times 10^2$ CFU of the Gabonese *B. pseudomallei* isolate showed markedly decreased bacterial loads in lung, liver, and blood 72 hours after intranasal inoculation when compared with the groups infected with 1026b strain (Figure 1D–F). Interestingly, bacterial loads of mice infected with the Gabonese *B. pseudomallei* isolate were similar to those in mice infected with the avirulent *B. thailandensis*. At this dose, none of the eight blood cultures of mice infected with the Gabonese *B. pseudomallei* isolate became positive. Only intranasal inoculation of the Gabonese isolate with a dose that was seven times higher than the *B. pseudomallei* 1026 dose resulted in equal bacterial loads in lung, liver, and blood between both strains 72 hours after infection (Figure 1D–F).
Figure 1  Comparative virulence of the different Burkholderia strains *ex vivo* and *in vivo*

(A) Whole blood, (B) peritoneal macrophages (PM), (C) and alveolar macrophages (AM) were isolated from naïve wild-type C57BL/6 mice and were stimulated overnight with medium, lipopolysaccharide of *Escherichia coli* (100 ng/mL), heat-killed *Burkholderia pseudomallei* 1026b (*B.ps*), or the Gabonese *B. pseudomallei* isolate (*B.gab*). After 20 hours of stimulation, supernatants were harvested and assayed for membrane-associated tumor necrosis factor-α (mTNFα); (*N* = 4 per group). Additionally, mice were infected with 7.5 × 10^2^ colony-forming units (CFU) of *B. pseudomallei*-1026b (*B.ps*), *B. thailandensis* (*B.th*), or the Gabonese *B. pseudomallei* isolate (*B.gab*), and were killed at 72 hours post-infection and bacterial loads were determined in (D) lung, (E) whole blood, and (F) liver. The dashed line marks the level of CFU detection. None of the eight blood cultures were positive for mice infected with the Gabonese *B. pseudomallei* isolate (*B.gab*). For the dose-finding experiment, mice were infected with 10^2^, 10^3^, or 5 × 10^3^ CFU of the Gabonese *B. pseudomallei* isolate (*B.gab*) and killed 72 hours post-infection. *N* = 8 per group. Kruskal–Wallis test was performed, followed by post hoc Dunn’s test, *P* < 0.05, ***P* < 0.001.
Local and systemic inflammation, as assessed by determination of chemokine (monocyte chemotactic protein-1) and cytokine (IL-6, IL-10, interferon-γ, and TNF-α) levels, was significantly reduced in mice infected with the Gabonese *B. pseudomallei* isolate when compared with the 1026b strain (Supplemental Table 1). Next, we determined the severity of organ inflammation in all groups. Mice infected with the Gabonese *B. pseudomallei* isolate showed markedly attenuated pulmonary and liver injury when compared with the strain 1026b (Figure 2). Evidence of profound organ damage was further reflected in increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) levels 72 hours after infection in all mice (Supplemental Table 1). However, in line with the pathology data, mice infected with the Gabonese *B. pseudomallei* showed strongly reduced ALT, AST, and LDH levels (Supplemental Table 1). In addition, we observed lower creatinine and blood urea nitrogen levels in Gabonese *B. pseudomallei*-infected mice, possibly due to better renal perfusion, since these mice appeared less septic (Supplemental Table 1). The inflammatory response elicited by the Gabonese *B. pseudomallei*, as determined by local and systemic cytokine production as well as pathology and markers of organ damage was comparable to that of mice infected with *B. thailandensis* (Supplemental Table 1).

