

## SHORT REPORT

### *Balamuthia mandrillaris* therapeutic mud bath in Jamaica

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#### SUMMARY

*Balamuthia mandrillaris* is an emerging cause of encephalitis in humans. The transmission dynamics are poorly understood due to the high fatality rate and the sporadic nature of cases. Seventy-two soil samples were collected from beaches and the banks of lagoons, rivers, ponds, mineral springs and streams from across Jamaica and assayed for the presence of *B. mandrillaris*. Seventy-nine sites were sampled and the mitochondrial 16S rDNA gene of *B. mandrillaris* was amplified and sequenced to confirm the presence of the amoeba. One isolate of *B. mandrillaris* was recovered from soil from mineral spring which hosts an informal therapeutic mud bath business. Although *B. mandrillaris* is less frequently isolated from soil than other free-living amoebae, rubbing mud containing the organism onto the skin increases the likelihood of exposure and infection. This first report on the isolation of *B. mandrillaris* in the Caribbean and its presence in soil where human contact is likely warrants further investigation using serological methods to elucidate exposure patterns.

**Key words:** *Balamuthia mandrillaris*, *Balamuthia* amoebic encephalitis, Caribbean, Jamaica.

*Balamuthia mandrillaris* is a free-living amoeba which is the causative agent of *Balamuthia* amoebic encephalitis (BAE). The amoeba enters the host through the olfactory neuroepithelium or respiratory track in dust or soil, or through a break in the skin, after which it may enter the CNS by haematogenous spread [1–3]. *Balamuthia* can infect both immunocompromised and immunocompetent individuals and may cause disease of the lungs, skin and sinuses in mostly immunocompromised individuals [4, 5]. BAE presents

similarly to granulomatous amoebic encephalitis (GAE) which may be caused by either *Acanthamoeba* sp. or *B. mandrillaris* [3–6]. There are no specific symptoms for BAE and the disease is usually diagnosed post-mortem [1, 3–6].

The first report of the isolation of *B. mandrillaris* was from the brain tissue of a female mandrill in San Diego Wild Animal Park in 1986 [4]. Since then more than 200 human cases of BAE have been reported [2–5]. To date *B. mandrillaris* has been isolated from soil and dust from USA, Peru, Mexico, Iran, and Costa Rica [5, 7–10]. Recently, the amoeba was isolated from well water in Guinea-Bissau [11].

In this study, the presence of *B. mandrillaris* in Jamaica was surveyed to determine whether this

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pathogen is present in the environment in Jamaica using culture and specific PCR approaches.

Seventy-two soil samples were collected from popular sites used for swimming, bathing, boating or garment laundering from the 14 parishes of Jamaica (Fig. 1). These sites included beaches, and the banks of lagoons, rivers, ponds, mineral springs and streams. Fifty-millilitre sterile centrifuge tubes (Corning Inc., USA) were used to collect ~10 g soil from each site. Samples were inoculated onto 2% non-nutrient agar (NNA) plates seeded with heat-killed *E. coli* and incubated at room temperature (~30 °C) which were examined daily for the presence of *Balamuthia*-like amoebae for up to 2 weeks.

DNA was extracted by placing 1–2 ml of amoebic cultures directly into a Maxwell® 16 Tissue DNA Purification kit sample cartridge (Promega, Spain). Amoebic genomic DNA was purified using the Maxwell 16 instrument as described in the kit's technical manual (no. TM284) (Promega). DNA yield and purity were determined using the NanoDrop® 1000 spectrophotometer (Fisher Scientific, Spain) as described previously [8, 10].

For the molecular identification of the putative *B. mandrillaris* strain, the 16S rDNA gene was amplified and sequenced as described previously [8, 10]. PCR products were purified using the Qiaquick® PCR purification kit (Qiagen, Germany) and sequenced using a MEGABACE 1000 automatic sequencer (Healthcare Biosciences, Spain) in the University of La Laguna Sequencing Services (Servicio de Secuenciación SEGAI, University of La Laguna). The obtained sequences were aligned using the Mega 5.0 software program (mega.software.informer.com/5.0/) and were compared to sequences available in the GenBank database. The sequence for the new isolate was deposited in the Genbank database under the accession number KM083743.

