Observations on the transmission of *Dientamoeba fragilis*: Ambiguity of the life cycle and the cyst stage.

Luke Hall[1], Varuni Munasinghe[1], Nicole Vella[2], Damien Stark[3], John Ellis[1]

1. School of Life Sciences, Faculty of Science, University of Technology Sydney, Broadway, NSW 2007, Australia
2. Macquarie University Microscopy Unit, Faculty of Science, Macquarie University, North Ryde, NSW 2109, Australia
3. Division of Microbiology, SydPath, St Vincent's Hospital, Darlinghurst, NSW 2010, Australia

INTRODUCTION

Life cycles depict a human acquiring an infection of *Dientamoeba fragilis* by ingesting either a: trophozoite, cyst, or pinworm ova with internalised *D. fragilis* [1]. In order for any of these stages to infect a new human host they must be able to survive passage through the stomach. The human stomach has a pH range of 1-2, and contents can remain in there for one to six hours [2]. Helminth ova and protozoan cyst/oocyst stages are traditionally considered to be the environmentally resistant stage to survive travel to a new host. The trophozoite of *D. fragilis* is considered to be fragile, hence the name, '*fragilis*', given to this organism when it was discovered [3].

HYPOTHESIS

The trophozoite of *Dientamoeba fragilis* is unable to survive in the acid environment of the human stomach making it unable to infect a new host.

METHODS

Acid Treatment

Cultures of trophozoites on Loeffler slopes were exposed to acidic conditions using a PBS overlay solution modified to have a pH in the range of two to seven.

Viability of these trophozoites was assessed by completing cell counts and checking their viability using a Trypan Blue stain.

Viability measurements were taken at the timepoints: 0, 2, 4 & 6 hours.

Electron Microscopy of Cysts

Cysts of *D. fragilis* were generated by infecting mice with a solution of cultured trophozoites.

Faecal pellets containing cysts were resuspended in PBS and centrifuged. The supernatant containing cysts was then fixed.

Cysts were then embedded in LR white resin and 70nm thick sections were visualised using a Philips CM10 Transmission Electron Microscope

RESULTS

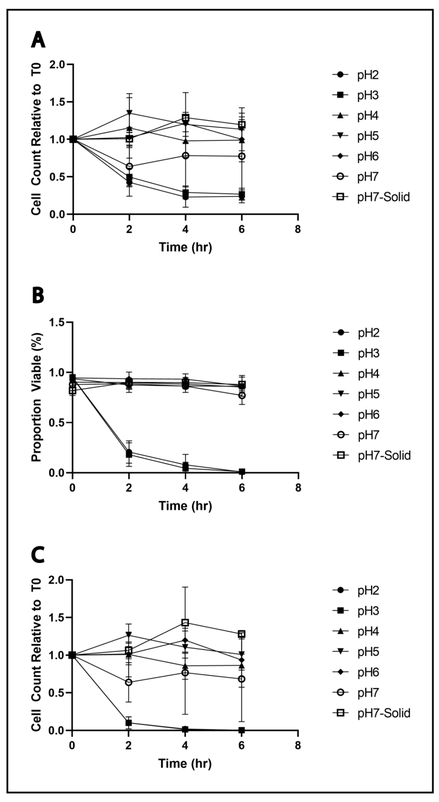

Fig 1 (left): Viability of *D. fragilis* in-vitro following incubation at acid pH. *Dientamoeba fragilis* trophozoites were incubated in PBS at different pH levels (2-7) for 0 to 6 hours. The graphs show the resulting total cell counts relative to Time 0 (A), viable proportion (B) and relative viable cells counts (C). The error bars represents standard error of mean cell counts, performed in triplicate.

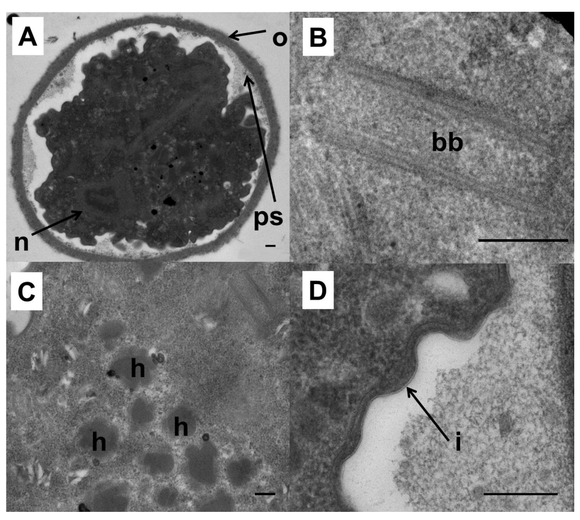

Fig 2 (above): Transmission electron micrographs of a *D. fragilis* cyst showing the cyst wall and other organelles. A, Transverse section across a mononucleated cyst showing the outer fibrillar cyst wall (o), peritrophic space (ps) and the nucleus of the encysted parasite (n). B, basal body structure (bb). C, developing hydrogenosomes which contain a dense core and a less electron dense outer layer (h). D, the inner membrane of the cyst wall (i). Scale bar represents 200nm.