To find the possible underlying cause of the observed decreased virulence of the Gabonese *B. pseudomallei* isolate when compared with *B. pseudomallei* 1026b, we used the Basic Local Alignment Search Tool algorithm to find regions unique to *B. pseudomallei* 1026b. The Gabonese *B. pseudomallei* isolate showed 13 chromosomal putative protein regions (A–M) that corresponded poorly to those of strain 1026b (Figure 3, Supplemental Table 2). Within these regions, most annotations classify the putative proteins into phage, integrase, transposase, and hypothetical proteins. Genomic inspection of the found regions, on potential causal proteins, showed one region, namely E (Figure 3), containing a fimbrial and adhesion virulence protein (Type IV fimbrial biogenesis protein PilY1).
Mice were infected with $7.5 \times 10^2$ colony-forming units of the Gabonese B. pseudomallei isolate (B.gab), B. pseudomallei 1026b (B.ps), or B. thailandensis (B.th). (A, E) Seventy-two hours post-infection, pulmonary and hepatic injury and inflammation were assessed by calculating pathology scores. Representative slides of hematoxylin and eosin–stained lungs and livers of mice infected with (B, F) B. pseudomallei (B.ps), (C, G) B. thailandensis (B.th), or (D, H) Gabonese B. pseudomallei isolate (B.gab) are shown (original magnification 10×). N = 8 per group. Kruskal–Wallis test was performed, followed by post hoc Dunn’s test, **P < 0.01, ***P < 0.001. The arrow indicates manifest inflammation.
Figure 3  Genome comparison of the Gabonese *Burkholderia pseudomallei* isolate with the reference strain *B. pseudomallei* 1026b: depiction of unique regions

*Burkholderia pseudomallei*

1026b

Thirteen chromosomal putative protein regions (A–M) of *B. pseudomallei* 1026b strain that have low correspondence are compared with the Gabonese *B. pseudomallei* genome. Within these regions, most annotations classify the putative proteins into phage, integrase, transposase, and hypothetical proteins. Genomic inspection of the found regions, on potential causal proteins, showed one region, namely E, containing a fimbrial and adhesion virulence protein (Type IV fimbrial biogenesis protein PilY3). Unique *B. pseudomallei* regions are shown with the location, length, and number of proteins.
Our experiments demonstrated that the Gabonese *B. pseudomallei* strain is just as virulent as the reference strain *B. pseudomallei* 1026b *ex vivo* but, more importantly, less virulent *in vivo*. This might in part be explained by the more complex interaction between host and bacterium in an *in vivo* model. In addition, the *in vitro* inflammatory response demonstrates only a portion of the complete immune response.

The majority of previously published studies about murine melioidosis are conducted with the “prototypical” strains 1026b or K96243 (isolated from 34-year-old Thai diabetic female). We conducted *in vivo* survival studies, which demonstrated equal lethality of both strains, when the same inoculation dose was used (data not shown). Following these experiments, we chose to compare the well-typed 1026b strain with the recently discovered *B. pseudomallei* isolate of a Gabonese patient. However, it is important to keep in mind that the pathogenicity of *Burkholderia* strains varies in animal models and does not always correlate with their virulence in humans, depending on host factors.

It is known that “serial passaging,” a transfer of a bacterium through a series of cultures or via experimental animals, may increase virulence of bacterial strains by selecting the most virulent bacteria. In addition, it is known that *B. pseudomallei* can rapidly genetically modify itself within the host. Nonetheless, the virulence of the Gabonese *B. pseudomallei* isolate did not differ before and after passaging, thereby remaining significantly less virulent than the 1026b strain. See Supplemental Material and Supplemental Figure 1 for more details.

Our dose-finding experiments demonstrate that a seven-fold higher dose of the Gabonese isolate is required to be equally virulent to the 1026b strain. It should be noted that the supposedly “avirulent” *B. thailandensis* can become as lethal as the 1026b strain when a 1000-fold higher dose is used in mice, demonstrating the potential harmfulness of even this “non-virulent” bacterium. We would like to stress that, in this study, we only compared a single African isolate with a single Asian isolate. In theory, one could hypothesize that, to cause melioidosis in humans, Gabonese isolates have to infect their host at a higher concentration when compared with their Asian counterparts. This could, in addition to severe under-reporting, explain in some part the low number of melioidosis cases reported from Gabon and Africa.