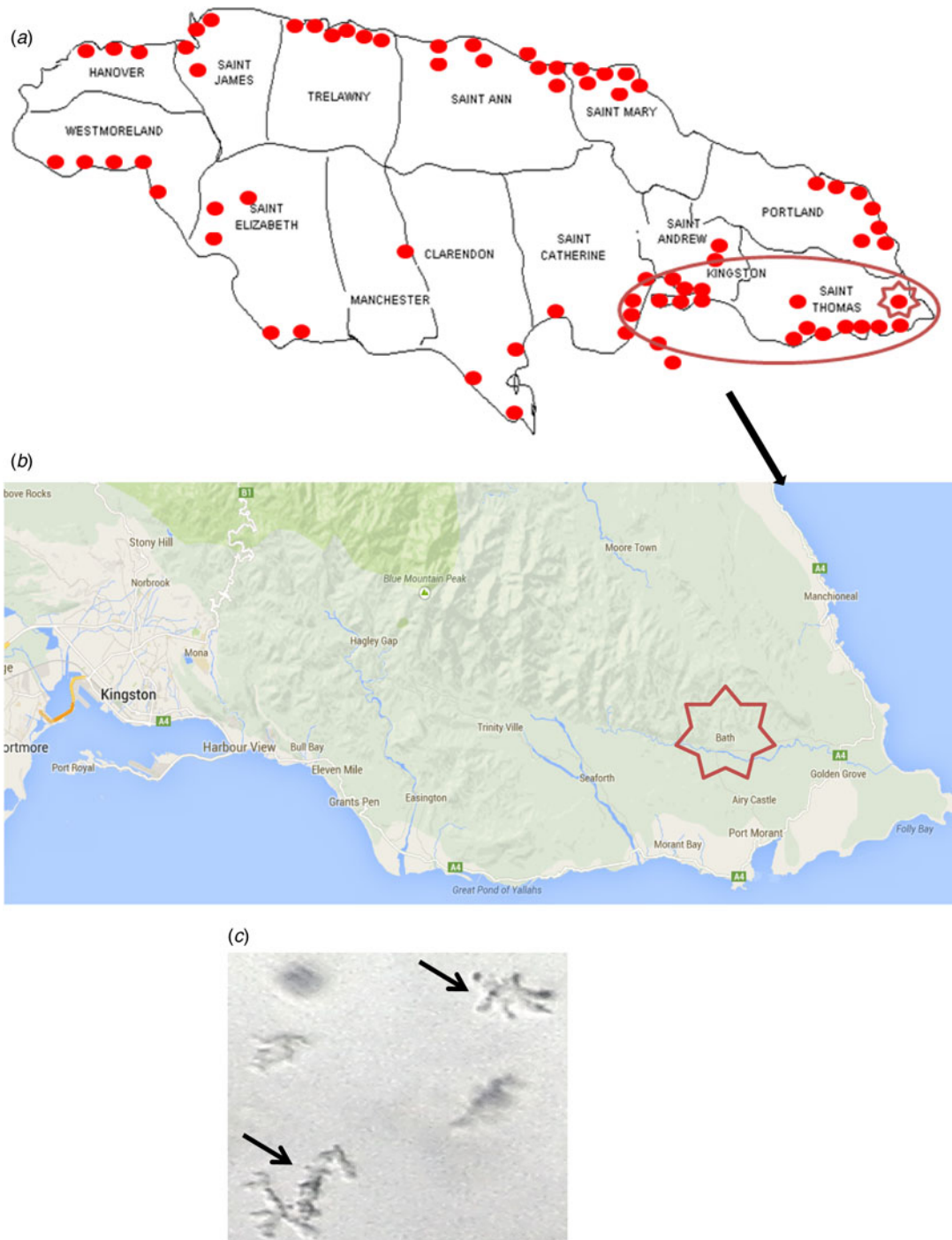
Seventy-two soil samples were assessed for the presence of *B. mandrillaris* (Fig. 1). The amoeba was isolated from a single soil sample collected from a mineral spring in the parish of St Thomas although not in the associated spring water. Furthermore, PCR of the 16S rDNA mitochondrial gene of *B. mandrillaris* confirmed the culture observation in this sample. The PCR product of this isolate showed sequence homology of 98% compared to the *B. mandrillaris* sequences available in Genbank. Unfortunately, axenicification of the isolate in 2% bactocastone or BM-3 medium was unsuccessful.