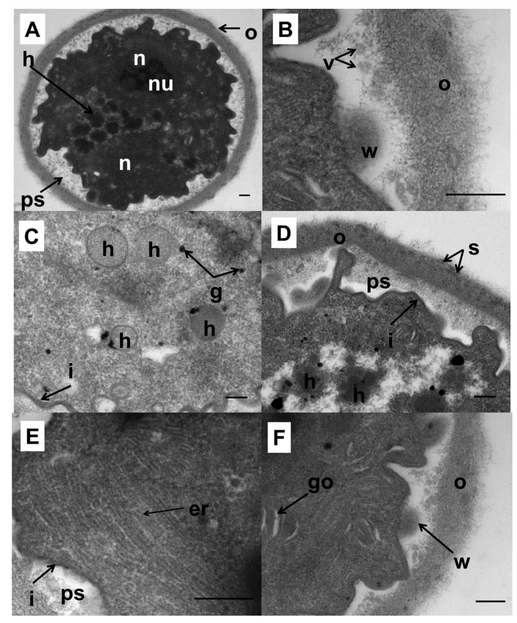

Fig 3 (right): Transmission electron micrographs of a *D. fragilis* cyst showing the whole cyst and other organelles and structures. A, Transverse section across a binucleated cyst showing the outer cyst wall (o), the peritrophic space (ps), the two nuclei (n) nucleolus (nu). Note the hydrogenosomes (h) that lie in the space between the margins of the two nuclei. B, a closer view of the outer cyst wall (o). Note the weakened area of the inner membrane (w) lying directly next to the excretory secretory vesicles (v). C, hydrogenosomes (h), glycogen granules (g) and the inner membrane of the cyst (i). D, electron micrograph of the outer cyst wall (o) showing the dark striations (s), the inner membrane (i), the peritrophic space (ps). Note the developing hydrogenosomes with the darker inner core and less electron dense outer layer (h). E, smooth endoplasmic reticulum (er), inner cyst membrane (i) and peritrophic space (ps), F, golgi complex of D. fragilis cyst: weakening of the inner membrane (w): outer cyst wall (o).

DISCUSSION

Compared to the healthy pH range of 1-2 in the stomach of humans [2] the pH range of the stomach in a mouse is 3.0-4.0 [4]. It is important to note that the pH range of the rat stomach is 3.2-3.9, which is similar to a mouse, so the pH of the stomach cannot solely explain the failure to establish an infection in a rat using trophozoites [4,5]. Mice have been shown to have a faster gastric empty rate than rats which may limit the exposure of *D. fragilis* trophozoites to acid in their stomach [6]. We speculate these differences have allowed for the establishment of the mouse model.

Our observations that trophozoites cannot withstand acid pH treatments are consistent with the mechanisms of transmission involving either the pre-cyst, cyst stage or an *E. vermincularis* vector. *Enterobious vermicularis* is human specific, with rare identifications in captive chimpanzees [7]. The identification of *D. fragilis* in a variety of non-human hosts [8,9] indicates that a model of transmission using *E. vermilcularis* as a vector is unlikely. The variety of *D. fragilis* hosts supports the importance of the cyst model of transmission. Further studies are required to ascertain the exact life cycle and mode of transmission of this parasite.

CONCLUSIONS

The current study showed that *D. fragilis* trophozoites cannot withstand extreme pH conditions present in the human stomach. This indicates faecal oral transmission via the trophozoite to humans is unlikely. Cyst forms are the dominant morphological form found in rodent faeces; it is possible that the cyst form plays an important role in zoonotic transmission. Transmission electron microscopic studies revealed the close similarity of the ultrastructure of *D. fragilis cysts* to other trichomonads especially *Histomonas,* which further highlights the presence of a cyst stage in the life cycle.

Acknowledgments

We thank Debra Birch (Macquarie University) for her advice and assistance in specimen processing and image interpretation. This work was supported by Sydpath, St. Vincent Hospital, Darlinghurst and the University of Technology Sydney.

References

1. CDC. (2019, 13 May 2019). Dientamoeba fragilis: Biology. Retrieved 22/03/2023 from https://www.cdc.gov/parasites/dientamoeba/biology.html
2. Hong, W., Jiao, W., Hu, J., Zhang, J., Liu, C., Fu, X., Shen, D., Xia, B., & Chang, Z. (2005). *Periplasmic protein HdeA exhibits chaperone-like activity exclusively within stomach pH range by transforming into disordered conformation.* J Biol Chem, **280**(29): 27029-27034.
3. Jepps, M. W., & Dobell, C. (1918). *Dientamoeba fragilis. A new Intestinal Amoeba from Man*. Parasitology **10**(3): 352-367.
4. McConnell, E.L., Basit, A.W., & Murdan, S. *Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments*. J Pharm Pharmacol, 2008. **60**(1): 63-70.
5. Munasinghe, V. S., Vella, N. G., Ellis, J. T., Windsor, P. A., & Stark, D. *Cyst formation and faecal-oral transmission of Dientamoeba fragilis--the missing link in the life cycle of an emerging pathogen.* Int J Parasitol 2013. **43**(11): 879-883.
6. Giron, R., Perez-Garcia, I., & Abalo, R. *X-ray analysis of gastrointestinal motility in conscious mice. Effects of morphine and comparison with rats.* Neurogastroenterol Motil, 2016. **28**(1): 74-84.
7. Murata, K., Hasegawa, H., Nakano, T., Noda, A., & Yanai, T. *Fatal infection with human pinworm, Enterobius vermicularis, in a captive chimpanzee.* J Med Primatol, 2002. **31**(2): 104-8.
8. Jirků, M., Kašparová, A., Lhotská, Z., Oborník, M., Brožová, K., P*A Cross-Sectional Study on the Occurrence of the Intestinal Protist, Dientamoeba fragilis, in the Gut-Healthy Volunteers and Their Animal*setrželková, K. J., Samaš, P., Kadlecová, O., Stensvold, C. R., & Jirků, K.. Int J Mol Sci 2022. **23**(23): 15407
9. Yildiz, İ., & Erdem Aynur, Z. *First detection and molecular characterization of Dientamoeba fragilis in cattle.* Zoonoses Public Health. 2022. **69**(8):897-903