Clinical *B. pseudomallei* isolates may have a broad genomic diversity. In a previous analysis, a large number of *B. pseudomallei* isolates from Thailand and Australia showed a strong genetic differentiation based on
geographical location and significant differentiation based on virulence potential.\textsuperscript{22} Recently, it has been shown that African strains have substantial genetic diversity, suggesting long-term \textit{B. pseudomallei} endemicity in this region.\textsuperscript{23} We compared the genomes of the Gabonese and the \textit{B. pseudomallei} 1026b strain and identified a region that contains a fimbrial and adhesion virulence protein (Type IV fimbrial biogenesis protein PilY1), which was absent in the Gabonese strain.\textsuperscript{17} Variation in this region might be responsible for observed differences in virulence between the African and Asian strain. However, a larger analysis on the in vivo virulence and genetic expression between African and Asian strains is required to further address this hypothesis.\textsuperscript{24}

In conclusion, we found that the Gabonese \textit{B. pseudomallei} isolate was significantly less virulent compared with a well-defined Thai isolate 1026b. This study is the first to compare the virulence of an African and Asian \textit{B. pseudomallei} strain both \textit{ex vivo} and \textit{in vivo}, thereby including genomic comparison. Recently, it has been predicted that the global burden of melioidosis is much higher than previously thought, with a worldwide mortality comparable with measles, dengue, and leptospirosis.\textsuperscript{9} This study highlights the need for increased insight into the virulence and expression patterns influencing pathogenicity of \textit{B. pseudomallei} isolates across the world.

\textit{Acknowledgments}

We thank Jacqueline Lankelma and Katja de Jong for their help in the laboratory, Marieke ten Brink and Joost Daalhuisen for their expert technical assistance during the animal experiments, and Sharon J. Peacock for help with the bacterial sequencing, and the colleagues of CERMEL who were involved in patient care and microbiological diagnosis, Abraham Alabi in particular.
REFERENCES


ETHICS STATEMENT


EX VIVO STIMULATION EXPERIMENTS

Pathogen-free wild-type (WT) C57BL/6 mice were purchased from Charles River (Leiden, the Netherlands). Age (8-10 weeks old) and sex-matched animals were used in all experiments. Whole blood, peritoneal macrophages and alveolar macrophages were harvested from naive wild-type mice as described (n=4 per group).1-4 Mice were anesthetized and sacrificed by bleeding from the vena cava inferior. Blood was transferred to V-bottom 96-well plates (Greiner Bio-One, Frickenhausen, Germany; 100 ul/well) and directly stimulated for 20 hours with 100 μl E.coli lipopolysaccharide (end concentration of 100 ng/ml, Invivogen, San Diego, CA), 107 CFU/ml of heat-killed B. pseudomallei 1026b, 107 CFU/ml of the Gabonese B. pseudomallei isolate or RPMI 1640 medium (Gibco, Waltham, CA).

Peritoneal macrophages were harvested by 5 ml peritoneal lavage with sterile PBS. Cells were resuspended in RPMI 1640 enriched with 1% L-glutamine (Gibco), 1% penicillin/streptomycin (Gibco), and 10% FCS (Gibco) and seeded in a F-bottom 96-well plate at a concentration of 0.5 x 106/ml. Alveolar macrophages were collected by bronchoalveolar lavage with 3 x 0.5 ml sterile PBS and suspended in the same medium. The cells were seeded at a concentration of 0.2 x 106/ml. The next day the cells were washed with RPMI 1640 to remove non-adherent cells and incubated with the same stimuli as used for whole blood; a MOI of 50 of heat-killed B. pseudomallei 1026b and the Gabonese isolate was used. Dose-response curves for these stimuli were performed prior to these experiments (data not shown). After 20 hours of stimulation, supernatants of blood and macrophages were harvested and assayed for Tumor Necrosis Factor alpha (TNF-α).