Although no free-living amoebae were isolated from the spring water of the established mineral bath, the presence of *B. mandrillaris* in the soil is a cause for concern. An informal mud bath business has developed outside the mineral bath which includes the application of mud from the area where *B. mandrillaris* was found to the skin for a few minutes which is then washed off with spring water. Some persons also consumed the water because they believe that it provided internal cleansing. These practices are potentially high risk for infection because they may provide direct entry portals for the amoebae through skin lesions.

*B. mandrillaris* has a >90% fatality rate in humans [3–5]. Most cases of BAE are reported from southwest USA and Latin America [2, 5]. An association was made between reported cases of BAE, sex and race, with most cases being reported in males and Hispanics [2, 5, 8]. Previous studies have reported that *B. mandrillaris* cases were linked to soil exposure and that Hispanics might be more exposed to the parasite because a large proportion of this population is employed in agriculture [2, 5]. Further, the soils from Southern California and Latin America are more likely to harbour the pathogen than other regions of the USA because they are usually warmer [12]. Whether these increased cases of amoebic encephalitis from the southwest USA and Latin America are due to environmental or genetic factors, remains unclear.

Diagnosis of BAE is usually made post-mortem because of the high fatality rates [2, 5]. The morphological features of *Acanthamoeba* and *Balamuthia* are similar when viewed in tissue sections; however, the latter can be differentiated if multiple nucleoli are present in the trophozoites [5]. Healthy human serum contains anti-*B. mandrillaris* antibodies and displays limited amoebicidal properties; preventing amoeba–host cell adherence and cytotoxicity [5, 12]. It is possible that the Jamaican population produces high levels of anti-*B. mandrillaris* antibodies and is therefore protected against infection due to the pathogen, which would for the lack of reported cases. However, BAE patients who display high antibody titres still succumb to their infection [3, 12].

According to Jackson *et al.* [13], exposure to *B. mandrillaris* in Arizona based on serological assays is much higher than the reported cases of BAE which remains a rare disease [5, 7, 13]. The presence of *B. mandrillaris* does not appear to be restricted to fertilized soil or specific soil types [7].



**Fig. 1.** Map of Jamaica showing (a) the 14 parishes and sampling points in this study (solid circles) and (b) the location (marked with a large star) of the sample site from which *Balamuthia mandrillaris* was isolated. (c) Trophozoites of *B. mandrillaris* in the sample from St Thomas parish (40×) (trophozoites are indicated by arrows).

The findings in this study are important because it is the seventh report on the environmental isolation of *B. mandrillaris* from the environment and the first report on the pathogen in the Caribbean. There are no reported cases of BAE from Jamaica or elsewhere in the Caribbean. This could be due to clinicians' lack

of awareness of the disease, misdiagnosis of cases or environmental, behavioural or genetic factors which limit transmission to humans. However, the finding of the organism in soil which is rubbed onto the skin presents a unique opportunity to study possible exposure of humans. Serological studies to determine

antibody prevalence in mud-bath workers and clients and carefully chosen controls would add value to our understanding of this cause of an important emerging disease.

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### DECLARATION OF INTEREST

None.

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