



Proceeding Paper

# Human Pathogenic Free-Living Amoebas in Faeces from Cows and Pigs from Bombali and Tonkolili Districts, Sierra Leone <sup>†</sup>

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<sup>†</sup> Presented at the 2nd International Electronic Conference on Microbiology, 1–15 December 2023; Available online: <https://ecm2023.sciforum.net>.

**Abstract:** Human pathogenic free-living amoebae (FLA), specifically *Acanthamoeba* spp., *Balamuthia mandrillaris* and *Naegleria fowleri*, are rarely studied in animals' gastrointestinal (GI) tract or their faeces as they do not have an obligate parasitic life cycle. However, FLA from different taxa have been recently recovered and identified in pigs' GI tract and their faeces. The presence of these FLA species was studied in faeces from cows and pigs monitored across Bombali and Tonkolili Districts, Sierra Leone (West Africa). Fresh faecal samples were aseptically collected, either from recent deposition or during defecation, from 12 pigs and 8 cows in Spring 2019. Fourteen samples were collected from five locations across Makeni city (Bombali District): animal market (5 cows, 1 pig), general and pig slaughterhouses (5 pigs), Lorrey Park (2 cows) and Comforti (1 cow). Additionally, 6 pigs were monitored in Royanka, within the Tonkolili District. Samples were processed by a triplex real-time TaqMan PCR assay after extracting DNA from pre-concentrated faecal samples using FastDNA<sup>®</sup> Spin Kit. Although all the faecal samples screened were negative, our results should be considered as inconclusive, owing to the limited number of animals and specific FLA species monitored. Moreover, we detected *Acanthamoeba* spp. in water reservoirs (wells and ponds) used for drinking by those animals from which samples were collected/screened. We also detected *B. mandrillaris* in the river in Royanka, which would confirm the presence of this emerging FLA in Tonkolili District, being the first time reported in the literature. Further monitoring studies would be required to understand the presence/circulation of these opportunistic FLA species in farm animals across these Sierra Leonean districts, to control the presence of foodborne pathogens.

**Keywords:** Bombali District; Tonkolili District; Sierra Leone; free-living amoebas; *Acanthamoeba* spp.; animal faeces

**Citation:** Peña-Fernández, A.; Wadoum, R.E.G.; Anjum, U. Human pathogenic Free-Living Amoebas in Faeces from Cows and Pigs from Bombali and Tonkolili Districts, Sierra Leone. *Biol. Life Sci. Forum* **2023**, *31*, x. <https://doi.org/10.3390/xxxxx>

Academic Editor(s): Name

Published: date



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## 1. Introduction

Free-living amoebae are a heterogeneous group of protozoa ubiquitously found in nature, which comprise different genera. However, some taxa can be opportunistic pathogens, with *Acanthamoeba* spp., *Naegleria fowleri*, and *Balamuthia mandrillaris* the most common free-living amoebae related with infections in different animals including humans [1]. Free-living amoebae present at least two developmental stages, a vegetative feeding form known as trophozoite, and an environmental resistant form known as cyst, which provides protection to harsh conditions, such as changes in temperature, pH or against biocides and other decontaminating substances [1,2]. These three human pathogenic free-living amoebae species (FLA) can act as opportunistic parasites on a wide range of

vertebrates, representing a serious risk to human health as they also can harbour endosymbionts, such as viruses, bacteria, yeasts and other protists, which could be also human pathogenic [1].

FLA have been found in different drinking, private/recreational and open water systems, including tap water, swimming pools, different fresh and salt water environments, and in different soil habitats globally [2,3]. Nevertheless, these human-pathogenic FLA species are rarely studied in animals' gastrointestinal (GI) tract or their faeces, as they do not have an obligate parasitic life cycle. However, FLA from different taxa have been recently recovered and identified in pigs' GI tract and their faeces, suggesting a potential transmission source for pathogenic FLA and their associated endosymbionts [4]. Moreover, although limited, there are a few works in the literature reporting the presence of *Acanthamoeba* in human faeces, which has been associated with low hygiene standards. Thus, meanwhile Mergerya [5] has reported a prevalence of 0.4% in German individuals, Moura et al. [6] has found a prevalence of 11.2% in faeces from individuals living in Brazil, and Zaman et al. [7] a prevalence of 10% in subjects from Karachi, Pakistan. Independently that these FLA were just passing through the GI or they were colonising the human intestinal tract, these works might suggest a novel potential via of entry into the human body [8], and/or a risks for these individuals due to their potential content of human-pathogenic endosymbionts.

The main aim was to determine the presence of these three specific FLA species in faeces from cows and pigs monitored across Bombali and Tonkolili Districts, Sierra Leone (SL, West Africa). SL supports high mortality and morbidity rates, which are the highest in the world for maternal and infant groups [9], and present high numbers of immunocompromised individuals including Ebola survivors and people living with HIV (human immunodeficiency virus) [10].

## 2. Material and Methods

No ethical approval was required for the described study. Endangered or protected species were not included in this work. Faecal samples were obtained after authorisation of the animal owners and the respective community leader as described in Peña-Fernández et al. [10]. No animals were harmed in the acquisition of faecal samples.

Fresh faecal samples were aseptically collected, either from recent deposition or during defecation, from 12 pigs and 8 cows in Spring 2019. Samples were individually packed in plastic containers and immediately stored in a  $-80^{\circ}\text{C}$  freezer. Fourteen samples were collected from five locations across Makeni city (situated in the Bombali District): animal market (5 cows, 1 pig), general and pig slaughterhouses (5 pigs), Lorrey Park (2 cows) and Comforti (1 cow). Additionally, 6 pigs were monitored in Royanka, within the Tonkolili District.