EXPERIMENTAL INFECTION

Intranasal infection was performed as previously described.1 Prior to infection, mice were lightly anesthetized by inhalation of isoflurane. In the dose-finding experiment wild-type mice were infected with either 102, 103 or 5 x 103 CFU/50 μl NaCl 0.9% of the Gabonese B. pseudomallei isolate intranasally (n=8 per group). Mice were observed for survival during 72 hours after infection, after which the mice were anesthetized with medetomidine/ketamine and sacrificed by bleeding from the vena cava inferior. Bacterial loads were determined in lungs, livers and blood. In the following experiment, wild-type mice were infected with either 7.5 x 104 CFU/50 μl NaCl 0.9% of B. pseudomallei 1026b, B. thailandensis E264 or the Gabonese B. pseudomallei (n=8 per group). At 72 hours post-infection mice were sacrificed and bacterial loads were determined in blood, lungs and livers. The second experiment has been repeated using the Gabonese B. pseudomallei isolate after serial passaging.
Serial passaging of the Gabonese B. pseudomallei isolate

Wild-type (WT) mice were intranasally infected with $0.5 \times 10^6$ CFU of the Gabonese B. pseudomallei isolate. The infected mice were sacrificed 72 hours after infection and the spleen was removed, homogenized, serially diluted in NaCl 0.9% and cultured on blood-agar plates for 24 hours at 37°C and 5% CO2. Isolated colonies were collected from the most diluted concentration in which they could be detected and grown in Luria Broth (Sigma-Aldrich, St. Louis, MO) overnight at 37°C. Sterile glycerol (Thermo Fisher Scientific, Waltsham, MA) was added to the bacterial culture at a 30% (vol/vol) final concentration and stocks were frozen at −80°C before being used for further experiments.

Assays

For cytokine measurements, lung homogenates were diluted 1:2 in lysis buffer containing 300 mM NaCl, 30 mM Tris, 2 mM MgCl2, 2 mM CaCl2, 1% Triton X-100 and a protease inhibitor cocktail (Roche, Indianapolis, IN) and incubated at 4°C for 30 min. Hereafter, homogenates were centrifuged at 1730xg at 4°C for 10 minutes. Supernatants were sterilized using 0.22 mm pore-size filters (Millipore, Billerica, MA) and stored at -20°C until further analysis. Interleukin (IL)-6, IL-10, IL-12p70, monocyte chemotactic protein-1 (MCP-1), Interferon-y (IFN-y), and TNF-α were measured in blood and lung homogenates by cytometric bead array (CBA) multiplex assay (BD Biosciences, San Jose, CA), in accordance with the manufacturers’ instructions. Lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT), and Blood Urea Nitrogen (BUN) were determined with the Cobas 8000 module c702 (Roche Diagnostics, Basel, Switzerland).

Pathology

Paraffin-embedded 4 µm tissue sections were stained with haematoxylin and eosin (H&E) and analyzed for inflammation and tissue damage as described. All slides were coded and scored by a pathologist blinded for the experimental groups. To score lung inflammation and damage, the entire lung surface was analyzed with respect to the following parameters: surface with pneumonia, necrosis/abscess formation, interstitial inflammation, endothelialitis, bronchitis, edema, thrombus formation and pleuritis. Each parameter was graded on a scale of 0-4 (0: absent; 1: mild; 2: moderate; 3: severe; 4: very severe). The total lung inflammation score was expressed as the sum of the scores for each parameter, the maximum being 32. Liver damage was scored based on inflammation, necrosis/abscess formation and thrombus formation using the scale given above. The maximum total liver pathology score was 12.

Sequencing

The complete genome alignment and annotation of B. pseudomallei strain 1026b (NC_017831.1 and NC_017832.1) have been obtained from GeneBank. The Gabonese B. pseudomallei strain has been sequenced and assembled at the Wellcome Trust Sanger Institute as previously described. Because the strains have been annotated with different methods both strains were annotated using RAST. The BLAST algorithm was used to find unique B. pseudomallei 1026b regions that could cause the difference in virulence. Regions of interest were those that showed less than 80 percent similarity between strains and were part of a region that contained at least more than two proteins. These genomic B. pseudomallei 1026b areas were converted back to the initial GeneBank notations and scanned for virulence genes.
Statistical analysis
Data were analyzed using GraphPad Prism for Windows (version 5.01; GraphPad Software). Differences between groups were analyzed by Mann-Whitney U test or Kruskal-Wallis with Dunn's post-test analysis where appropriate. Values are expressed as means ± SEM. P-values <0.05 were considered statistically significant.

