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Viable *Blastocystis* Cysts in Scottish and Malaysian Sewage Samples

K. Suresh,[1,2]* H. V. Smith,[1] and T. C. Tan[2]

Scottish Parasite Diagnostic Laboratory, Stobhill Hospital, Glasgow G21 3UW, Scotland,[1] and Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia[2]

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***Blastocystis* cysts were detected in 38% (47/123) (37 Scottish, 17 Malaysian) of sewage treatment works. Fifty percent of influents (29% Scottish, 76% Malaysian) and 28% of effluents (9% Scottish, 60% Malaysian) contained viable cysts. Viable cysts, discharged in effluent, provide further evidence for the potential for waterborne transmission of *Blastocystis*.**

Over the last decade, public health interest in the human intestinal protozoan parasite *Blastocystis hominis* has increased (17) as further evidence of its pathogenicity is identified (1, 3, 6, 20). *Blastocystis* is a polymorphic parasite and can present, under various conditions, a perplexing array of morphologies, including vacuolar, multivacuolar, avacuolar, granular, amoeboid, and cystic forms (14, 19). Only in the last decade has a detailed description of the small (6.65 to 12.65 μm) transmissive cystic stage been published (12, 23), and although avacuolar forms in recently voided feces have only been reported twice in the literature (13, 24), they are thought to be the form of *B. hominis* found in the human intestine (13). This variability of forms found in stools can also account for the unpredictability of detection in clinical parasitology laboratories.

Transmission is by the fecal-oral route, and evidence for experimental anthropozoonotic transmission using *B. hominis* cysts is available for a variety of nonhuman recipients (e.g., Wistar rats [15], mice [5], and gnotobiotic guinea pigs [9]). The size range of *B. hominis* cysts (12.65 μm, with their outer membranous coat; 6.65 μm, without their outer membranous coat [23]) is within the size range of both *Cryptosporidium* oocysts and *Giardia* cysts, which are etiological agents of waterborne disease. The description of two suspected waterborne outbreaks of blastocystosis (7, 8) together with the chlorine insensitivity of *B. hominis* cysts implicate chlorinated drinking water as a potentially significant transmission route (21).

Wastewater reuse is associated with parasitological health risks. Habbari et al. (4) demonstrated that children living in wastewater reuse regions of Morocco had an increased risk of parasite infections. One or more parasite infections were identified in 50.8% of children living in the wastewater reuse regions compared with only 8.2% of children living in regions with no wastewater reuse (n = 1,343 children). As for the enteric protozoan pathogens, *Cryptosporidium* and *Giardia*, the potential for environmental contamination with *Blastocystis* depends upon a variety of factors, including the number of infected human and nonhuman hosts, seasonal influences and duration of infection, the number of transmissive stages excreted, agricultural practices, host behavior and activity, socioeconomic and ethnic differences in human behavior, geographic distribution, sanitation, and climate and hydrogeology of the area (11).

An insight into the potential for contaminating surface waters used for abstraction of potable water can be provided by determining the presence of viable *B. hominis* cysts in sewage effluent. Apart from a report of the isolation of *Blastocystis* from sewage (22), no further studies have investigated its presence and viability in sewage.

The increasing reports of human blastocystosis and the lack of information on its waterborne occurrence together with our knowledge of the role that sewage effluent acts as a contributor to waterborne *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts (2) indicate that the original finding of Zaman and Khan (22) should be confirmed. Furthermore, the occurrence of viable *Blastocystis* cysts in sewage treatment plants should be established so that the potential significance of the waterborne route of transmission can be determined.

Here, we analyzed sewage samples from Scotland, United Kingdom, and Kuala Lumpur, Malaysia, for the presence of *Blastocystis* cysts. A total of 73 1-liter samples from 37 Scottish sewage treatment plants (31 influent and 42 effluent samples) and 50 samples from 17 sewage treatment plants around Kuala Lumpur, Malaysia (25 influent and effluent samples, respectively), were collected. Influent and effluent samples were collected separately from different sewage treatment plants. The sewage samples were centrifuged (1,800 × *g*, 15 min at room temperature) and reduced in volume to ~40 ml, ensuring that pellets were always retained. Each sample was further centrifuged (as above), the supernatant was discarded, and the pellet was resuspended to 100 μl, of which 10 μl of resuspended pellet was placed on a glass microscope slide to which a coverslip was added, and the wet film was examined by light microscopy at ×400 magnification for *Blastocystis* life cycle stages (vacuolar, multivacuolar, avacuolar, granular, amoeboid, and cystic). The remainder of the resuspended pellet (90 μl) was cultured in vitro to augment viable forms. The recovery efficiency of this method for concentrating *Blastocystis* cysts was 12.1 ± 2.9% (n = 3) when 1-liter sewage effluent samples were seeded with 10,000 cysts.

