Free-living Amoebae Recovered from Human Stool in *Strongyloides* Agar Culture

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To the editor,

Our laboratory in The Gambia, West Africa, performs Koga agar culture (1.5% bacteriological agar, 0.5% sodium chloride, 0.5% meat extract, 0.1% bacteriological peptone) for larvae of strongyle nematodes on human faeces samples for which parasitological investigation has been requested. We have recovered FLA from human faecal specimens on two occasions over a period of nine months (representing 130 individual faecal cultures) on this agar. The amoebae in both cases were identified as Hartmannella species based upon the morphology of trophozoites and cysts in agar culture (figures 1,2) and their incapacity to enflagellate in distilled water after eight hours incubation at 37°C. No other types of free-living amoebae (FLA) have been recovered. Due to resource constraints, sequencing of the partial 18S rRNA gene and ITS region could not be performed and this identification must remain presumptive. Specimens were collected into sterile containers and Koga culture plates are sterile and sealed with parafilm prior to incubation at 30°C for five days and therefore environmental contamination with FLA was very unlikely. Repeat specimens could not be obtained from subjects, and so it was not possible to determine if these findings represent transient passage of FLA or true intestinal colonization.

It has been postulated that FLA provide a vehicle for bacterial pathogens to gain entry into the human respiratory tract (Khan 2006). That free-living amoeba may have a similar role in the introduction of bacterial pathogens into the human intestinal tract (and particularly defence against stomach acid during transit to the duodenum) should be further considered given these findings. FLA are potential environmental reservoirs of several important pathogens of the human intestinal tract, including Salmonella spp., Shigella spp., Campylobacter spp. and enterohemorrhagic Escherichia coli (Wildschutte 2007; Bui 2012; Jeong 2007; Chekabab 2012). Specifically, due to the large infective dose of Vibrio cholerae...
(10^3-10^9 cells), it has been suggested that Acanthamoebae may act as both an environmental host and intracellular multiplier of this organism, allowing it to grow sufficiently to be able to cause human infection (Abd 2011).

Human respiratory (Madrigal 1989; Rivera 1984) and urinary tract (Santos 2009) colonization with *Acanthamoeba* spp. may occur. Recovery of FLA, including *Hartmannella* spp. from the intestinal tract of rodents (Kedraon 1986), reptiles (Hassl 2000), fish and birds (Franke 1982) has been previously reported. Only one previous reference in the literature describes the recovery of FLA from the human intestinal tract (de Moura 1985).

The potential for transient passage or true colonization of human the human intestinal tract with FLA may also provide a novel potential portal of entry into the human body for FLA causing primary amoebic meningitis (PAM) and granulomatous amoebic encephalitis (GAE). These findings may also require a review of safety practices when dealing with such cultures, including the use of biosafety cabinets when manipulating Koga agar cultures to avoid cultures potentially containing *Naegleria fowleri* being inappropriately manipulated on a bench top in the laboratory.

In summary, we report the recovery of FLA in Koga agar culture of faeces from two separate individuals in West Africa. It is unknown if this represents transient passage or true intestinal colonization. These findings demonstrate an important novel mechanism of entry for bacterial intestinal pathogens and possibly pathogenic FLA themselves into the human body. They also raise the need for awareness of the potential for FLA to be recovered in Koga agar culture, in order to ensure the health and safety of laboratory staff performing such cultures.
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References


Figure 1: Tracks of presumptive *Hartmannella* species on the surface of Koga agar culture of human faeces after five days incubation at 30°C.
Figure 2: Characteristic morphology of presumptive Hartmannella cysts and trophozoites on agar culture.