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SHORT COMMUNICATION

Isolation of *Balamuthia mandrillaris* from urban dust, free of known infectious involvement

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Abstract The free-living amoeba *Balamuthia mandrillaris* can cause fatal encephalitis in humans and other mammals. The organism is associated with soils, and soil exposure has been identified as a risk factor for this pathogen. However, *B. mandrillaris* has been isolated only once from soils believed to be the source of the infection in child from California, USA who died of *Balamuthia* amoebic encephalitis and once from another unrelated soil source. We report for a third time the isolation of *B. mandrillaris* from the environment and for the second time its isolation from a sample not known to be involved with pathogenicity. We have established the new clonal *B. mandrillaris* strain (ID-19) in axenic media. The identity of our isolate was originally by morphology using a light microscope and this has been confirmed by 16S rRNA gene PCR. The new strain ID-19 groups with others of the species. The fact that our isolate came from dust particles deposited on surfaces from the air in an urban environment may suggest that it is not just soil exposure that constitutes a risk factor for *Balamuthia* infection. This is the first report of this organism from Iran.

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Introduction

Free-living amoebae from a number of genera are known to be pathogenic in animals and humans. One of these, *Balamuthia mandrillaris*, is a pathogen whose importance is becoming ever more apparent (Maciver 2007; CDC Centers for Disease Control and Prevention 2008; Matin et al. 2006; Schuster et al. 2009). Since its first discovery in 1990 (Visvesvara et al. 1990), about 150 cases have been reported (CDC Centers for Disease Control and Prevention 2008). *Balamuthia* amebic encephalitis due to *B. mandrillaris* (BAE) has most often been diagnosed postmortem as the disease is not well known and the symptoms are variable; however, some patients have been successfully treated (Schuster et al. 2009). *B. mandrillaris* infects both the immunocompromised and apparently immunocompetent individuals and seems to be present in the warmer countries (Schuster et al. 2009) with the exception of the previously reported case of BAE in the Czech Republic (Kodet et al. 1998). More male BAE victims than females have been discovered, and there seems to be a tendency for Hispanic people to be more at risk (Schuster et al. 2009). Exposure to soils in these warmer countries seems to be a risk factor (Schuster et al. 2009), but the organism has only once been isolated from soils believed to be the source of the infection (Schuster et al. 2003) and once from another unrelated soil source (Dunnebacke et al. 2003).

We report for the third time isolation of *B. mandrillaris* from the environment and for the second time isolation

from a sample not known to be involved with pathogenicity. The fact that our isolate came from dust in an urban environment may suggest that it is not just soil exposure that constitutes a risk factor for *Balamuthia* infection.

Results

The amoeba was isolated from a dust sample that was collected in a large public building in the city of Tehran in Iran. The sample was collected with a sterile cotton pad, which was subsequently streaked out on *Escherichia coli*-coated, non-nutrient agar plates. The plates were then sealed, incubated at room temperature and checked intermittently for the presence of free-living amoebae. *Acanthamoeba*, vahlkampfids and *Thecamoeba* were observed after a few days, but also an amoeba that we identified microscopically as *Balamuthia* appeared later. As the plates developed, we observed that *Acanthamoeba* and vahlkampfids were being preyed upon by both *Thecamoeba* and *Balamuthia*. However, it was also apparent that the *Balamuthia* were also being phagocytosed by the larger *Thecamoeba*. In order to rescue the *Balamuthia* strain, we removed it from the primary plate and cloned it from *Balamuthia* cysts. This clone was then introduced to axenic culture (RPMI 1640 with 2 μg/ml gentamicin and 10% foetal calf serum). This new *Balamuthia* strain ID-19 took to this media readily.

We confirmed our identification of strain ID-19 as being *Balamuthia* by PCR based on primers of 16S rRNA gene (Booton et al. 2003; 5′ Balspec16S 5′-CGCATGTATGAA GAAGACCA-3′ and 3′ Balspec16S 5′-TTACCTATA TAATTGTCGATACCA-3′). PCR was carried out (using a positive control of a reference *B. mandrillaris* strain from Dr Naveed Khan), our isolate (ID-19) was positive for PCR and sequencing of the product revealed high homology (93–96%) with CDC *B. mandrillaris* strains from Genbank confirming the identification of the amoeba (Fig. 1a). Microscopic observation (Fig. 1b) showed that strain ID-19 was similar in appearance to other *B. mandrillaris* and had typical polyaxial morphology. The cysts (Fig. 1c) also presented the typical appearance with a loose outer wall. Strain ID-19 was observed to move rapidly in culture compared to the reference strain but it is possible that this is due to the fact that the reference strain has been in axenic culture longer.