For in vitro culture, 30 μl of each resuspended pellet was dispensed into a sterile bijou bottle containing 3 ml of Jones' medium (16, 18, 22), incubated for 48 h at 37°C, and examined for the presence of various morphological forms of *Blastocystis*

* Corresponding author. Mailing address: Scottish Parasite Diagnostic Laboratory, Stobhill Hospital, 133 Balornock Road, Glasgow G21 3UW, Scotland. Phone: 44 (0)141 201 3028. Fax: 44 (0)141 201 3029. E-mail: huw.smith@northglasgow.scot.nhs.uk.

under the light microscope at ×400. Three replicates were performed for each sewage sample, and samples were regarded as positive when *Blastocystis* was observed in any of the three replicate cultures. The majority of forms seen were vacuolar and increased in density when reexamined after 5 days of incubation. Culture tubes negative for *Blastocystis* after 48 h remained negative at 5 days.

No morphological forms of *Blastocystis* were seen following direct microscopy of wet films prior to in vitro culture. In Scottish sewage samples cultured in vitro, 29% (9/31) and 9.2% (4/42) of influent and effluent samples, respectively, were positive for *Blastocystis*, while 76% (19/25) and 60% (15/25) of in vitro-cultured influent and effluent samples, respectively, were positive in the Malaysian sewage samples. Because influent and effluent samples were unmatched and collected separately from different sewage treatment plants, both in Scotland and in Malaysia, we are unable to determine the removal efficiencies of the sewage treatment process for *Blastocystis* cysts. However, being similar in size to *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts, we would extrapolate from our previous work that the removal efficiencies of primary and secondary sewage treatments for *Blastocystis* cysts should range between 15 and 99% (10).

Zaman and Khan (22) stated that all four sewage influent and effluent samples in Pakistan were positive for *Blastocystis*, indicating that treatment does not completely remove *Blastocystis* cysts. In the present study, 9% of Scottish and 60% of Malaysian sewage effluent samples were *Blastocystis* positive, indicating that the sewage treatment works investigated neither completely remove cysts nor render the cysts discharged in effluent nonviable. Our data confirm the robustness of *Blastocystis* cysts to wastewater treatment processes.

Human contributors to the pool of *Blastocystis* cysts in sewage have been reported in both Scotland and Kula Lumpur. *Blastocystis* prevalence in humans with gastrointestinal symptoms is 3.9% in Scotland and 5.6% in Kuala Lumpur, Malaysia (18, 20). In the Scottish cases, 20.5% excreted cysts (18).

In vitro culture is useful for detecting *Blastocystis* in human stool samples and increases the percent positivity rate compared to direct microscopy (16, 18, 22), as viable organisms increase exponentially in culture medium, making them more easily identifiable by bright-field microscopy. The ability to culture *Blastocystis* cysts from sewage concentrates and to augment their numbers within 48 h to detectable levels confirms that this in vitro culture technique is also a useful adjunct for environmental samples. This is the first report of culturing viable *Blastocystis* from Scottish and Malaysian sewage samples. The study was designed to include sewage samples from geographically disparate and temperate and tropical countries using the same detection methods. It establishes that *Blastocystis* cysts can be found readily in sewage samples and that sewage treatment plants in Scotland and Malaysia are ineffective in removing or killing all *Blastocystis* cysts.

These findings have important implications and provide further evidence for the potential for waterborne transmission of *Blastocystis*, given the increasing reports of the parasite's pathogenicity. To determine the levels of contamination and the potential routes of transmission, *Blastocystis* should be included as a further parameter when investigating parasites in wastewater and other environmental samples.

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