Faecal samples were washed and pre-concentrated with PBS-EDTA and centrifuged at 2500 rpm for 15 min to remove inhibitors. DNA was extracted from appropriately pre-concentrated faecal samples using FastDNA<sup>®</sup> Spin Kit (MP Biomedicals, Solon, OH, USA), following the manufacturer protocol with some modifications as described in Gomes et al. [1]. Briefly, an additional 1/4 inch ceramic sphere was added in each Fastprep tube and the lysing cycles were performed in triplicate in an homogeniser FastPrep-24<sup>™</sup> 5G (MP Biomedicals, Solon, OH, USA). DNA materials were purified with NucleoSpin<sup>®</sup> Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany), following the manufacturers' instructions. Extracts were screened for FLA using a triplex real-time TaqMan PCR assay which simultaneously identifies these three human pathogenic amoebae, as described previously by Qvarnstrom et al. [11]. Positive controls were used for each amoeba, as follows. Validated positive control for *Acanthamoeba* spp. and *B. mandrillaris* were kindly provided by the Laboratory of Parasitology at the University of San Pablo CEU, as previously used by Magnet et al. [12]. *Naegleria fowleri* Carter (ATCC<sup>®</sup> 30174<sup>™</sup>, isolated from spinal fluid; <https://www.atcc.org/products/30174>) was used as positive control for this species.

### 3. Results and Discussion

All the faecal samples screened were negative. However, our results should be considered as inconclusive, owing to the limited number of animals and specific FLA species monitored. Thus, other similar studies report the presence of *Acanthamoeba* in cow and pig faeces [4]. Niyyati et al. [13] has detected the presence of genotype T4 *Acanthamoeba* in cow faeces from Teheran city, described as the predominant genotype responsible for *Acanthamoeba* infections in humans in the literature [14]. Moreover, *Acanthamoeba* may have veterinary significance as has been reported in diseased or dead cows, pigs and other farm animals previously [15]. The investigated FLA species have been identified in different animals, including domestic and wild animals. Moreover, *Acanthamoeba* and *Naegleria* have been isolated from the GI tract, faeces and brains of reptiles, which could represent a risk when kept as domestic pets [16].

Moreover, we detected *Acanthamoeba* spp. in water reservoirs (wells and ponds) used for drinking by those animals from which samples were collected/screened in Sierra Leone—these initial results have been presented at the XI Congress of the Spanish Society of Tropical Medicine and International Health (SEM-TSI) in 2019 [17]. We also detected *B. mandrillaris* in the river in Royanka (*data not published yet*), which would confirm the presence of this emerging FLA in Tonkolili District. To the best of our knowledge, this is the first time that the presence of this emerging opportunistic FLA in being reported in the literature. The presence of FLA in the monitored water environments may pose a risk within susceptible people, highlighting the need of performing a largest monitoring study in farm animals in Sierra Leone to protect immunocompromised individuals including Ebola survivors.

In addition, the pre-concentration technique used might have not facilitated the detection of these FLA species, especially if they have a very low presence, as other recovery methods (e.g., filtration, sedimentation) have been described as more appropriate for recovering and culturing free-living protozoa from porcine faeces [4]. Further monitoring studies, which also include non-pathogenic FLA taxa, would be required to understand the presence/circulation of these pathogenic/opportunistic species, particularly *Acanthamoeba* spp., in farm animals across these Sierra Leonean districts, to control the presence of foodborne pathogens.

### 4. Conclusions

We have not detected the presence of FLA in any of the fresh faecal samples collected from cows and pigs from Bombali and Tonkolili Districts. Contrarily, and to the best of our knowledge, this is the first report reporting the presence of *Acanthamoeba* spp. in wells and ponds that farm animals use to drink water in farms from Bombali District in Sierra Leone; and the first time that *B. mandrillaris* has been isolated in a river in the Tonkolili District (SL). A better understanding of the potential interaction and medical significance of the presence of FLA in mammals' intestinal tract, particularly for *Acanthamoeba* spp., would be needed to tackle their opportunistic infections and prevent and minimise human exposure. Finally, genotyping of the FLA species identified in the different water environments monitored in these Sierra Leonean districts would be needed to establish appropriate interventions to prevent their infections.

**Author Contributions:** Conceptualization, A.P.-F.; methodology, A.P.-F., R.E.G.W. and U.A.; validation, A.P.-F. and U.A.; formal analysis, A.P.-F., R.E.G.W. and U.A.; investigation, A.P.-F., R.E.G.W. and U.A.; resources, A.P.-F.; data curation, A.P.-F. and U.A.; writing—original draft preparation, A.P.-F.; writing—review and editing, A.P.-F., R.E.G.W. and U.A.; visualization, A.P.-F., R.E.G.W. and U.A.; supervision, A.P.-F.; project administration, A.P.-F.; internal funding acquisition, A.P.-F. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research has been funded by the DMU (De Montfort University, England) Quality Research Global Challenges Research Fund (QR GCRF) 2018-19, awarded to Dr Peña-Fernández.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Faecal samples were obtained after authorisation of the animal owners and the respective community leader.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author. The data are not publicly available due to further processing for a future submission as a manuscript.

**Acknowledgments:** This project has been also partially supported with other internal funds from Leicester School of Allied Health Sciences, De Montfort University.

**Conflicts of Interest:** The authors declare no conflict of interest.

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