AKADÉMIAI KIADÓ

European Journal of Microbiology and Immunology

11 (2021) 3, 57–61

DOI:
10.1556/1886.2021.00014

SHORT COMMUNICATION

No hints for abundance of *Bacillus anthracis* and *Burkholderia pseudomallei* in 100 environmental samples from Cameroon

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Received: July 29, 2021 • Accepted: August 14, 2021
Published online: September 2, 2021

ABSTRACT

Background: Little is known on the abundance of the pathogens *Bacillus anthracis* and *Burkholderia pseudomallei* in environmental samples in Cameroon. Therefore, 100 respective samples were assessed in a proof-of-principle assessment. *Methods:* DNA residuals from nucleic acid extractions of 100 environmental samples, which were collected between 2011 and 2013 in the Mapé Basin of Cameroon, were screened for *B. anthracis* and *B. pseudomallei* by real-time PCR. The samples comprised soil samples with water contact ($n = 88$), soil samples without water contact ($n = 6$), plant material with water contact ($n = 3$), water ($n = 2$), and soil from a hospital dressing room ($n = 1$). *Results:* *B. anthracis* and *B. pseudomallei* were detected in none of the samples assessed. *Conclusion:* The results indicate that at least a quantitatively overwhelming, ubiquitous occurrence of *B. anthracis* and *B. pseudomallei* in the environment in Cameroon is highly unlikely. However, the number and choice of the assessed samples limit the interpretability of the results.

KEYWORDS

Bacillus anthracis, Burkholderia pseudomallei, soil, environmental, PCR, Cameroon

INTRODUCTION

Bacillus anthracis, the causative agent of anthrax [1] and *Burkholderia pseudomallei*, the causative agent of melioidosis [2], are pathogens of concern with still insufficient epidemiological awareness in Subsaharan Africa. In Cameroon, at least anthrax is a well-known issue of concern. *B. anthracis* as well as *Bacillus cereus* Biovar Anthracis are constant threats to Cameroon's wildlife and cattle breeding [3–7]. In contrast, melioidosis has not been reported from Cameroon so far, although at least strains of the *Burkholderia cepacia* complex seem to be commonly isolated in local hospitals [8]. As *B. pseudomallei* can be easily confused with other *Burkholderia* spp. [9], local occurrence of this species cannot be excluded. Further efforts on targeted screening for *B. pseudomallei* in Subsaharan Africa have been recently suggested [10].

The geographic proximity of Cameroon to Nigeria and Chad, which were characterized as probable sites of endemicity of *B. pseudomallei* [11] based on ecological characteristics, makes the occurrence of this pathogen in Cameroon highly likely. In particular, humid and windy conditions in association with warm weather facilitate the spread of melioidosis in the tropics [12, 13].

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As both *B. anthracis* and *B. pseudomallei* are soil-associated pathogens, the screening of environmental samples can provide a first overview on the local abundance. In this study, we performed a molecular screening for DNA of the two species from a total of 100 environmental samples from Cameroon.

MATERIALS AND METHODS

Samples

At total of 100 environmental samples collected in the Mapé Basin of Cameroon from a previous study on the abundance of *Mycobacterium ulcerans* [14] was included in the assessment. The samples had been collected between 2011 and 2013 and comprised soil samples with water contact (n = 88), soil samples without water contact (n = 6), plant material with water contact (n = 3), water (n = 2), and soil from a hospital dressing room (n = 1). The specimens had been taken in areas of high prevalence for *M. ulcerans*. Information on the sampling strategy and the precise sampling spots are extensively detailed elsewhere, same as the nucleic acid extraction procedure using the Fast DNA Spin Kit for Soil (MP Biomedicals, Eschwege, Germany, product number 116560-200) [14]. Typical sampling sides are shown in Fig. 1.

PCR assessment

From the nucleic acid extractions, real-time PCR targeting a species-specific 115-base-bair-sequence of the *orf2* gene of the type III secretion system of *B. pseudomallei* was performed as described elsewhere [15]. The detection of *B. anthracis* was based on the parallel amplification of specific sequence segments from three different coding regions: on plasmid pXO1 (*cya* gene coding for an anthrax toxin component), on plasmid pXO2 (*capB* gene coding for a component necessary for polyglutamate synthesis) as well as on the bacterial chromosome (pro-phage lambda Ba03-PL3). Again, real-time PCR was performed as described elsewhere [16].

Ethical statement

Not applicable, because no patient data or primary human sample materials were used.

RESULTS AND DISCUSSION

Neither DNA of *B. pseudomallei* nor of *B. anthracis* was identified in any of the samples. This finding suggests that those pathogens are at least not ubiquitously abundant at high titers in environmental samples in this area. The absence *of B. pseudomallei* in moist soil is well in line with lacking reports on melioidosis in Cameroon. Nevertheless, it might be interesting to further differentiate the *B. cepacia* complex strains that were reported from hospital environments in Cameroon [8] to exclude misidentified *B. pseudomallei* stains.

Focusing on anthrax, which is reported to be prevalent in Cameroon [3–7], the data suggest that soil from water sources, where dilution has to expected, is obviously no adequate material for PCR-based screening for *B. anthracis*, although it is likely that some of the sampling sites were used as drinking places by wild and domestic animals. Areas where excretions of infected animals have contaminated the environment or where infected animals have died and have

Fig. 1. Sampling sites in Cameroon

been buried are the more likely places of high spore density. On the other hand, it can be concluded from the presented results that the spore density is at least not high enough to even allow positive findings in PCR from soil with contact to water.

