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Proceeding Paper

Human Pathogenic Free-Living Amoebas in Faeces from Cows and Pigs from Bombali and Tonkolili Districts, Sierra Leone †

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Abstract: Human pathogenic free-living amoebae (FLA), specifically *Acanthamoeba* spp., *Balamuthia mandrillaris* and *Naegleria fowleri*, are rarely studied in animals' gastrointestinal (GI) tracts 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 tracts 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 eight cows in Spring 2019. Fourteen samples were collected from five locations across Makeni city (Bombali District): an animal market (five cows, one pig), general and pig slaughterhouses (five pigs), Lorrey Park (two cows) and Comforti (one cow). Additionally, six 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 a FastDNA® 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, with this being the first time it has been 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

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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* being the most common free-living amoebae related to infections in different animals, including humans [1]. Free-living amoebae present at least two developmental stages: a vegetative feeding form known as a trophozoite and an environmentally resistant form known as a cyst, which provides protection to harsh conditions, such as changes in temperature, pH, and against biocides and other decontaminating substances [1,2]. These three human pathogenic free-living amoebae (FLA) species 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 pathogenic to humans [1].

FLA have been found in different drinking, private/recreational and open water systems, including tap water, swimming pools, different fresh and saltwater environments, and in different soil habitats globally [2,3]. Nevertheless, these human-pathogenic FLA species are rarely studied in animals' gastrointestinal (GI) tracts 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 tracts 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, while Mergerya [5] reported a prevalence of 0.4% in German individuals, Moura et al. [6] found a prevalence of 11.2% in faeces from individuals living in Brazil, and Zaman et al. [7] found a prevalence of 10% in subjects from Karachi, Pakistan. Independent of whether these FLA were just passing through the GI or they were colonising the human intestinal tract, these works might suggest a novel potential method of entry into the human body [8] as well as the risks for these individuals due to their potential carrying 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 has high mortality and morbidity rates, which are the highest in the world for maternal and infant groups [9], and presents 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 twelve pigs and eight cows in Spring 2019. Samples were individually packed in plastic containers and immediately stored in a −80 °C freezer. Fourteen samples were collected from five locations across Makeni city (situated in Bombali District): an animal market (five cows, one pig), general and pig slaughterhouses (five pigs), Lorrey Park (two cows) and Comforti (one cow). Additionally, six pigs were monitored in Royanka, which is within 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 a FastDNA® 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 a homogeniser FastPrep-24™ 5G (MP Biomedicals, Solon, OH, USA). DNA materials were purified with NucleoSpin® Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany), following the manufacturers' instructions. The 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 controls 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. [3]. *Naegleria fowleri* Carter (ATCC® 30174™, isolated from spinal fluid; American Type Culture Collection (ATCC), Manassas, VA, USA https://www.atcc.org/products/30174 [accessed on 1 February 2024]) was used as the 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. [12] 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 [13]. Moreover, *Acanthamoeba* may have veterinary significance as has been reported in diseased or dead cows, pigs and other farm animals previously [14]. 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 [15].

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 (SEMTSI) in 2019 [16]. To the best of our knowledge, this is the first time that the presence of this emerging opportunistic FLA is being reported in the literature. The presence of FLA in the monitored water environments may pose a risk for susceptible people, highlighting the need to perform a large-scale monitoring study on farm animals in Sierra Leone to protect immunocompromised individuals, including Ebola survivors.

In addition, the pre-concentration technique used might not have facilitated the detection of these FLA species, especially if they have a very low presence, as other recovery methods (e.g., filtration and 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 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, the 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.

