biology and life sciences forum

MDPI

Proceeding Paper

Human Risks Due to *Acanthamoeba* spp. in Grass from Public Parks Across Leicester City, England †

Umar Anjum [1], Angela Magnet [2], Fernando Izquierdo [2] and Antonio Peña-Fernández [1, 3, *]

[1] Leicester School of Allied Health Sciences, De Montfort University, Leicester, LE1 9BH, UK.
[2] Leicester School of Allied Health Sciences, De Montfort University, Leicester, LE1 9BH, UK.
[3] Department of Surgery, Medical and Social Sciences, Faculty of Medicine and Health Sciences, University of Alcalá, Ctra. Madrid-Barcelona, Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain.
* Correspondence: antonio.penafer@uah.es.
† Presented at the 2nd International Electronic Conference on Microbiology, 1-15 December 2023.

Abstract: *Acanthamoeba* spp., an emerging opportunistic human parasite, has been previously detected in open-water systems across Leicester city and surrounding rural areas (Leicestershire, England), suggesting a potential risk for the population. 190 grass samples were collected over a year (June 2017 to August 2018) to study temporal variation, in and around Leicester city and adjacent rural areas. After appropriate pre-treatment to resuspend potential cysts/amoebas present in the grass avoiding potential soil contamination, DNA was extracted from each pre-concentrated sample using the Fast DNA® Spin kit. *Acanthamoeba* spp. was detected using a triplex real-time TaqMan PCR assay in 22 grass samples (11.57%). The seasonal study revealed the following prevalence percentages from Summer 2017 to Summer 2018: 15.7, 10.5, 13.1, 7.89 and 10.5%. To our knowledge, this is the first report reporting the presence and seasonal distribution of *Acanthamoeba* spp. in grass across Leicestershire.

Keywords: Free-living amoebas; *Acanthamoeba* spp.; grass; public parks; Leicester; England

Citation: Anjum, U.; Magnet, A.; Izquierdo, F.; Peña-Fernández, A. Human Risks Due to *Acanthamoeba* spp. in Grass from Public Parks Across Leicester City, England. **2023**, *3*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Published: date

1. Introduction

Acanthamoeba spp., an emerging opportunistic free-living heterotrophic protist that can affect humans [1], is the most predominant amoeba in diverse ecological habitats from an ample range of climatic regions [2]. *Acanthamoeba*'s life cycle consists of an active and feeding trophozoite and a dormant double-walled polygonal cyst, which are highly resistant to environmental harsh conditions and can remain viable for many years (for more than twenty years) [2,3]. As a result, *Acanthamoeba* has been found in a myriad of different environments, including in soils, sewage, domestic water supplies, ponds, seawater, and water reservoirs across the world. Moreover, Fatemi et al. [4] has recently identify the presence of the highly pathogenic genotypes of T4, T5 and T9 in garden cress, chives, mint, parsley, and basil collected from municipal public markets.

Owing to the increasing relevance of *Acanthamoeba* spp. as an emerging human concern in recent years, our group is carrying out a comprehensive environmental monitoring study to identify the presence and circulation of these amoebas in different environmental habitats across Leicester city and surrounding rural areas (Leicestershire, England). We initiated this study because of the reported high incidence of *Acanthamoeba* keratitis (AK) in the United Kingdom, which is about 15 times that recorded in the United States and seven times that in the Netherlands [5]. These authors have related the high incidence with the presence of water storage tanks, which might promote colonisation of domestic water this amoebas, although a preliminary study carried out by our group recently has not detected the presence of any human-pathogenic free living amoebas in the tap water in Leicester [6]. However, we have isolated *Acanthamoeba* spp. in different open

water systems across Leicestershire, including the River Soar, which crosses Leicester city and attracts large numbers of users [7]. Thus, the main aim was to determine the presence, distribution/circulation of this emerging opportunistic pathogen in grass from public parks and recreational areas in and around Leicester city and adjacent rural areas, and to identify potential risks to their citizens and users.

2. Material and methods

No ethical approval was required for the described study. A total of 190 grass samples were collected over a year (June 2017 to August 2018), specifically 38 samples during 5 seasons. Grass samples were collected by cutting the top portion (5-10 cm) to avoid contamination with the topsoils and stored in 50 ml Falcon tubes. Each sample was washed with 15-20 mL PBS (1% sterile) during 24h in agitation to obtain a homogenised supernatant, and resuspend any potential cyst or amoeba present. Supernatants were collected in sterile 50 ml Falcon tubes and centrifuged at 3000 rpm for 30 minutes. Pellets were collected and suspended in 5 mL of sterile PBS (1%) for the detection of these amoebas.

DNA was extracted from appropriately pre-concentrated grass samples using FastDNA® Spin Kit (MP Biomedicals, Solon, OH, USA), following the manufacturer protocol with the modifications described by Gomes et al. [1]. These modifications included the introduction of an additional 1/4 inch ceramic sphere in each Fastprep tube and the lysing cycles were performed in triplicate in an homogeniser FastPrep-24™ 5G (MP Biomedicals, Solon, OH, USA). DNA materials were then purified with NucleoSpin® Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany), following the manufacturers' instructions.

The triplex real-time TaqMan PCR assay described by Qvarnstrom et al. [8] was used to molecularly detect the presence of *Acanthamoeba* spp. in the collected grass samples. Positive controls were used as described previously by our group in Peña-Fernández et al. [9].