Basically, detection of *B. pseudomallei* from soil samples [17–20] is feasible, same as detection of *B. anthracis* [21]. Therefore, at least for *B. anthracis*, the choice of the assessed samples is likely to have led to the observed negative diagnostic results. Even a guideline for environmental sampling of *B. pseudomallei* exists [11]. Due to the fact that the samples were originally not sampled for a screening for *B. pseudomallei*, no attention had been paid to adherence with this guideline [14], an undeniable limitation of the study. Identification of *B. pseudomallei* from soil samples even by molecular approaches is not trivial [22] and broth enrichment techniques are recommended [23] which were not applied in this assessment. Next to this, physico-chemical properties of soil affect the likelihood of detecting *B. pseudomallei* in endemic areas, with best chances of successful isolations in rice fields [24, 25]. Accordingly, the non-specific choice of sampling sites is considered as the most important limitation of the here presented screening approach, reducing the interpretability of the obtained data.

However, there are also considerations which justify the way how the assessment was performed. The applied *B. pseudomallei* real-time PCR protocol [15] has been successfully evaluated with soil samples before. This evaluation suggested detection limits of less than 10 genome equivalents [26]. In a similar way, real-time PCR for pXO1 and pXO2 plasmid sequences of *B. anthracis* have been shown to detect about 1 bacterial cell per gram soil [27, 28], so high sensitivity can be assumed. As specificity of PCR depends on the abundance of phylogenetically closely related microorganisms which may regionally vary [29, 30], it was difficult to a priori predict the assays' specificity if applied with soil samples from Cameroon. However, high specificity is suggested by the fact that not a single positive result has been recorded in the study, although the low number of assessed environmental samples does not allow precise estimations.

Admittedly, negative results may have resulted from sample inhibition rather than from the absence of target DNA as well. However, as the applied multiplex real-time PCR protocol for *B. anthracis* [16] contains an internal control PCR targeting the *B. thuringiensis cry1* gene, it can be assumed that severe sample inhibition would not have gone undetected. The applied sample dilution strategy in case of inhibited samples has been described previously in the study on *M. ulcerans* detection within the same samples [14]. Of course, the additional application of positive control PCRs like 16S rRNA gene specific assays [31] or additional spiking experiments with positive control DNA would have even increased the expressiveness of the quality control assessments. So, it is an admitted limitation that respective analyses were not performed. The same applies to the lack of parallel culture-based assessments.

Finally, lacking pathogen-specific optimization of storage and transport of the samples is another admitted limitation which might have been associated with a potential reduction of target sequence quantities within the samples. In fact, the samples were transported exposed to variable environmental conditions and stored at 4 °C as described elsewhere [14] before nucleic acids could be extracted and stored frozen at −80 °C. So, it is theoretically possible that the abundance of small amounts of target DNA may have gone undetected. In addition, the quality of extracted DNA has not been controlled by quantification of the DNA content within the eluates. As nucleic acid extraction was performed with a standardized and well characterized assay explicitly developed for soil samples [32, 33], however, acceptable yields of DNA were considered as likely.

Based on the abovementioned reasons and considering the admitted intrinsic limitations of the assessment, the study excludes the abundance of small target DNA amounts neither in the samples nor in the assessed region of Cameroon. However, as well standardized methods were nevertheless applied, it seems at least justified to assume that the presence of high quantities of the targeted bacteria was virtually excluded by the approach. In particular for *B. anthracis*, which is an ongoing menace in various tropical settings [34], soil-borne transmission to humans in case of low pathogen density can be considered as unlikely [35], also in the Mapé Basin of Cameroon.

CONCLUSIONS

The assessment suggested, if any, only a low abundance of *B. anthracis* and *B. pseudomallei* below the diagnostic detection threshold in the assessed environmental samples. Although the limited sample size and regional coverage considerably limit the interpretability of the results, at least an overwhelming and ubiquitous occurrence of these pathogens in the assessed environment of the Mapé Basin of Cameroon can be widely excluded. This is in line with the lack of described melioidosis cases in Cameroon.

Due to the limitations of the study, further investigations are reasonable. Due to limited resources available for these neglected pathogens and given the considerable effort for sample collection and DNA isolation, the approach of creating synergisms and using samples multiple times makes sense in principle and should be taken into account when collecting environmental samples. In future projects, particular care should be taken to collect samples for multiple studies from the outset. To do so, it would be desirable to have more cooperation between different research disciplines in projects with elaborate sample collections, an issue that is not taken into account enough in current focused research funding.

Conflict of interest: Nothing to declare.

Funding sources: No financial funding was received for this study.

Authors' contributions: S.P. planned the study and provided the data. H.F. and S.P. jointly wrote and revised the manuscript.

ACKNOWLEDGEMENTS

We highly appreciate the contributions and support by our collaborators in Basel, Switzerland, in Cameroon as well as in Jena, Germany, who generously shared the samples and provided the PCRs targeting *B. pseudomallei*, respectively, and thereby made this work possible.

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