Discussion

The appearance of *Balamuthia* on plates swabbed with sample material after the other amoebae which served as food organisms had emerged is in agreement with previous reports of *Balamuthia* isolation (Schuster et al. 2003; Dunnebacke et al. 2003). Unlike most other genera of free-living amoebae, *Balamuthia* does not seem to consume bacteria (Visvesvara et al. 1990; Matin et al. 2006); instead, *Balamuthia* eats other amoeba. Our study is the second to isolate *Balamuthia* from samples which had not previously been suspected to be the source of a *Balamuthia* infection. However, our strain groups within *B. mandrillaris* strains known to be pathogenic, and so it too is likely to be a human pathogen. The genus *Acanthamoeba* contains pathogenic strains especially within the T4 subgroup but not all T4s are pathogenic despite the similarity of the SSU rDNA gene. It is a formal possibility that like *Acanthamoeba*, not all *Balamuthia* are pathogenic; this would explain why most individuals have a measureable titre of anti-*Balamuthia* antibodies (Schuster et al. 2002) without infection despite the apparent high infective potential of this organism even in immunocompetent individuals. The existence of non-pathogenic *Balamuthia* in the environment may have ramification especially if abundant, as contact with non-pathogenic strains may afford an element of immunity to pathogenic strains.

Finding *Balamuthia* in Tehran, Iran is not unexpected as this organism has been found throughout the world, most often in hotter countries. So far it has not been recorded in temperate regions with the exception of a reported case of

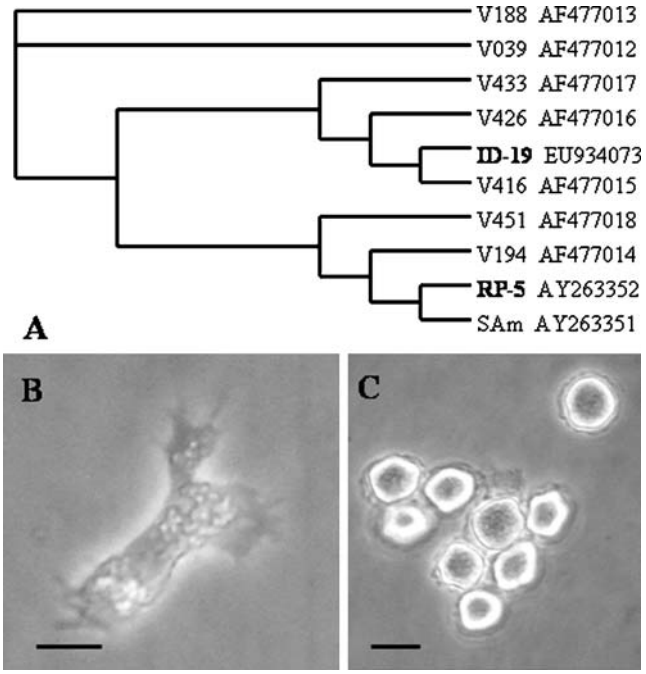

Fig. 1 **a** Relationship between the characterised *Balamuthia* strains for which sequence data are known. The new sequence data for ID-19 have been deposited in GenBank as EU934073. Alignment of sequences and tree construction was performed using Clustal X and TreeView software, and similar results were obtained using MEGA. **b** Phase contrast microscopy of *Balamuthia* ID-19 trophozoite and **c** cysts (*bar* is 10 μm in both **b** and **c**)

BAE in the Czech Republic (Kodet et al. 1998). A patient in New York state found to be infected with a *Balamuthia* infection had returned from visits to Texas and Arizona where there was reason to presume that she was infected (Jung et al. 2004). In addition, the single reported UK case was from a patient who had recently arrived from Bolivia (Jayasekera et al. 2004).

The fact that we have isolated *B. mandrillaris* from the environment free from any known infection concurs with the suggestion that this organism is free living. *Balamuthia* has previously been isolated on two occasions from soil, and infection has often been associated with exposure to soils and this is assumed to be a risk factor (Jung et al. 2004; Maciver 2007). However, we have isolated *Balamuthia* from dust in an urban setting, suggesting that the potential infection risk is not limited to soil. The *Balamuthia* cyst is known to be particularly resistant (Matin et al. 2008; Siddiqui et al. 2008), and it is assumed that our strain was initially present within the dust sample as a cyst. It is not yet know if the cyst stage is directly capable of causing human infection.

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