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

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Gomes, T.S.; Vaccaro, L.; Magnet, A.; Izquierdo, F.; Ollero, D.; Martinez-Fernandez, C.; Mayo, L.; Moran, M.; Pozuelo, M.J.; Fenoy, S.; et al. Presence and interaction of free-living amoebae and amoeba-resisting bacteria in water from drinking water treatment plants. *Sci. Total Environ.* **2020**, *719*, 137080. [CrossRef] [PubMed]
2. Angelici, M.C.; Walochnik, J.; Calderaro, A.; Saxinger, L.; Dacks, J.B. Free-living amoebae and other neglected protistan pathogens: Health emergency signals? *Eur. J. Protistol.* **2021**, *77*, 125760. [CrossRef]
3. Magnet, A.; Fenoy, S.; Galván, A.L.; Izquierdo, F.; Rueda, C.; Vadillo, C.F.; Del Aguila, C. A year long study of the presence of free living amoeba in Spain. *Water Res.* **2013**, *47*, 6966–6972. [CrossRef] [PubMed]
4. Chavatte, N.; Lambrecht, E.; Van Damme, I.; Sabbe, K.; Houf, K. Free-living protozoa in the gastrointestinal tract and feces of pigs: Exploration of an unknown world and towards a protocol for the recovery of free-living protozoa. *Vet. Parasitol.* **2016**, *225*, 91–98. [CrossRef] [PubMed]
5. Mergeryan, H. The prevalence of *Acanthamoeba* in the human environment. *Rev. Infect. Dis.* **1991**, *13* (Suppl. 5), S390–S391. [CrossRef] [PubMed]
6. Moura, H.D.; Salazar, H.C.; Fernandes, O.; Lisboa, D.C.; Carvalho, F.G.D. Amebas de vida livre no intestino humano: Evidências de parasitismo. *Rev. Do Inst. De Med. Trop. De São Paulo* **1985**, *27*, 150–156. [CrossRef] [PubMed]
7. Zaman, V.; Zaki, M.; Manzoor, M. *Acanthamoeba* in human faeces from Karachi. *Ann. Trop. Med. Parasitol.* **1999**, *93*, 189–191. [CrossRef] [PubMed]
8. Bradbury, R.S. Free-living amoebae recovered from human stool samples in Strongyloides agar culture. *J. Clin. Microbiol.* **2014**, *52*, 699–700. [CrossRef] [PubMed]
9. World Health Organization, Regional Office for Africa. WHO country cooperation strategy 2017–2021: Sierra Leone. World Health Organization. Regional Office for Africa. 2017. Licence: CC BY-NC-SA 3.0 IGO. Available online: https://iris.who.int/handle/10665/258610 (accessed on 1 February 2024).
10. Peña-Fernández, A.; Anjum, U.; Wadoum, R.E.G.; Koroma, S.; Berghs, M. Competing ethics in a pilot strategy to implement parasitology training and research in post-Ebola Sierra Leone. *Int. Health* **2020**, *12*, 509–514. [CrossRef] [PubMed]
11. Qvarnstrom, Y.; Visvesvara, G.S.; Sriram, R.; da Silva, A.J. Multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., Balamuthia mandrillaris, and Naegleria fowleri. *J. Clin. Microbiol.* **2006**, *44*, 3589–3595. [CrossRef] [PubMed]
12. Niyyati, M.; Lorenzo-Morales, J.; Rezaie, S.; Rahimi, F.; Mohebali, M.; Maghsood, A.H.; Motevalli-Haghi, A.; Martín-Navarro, C.M.; Farnia, S.; Valladares, B.; et al. Genotyping of *Acanthamoeba* isolates from clinical and environmental specimens in Iran. *Exp. Parasitol.* **2009**, *121*, 242–245. [CrossRef] [PubMed]
13. Lorenzo-Morales, J.; Lopez-Darias, M.; Martínez-Carretero, E.; Valladares, B. Isolation of potentially pathogenic strains of *Acanthamoeba* in wild squirrels from the Canary Islands and Morocco. *Exp. Parasitol.* **2007**, *117*, 74–79. [CrossRef] [PubMed]
14. Siddiqui, R.; Khan, N.A. Biology and pathogenesis of *Acanthamoeba*. *Parasites Vectors* **2012**, *5*, 6. [CrossRef] [PubMed]
15. Schuster, F.L.; Visvesvara, G.S. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. *Int. J. Parasitol.* **2004**, *34*, 1001–1027. [CrossRef] [PubMed]
16. Peña-Fernández, A.; Magnet, A.; Guetiya Wadoum, R.E.; Koroma, S.; Acosta, L.; Anjum, U. First detection of *Acanthamoeba* spp. in different water reservoirs in Sierra Leone, West Africa. In Proceedings of the XI Congress of the Spanish Society of Tropical Medicine and International Health (SEMTSI), Avila, Spain, 28–30 October 2019; Available online: https://revistacientificasanum.com/congresos.php (accessed on 1 February 2024).