REFERENCES
**Supplemental Table 1**  Cytokine response in plasma and lung homogenates and organ injury markers in mice infected with *B. pseudomallei* 1026b, *B. thailandensis* or the Gabonese *B. pseudomallei* isolate

Cytokine levels and markers for systemic organ injury were measured in plasma and/or lung homogenate 72 hours after intranasal infection. Mice were infected with $7.5 \times 10^2$ CFU of *B. pseudomallei* 1026b (*B.ps*), *B. thailandensis* (*B.th*), or the Gabonese *B. pseudomallei* isolate (*B.gab*). Data are represented as means ± SEM. N = 8 per group. TNF-α = tumor necrosis factor-α; IL = interleukin; MCP-1 = monocyte chemoattractant Protein-1; IFN-γ = interferon-γ; KC = keratinocyte chemoattractant; BUN = blood urea nitrogen; AST = aspartate aminotransferase; ALT = alanine aminotransferase; LDH = lactate dehydrogenase; *P<0.05; **P<0.01; ***P<0.001, when compared to mice infected with *B. pseudomallei* 1026b (Kruskal-Wallis test; followed by separate Mann-Whitney *U* tests).

<table>
<thead>
<tr>
<th></th>
<th><em>B.ps</em> T=72h</th>
<th><em>B.th</em> Lung</th>
<th><em>B.gab</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TNF-α (pg/mL)</strong></td>
<td>18521 ± 1176</td>
<td>707.2 ± 93.52***</td>
<td>1471 ± 261.4***</td>
</tr>
<tr>
<td><strong>IL-6 (pg/mL)</strong></td>
<td>40000 ± 267.3</td>
<td>784.8 ± 100.1***</td>
<td>1359 ± 222.3***</td>
</tr>
<tr>
<td><strong>KC (pg/mL)</strong></td>
<td>60000 ± 267.3</td>
<td>758.9 ± 115.5***</td>
<td>2922 ± 380.7***</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th><em>B.ps</em> T=72h</th>
<th><em>B.th</em> Plasma</th>
<th><em>B.gab</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TNF-α (pg/mL)</strong></td>
<td>1704 ± 487.9</td>
<td>3.2 ± 0.3**</td>
<td>5.7 ± 0.5**</td>
</tr>
<tr>
<td><strong>IL-6 (pg/mL)</strong></td>
<td>10000 ± 0.1</td>
<td>17.5 ± 4.6***</td>
<td>20 ± 4.8***</td>
</tr>
<tr>
<td><strong>MCP-1 (pg/mL)</strong></td>
<td>3606 ± 1052</td>
<td>12.3 ± 1.5**</td>
<td>26 ± 2.8***</td>
</tr>
<tr>
<td><strong>IFN-γ (pg/mL)</strong></td>
<td>3424 ± 822.5</td>
<td>1.4 ± 0.2**</td>
<td>7.1 ± 1***</td>
</tr>
<tr>
<td>**IL-12p70 (pg/mL)</td>
<td>76.5 ± 55</td>
<td>3.7 ± 1.5**</td>
<td>6.7 ± 2.4**</td>
</tr>
<tr>
<td><strong>IL-10 (pg/mL)</strong></td>
<td>12.7 ± 3.4</td>
<td>3.5 ± 2.8*</td>
<td>1.1 ± 0.8**</td>
</tr>
<tr>
<td><strong>BUN (mmol/L)</strong></td>
<td>30.3 ± 1.5</td>
<td>7.8 ± 0.4</td>
<td>7.3 ± 0.3**</td>
</tr>
<tr>
<td><strong>Creatinine (µmol/L)</strong></td>
<td>50 ± 9.2</td>
<td>7 ± 0.3***</td>
<td>6.4 ± 0.4**</td>
</tr>
<tr>
<td><strong>AST (U/L)</strong></td>
<td>659.2 ± 175.6</td>
<td>80.9 ± 14**</td>
<td>58.9 ± 6.8**</td>
</tr>
<tr>
<td><strong>ALT (U/L)</strong></td>
<td>216.4 ± 91.7</td>
<td>32.1 ± 6.5**</td>
<td>27.7 ± 2 ±**</td>
</tr>
<tr>
<td><strong>LDH (U/L)</strong></td>
<td>1857 ± 161.9</td>
<td>273 ± 42.5**</td>
<td>234.7 ± 8.2***</td>
</tr>
</tbody>
</table>
Supplemental Table 2  Different genomic regions in the Gabonese \textit{B. pseudomallei} isolate when compared with \textit{B. pseudomallei} 1026b