3. Results and discussion

Acanthamoeba spp. was detected in 22 grass samples (11.57%). The seasonal study revealed the following prevalence percentages: 15.7 (Summer 2017), 10.5 (Autumn 2017), 13.1 (Winter 2017-18), 7.89 (Spring 2018) and 10.5% (Summer 2018). The highest frequency of *Acanthamoeba* spp. (15.7%; 8/38) was determined in Summer 2017, meanwhile the lowest prevalence was observed in Spring 2018 (7.9%; 3/38). The seasonal variations in the prevalence detected did not showed statistical differences. To our knowledge, this is the first report reporting the presence and seasonal distribution of *Acanthamoeba* spp. in grass across different public parks and recreational green areas in Leicester city and surroundings rural areas in Leicestershire. The moderate to high seasonal prevalence found would be logical owing to the high environmental resistance of their cysts [1-5]. Our results would suggest a potential unknown source for *Acanthamoeba* spp. in Leicester city/Leicestershire, which could represent a serious hazard to human health that should be appropriately tackled, to prevent and minimise the exposure to the users of these public and recreational areas in the East Midlands region explored.

Although data on the presence and/or prevalence of *Acanthamoeba* spp. and other free living amoebas in grass is limited in the literature, different recent studies have reported their presence in a wide range of vegetables including lettuce, carrots, cauliflower, radishes, onions, spinach, and tomatoes [4], which might explain the presence found in the grass.

The presence and seasonal circulation of *Acanthamoeba* spp. found in the grass could be explained by their presence in topsoils. However, and although our group has detected their presence in some topsoils sampled in a pilot study carried out on different public parks in Leicester, further monitoring studies would be needed to fully understand the potential role of the (top)soils on the presence and distribution of *Acanthamoeba* spp. found

in the grass samples. Moreover, although their interactions with domestic and wild animals is not well understood, a potential zoonotic reservoir might explain the moderate to high distribution found in a similar fashion as for other parasites. Thus, although limited, *Acanthamoeba* species have been found in domestic and wild animals [10].

4. Conclusions

This is the first report reporting the presence and seasonal distribution of *Acanthamoeba* spp. in grass collected from public parks/recreational areas in Leicester city and in different rural areas across Leicestershire, suggesting a moderate to high seasonal prevalence, which highlight a concern for public health. Leicestershire's citizens could be exposed when playing sports, or by contact of cornea or tissue wounds with the grass. Further genotyping studies are required for a better characterisation of the distribution and circulation of *Acanthamoeba* spp. in Leicestershire, and to identify appropriate public health interventions and decontamination techniques to minimise the risks identified, especially to protect individuals with their immune system compromised.

Author Contributions: Conceptualization, APF; methodology, UA, AM, FI, APF; validation, UA, APF; formal analysis, UA, APF; investigation, UA, AM, FI, APF; resources, AM, FI, APF; data curation, UA, APF; writing—original draft preparation, APF; writing—review and editing, UA, AM, FI, APF; visualization, UA, AM, FI, APF; supervision, APF; project administration, APF; internal funding acquisition, APF. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

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.

References

1. Gomes, T. S., Vaccaro, L., Magnet, A., Izquierdo, F., Ollero, D., Martinez-Fernandez, C., ... & Del Águila, C. (2020). Presence and interaction of free-living amoebae and amoeba-resisting bacteria in water from drinking water treatment plants. Science of the Total Environment, 719, 137080.
2. Rayamajhee, B., Williams, N. L., Siboni, N., Rodgers, K., Willcox, M., Henriquez, F. L., ... & Carnt, N. (2023). Identification and quantification of Acanthamoeba spp. within seawater at four coastal lagoons on the east coast of Australia. Science of the Total Environment, 901, 165862.
3. Sriram, R., Shoff, M., Booton, G., Fuerst, P., & Visvesvara, G. S. (2008). Survival of Acanthamoeba cysts after desiccation for more than 20 years. Journal of clinical microbiology, 46(12), 4045-4048.
4. Fatemi, M., Niyyati, M., Rouhani, S., Karamati, S. A., Mirjalali, H., & Karanis, P. (2023). Contamination of fresh vegetables in municipal stores with pathogenic Acanthamoeba genotypes; a public health concern. International Journal of Environmental Health Research, 33(10), 1010-1021.
5. Kilvington, S., Gray, T., Dart, J., Morlet, N., Beeching, J. R., Frazer, D. G., & Matheson, M. (2004). Acanthamoeba keratitis: the role of domestic tap water contamination in the United Kingdom. Investigative ophthalmology & visual science, 45(1), 165-169.
6. Anjum, U., Acosta, L., & Peña-Fernández, A. (2022, September). Determination of human-pathogenic free living amoebas in drinking water supplies in Leicester, UK. In ISEE Conference Abstracts (Vol. 2022, No. 1).
7. Anjum, U., & Peña Fernández, A. (2021, August). Annual presence and distribution of human-pathogenic {Acanthamoeba} spp. in River Soar, Leicester, UK. In ISEE Conference Abstracts (Vol. 2021, No. 1).
8. Qvarnstrom, Y., Visvesvara, G. S., Sriram, R., & da Silva, A. J. (2006). Multiplex real-time PCR assay for simultaneous detection of Acanthamoeba spp., Balamuthia mandrillaris, and Naegleria fowleri. Journal of clinical microbiology, 44(10), 3589-3595.
9. Peña-Fernández A., Guetiya Wadoum RE., 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; *submitted for publication*.

10. Schuster, F. L., & Visvesvara, G. S. (2004). Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. International journal for parasitology, 34(9), 1001-1027.