<table>
<thead>
<tr>
<th>Region</th>
<th>Start</th>
<th>Stop</th>
<th>Number of nucleotides</th>
<th>Proteins in the regions*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chr1 A</td>
<td>135537</td>
<td>142823</td>
<td>7286</td>
<td>BP1026B.I0124 – BP1026B.I0140</td>
</tr>
<tr>
<td>Chr1 B</td>
<td>1363987</td>
<td>1372779</td>
<td>8792</td>
<td>BP1026B.I1266 – BP1026B.I1273</td>
</tr>
<tr>
<td>Chr1 C</td>
<td>1710172</td>
<td>1728095</td>
<td>17923</td>
<td>BP1026B.I1581 – BP1026B.I1601</td>
</tr>
<tr>
<td>Chr1 D</td>
<td>1760564</td>
<td>1772124</td>
<td>11560</td>
<td>BP1026B.I1623 – BP1026B.I1631</td>
</tr>
<tr>
<td>Chr1 E</td>
<td>1784561</td>
<td>1801183</td>
<td>16622</td>
<td>BP1026B.I1642 – BP1026B.I1661</td>
</tr>
<tr>
<td>Chr1 F</td>
<td>2333701</td>
<td>2378572</td>
<td>44871</td>
<td>BP1026B.I2083 – BP1026B.I2142</td>
</tr>
<tr>
<td>Chr1 G</td>
<td>2383163</td>
<td>2389088</td>
<td>5925</td>
<td>BP1026B.I2152 – BP1026B.I2161</td>
</tr>
<tr>
<td>Chr1 H</td>
<td>2881096</td>
<td>2889116</td>
<td>8020</td>
<td>BP1026B.I2595 – BP1026B.I2600</td>
</tr>
<tr>
<td>Chr1 I</td>
<td>3932807</td>
<td>3938403</td>
<td>5596</td>
<td>BP1026B.I3562 – BP1026B.I3568</td>
</tr>
<tr>
<td>Chr1 J</td>
<td>3985379</td>
<td>3994236</td>
<td>8857</td>
<td>BP1026B.I3623 – BP1026B.I3636</td>
</tr>
<tr>
<td>Chr2 K</td>
<td>522030</td>
<td>535839</td>
<td>13809</td>
<td>BP1026B.II0422 – BP1026B.II0433</td>
</tr>
<tr>
<td>Chr2 L</td>
<td>2729852</td>
<td>2731965</td>
<td>2113</td>
<td>BP1026B.II2199 – BP1026B.II2201</td>
</tr>
<tr>
<td>Chr2 M</td>
<td>2755687</td>
<td>2783156</td>
<td>27469</td>
<td>BP1026B.II2213 – BP1026B.II2239</td>
</tr>
</tbody>
</table>

* GeneBank gene locus_tag numbers
Mice were infected before (pre) and after (post) serial passaging, with $7.5 \times 10^2$ CFU of either *B. pseudomallei* 1026b (*B.ps*), *B. thailandensis* (*B.th*) or the Gabonese *B. pseudomallei* isolate (*B.gab*) and were sacrificed at 72 hours post-infection and bacterial loads were determined in (A) lung, (B) whole blood, (C) and liver. The dashed line marks the level of CFU detection. $N=8$ per group. Kruskal Wallis test was performed, followed by post-hoc Dunn’s test, *p < 0.05, **p < 0.01, ***p < 0.001.