Chan, D., Barratt, J., Roberts, T., Phillips, O., Šlapeta, J., Ryan, U., Marriott, D., Harkness, J., Ellis, J. and Stark, D. (2016) Detection of Dientamoeba fragilis in animal faeces using species specific real time PCR assay. Veterinary Parasitology, 227. pp. 42-47.

Title: Detection of *Dientamoeba fragilis* in animal faeces using species specific real time PCR assay

Author: Douglas Chan Joel Barratt Tamalee Roberts Owen Phillips Jan Šlapeta Una Ryan Deborah Marriott John Harkness John Ellis Damien Stark

PII: S0304-4017(16)30284-9
DOI: http://dx.doi.org/doi:10.1016/j.vetpar.2016.07.025
Reference: VETPAR 8098

To appear in: *Veterinary Parasitology*

Received date: 1-4-2016
Revised date: 18-6-2016
Accepted date: 19-7-2016

Please cite this article as: Chan, Douglas, Barratt, Joel, Roberts, Tamalee, Phillips, Owen, Šlapeta, Jan, Ryan, Una, Marriott, Deborah, Harkness, John, Ellis, John, Stark, Damien, Detection of Dientamoeba fragilis in animal faeces using species specific real time PCR assay.Veterinary Parasitology http://dx.doi.org/10.1016/j.vetpar.2016.07.025

Detection of *Dientamoeba fragilis* in animal faeces using species specific real time PCR assay

Douglas Chan[1,2,3], Joel Barratt[2,3], Tamalee Roberts[1],Owen Phillips[1],Jan Šlapeta[4], Una Ryan[5], Deborah Marriott[1], John Harkness[1], John Ellis[3], Damien Stark[1,3*]

[1]Department of Microbiology, SydPath, St. Vincent's Hospital, Victoria St, Darlinghurst, N.S.W, Australia

[2]i3 Institute, University of Technology, Sydney, Ultimo, N.S.W, Australia

[3]School of Life Sciences, University of Technology, Sydney, Ultimo, N.S.W, Australia

[4]School of Life and Environmental Sciences, Faculty of Veterinary Science, The University of Sydney, N.S.W., Australia

[5]School of Veterinary and Life Sciences, Murdoch University, Western Australia, Australia

[*]Corresponding author: Damien.Stark@svha.org.au

Tel: +[61 2] 8382 9266

Highlights

- Evaluation of three molecular diagnostic assays for detecting *Dientamoeba fragilis*
- *Dientamoeba fragilis* was detected in domestic dog and cat faecal samples
- No *D. fragilis* was found in pigs

Abstract

Dientamoeba fragilis is a potentially pathogenic enteric protozoan parasite with a worldwide distribution. While clinical case reports and prevalence studies appear regularly in the scientific literature, little attention has been paid to this parasite's biology, life cycle, host range, and possible transmission routes. Overall, these aspects of *Dientamoeba* biology remain poorly understood at best. In this study, a total of 420 animal samples, collected from Australia, were surveyed for the presence of *Dientamoeba fragilis* using PCR. Several PCR assays were evaluated for sensitivity and specificity. Two previously published PCR methods demonstrated cross reactivity with other trichomonads commonly found in animal samples. Only one assay exhibited excellent specificity. Using this assay *D. fragilis* was detected from one dog and one cat sample. This is the first report of *D. fragilis* from these animals and highlights the role companion animals may play in the transmission. This study demonstrated that some published *D. fragilis* molecular assays cross react with other closely related trichomonads and as such are not suitable for animal prevalence studies.

Keywords: *Dientamoeba fragilis, real-time PCR, Animal reservoirs*

1 Introduction

Dientamoeba fragilis is an enteric trichomonad parasite that is found within the gastrointestinal tracts of humans and has been linked to various clinical manifestations (Barratt et al., 2011a). However the parasite's pathogenic potential is yet to be fully resolved, (Barratt et al., 2011a; Barratt et al., 2011b; Röser et al., 2013; Sarafraz et al., 2013; Stark et al., 2010). The

parasite's biology, life cycle, animal reservoirs and transmission pathways are all poorly defined (Barratt et al., 2011a).

Although the mode of transmission of *D. fragilis* is yet to be determined, there are two theories proposed; transmission via a helminth transport vector (*Enterobius vermicularis*) or direct transmission via the faecal-oral route (Barratt et al., 2011a). Animal hosts have been implicated in the transmission of enteric protozoa and are possible sources of human infections (Esch and Petersen, 2013; Ruaux and Stang, 2014; Smith et al., 2009). *Dientamoeba* has been reported in several animal species with the first report outside of humans documented in wild monkeys from the Philippines in 1930 (Hegner and Chu, 1930). Later studies found *D. fragilis* in captive macaques (Knowles and DasGupta, 1936), sheep (Noble and Noble, 1952) and baboons (Myers and Kuntz, 1968). Recent studies have also reported the parasite in several non-human primates, pigs, sheep and rodents (Cacciò et al., 2012; Helenbrook et al., 2015; Ogunniyi et al., 2014; Stark et al., 2011; Stark et al., 2008). However, only two of these studies utilised molecular techniques (Cacciò et al., 2012; Stark et al., 2008).

Traditionally, diagnostic methods for detection of *D. fragilis* have relied on microscopic examination of faecal material. However, several conventional and real-time PCR (RT-PCR) assays have been described in the literature that are considered more sensitive and specific, and are now considered the gold-standard for detecting *D. fragilis* (Röser et al., 2013; Stark et al., 2011).

The aim of this study was to evaluate two RT-PCR assays and a nested conventional PCR assay with particular attention to assay specificity, for the detection of *D. fragilis* in animal samples (Cacciò et al., 2012; Stark et al., 2014; Verweij et al., 2007).

2 Materials and methods

2.1 Sample Collection and DNA extraction

This study was performed at St Vincent's Hospital, Sydney. A total of 420 animal stool samples were collected and DNA extracted using a Bioline Isolate faecal DNA kit (Bioline, Australia) as previously described (Roberts et al., 2013). Animal faecal samples included 37

distinct animal species collected from several different locations in Australia over a two-year period (Roberts et al., 2013). Additionally, pig faecal samples collected from Western Australia used in a previous study were also included (Armson et al., 2009). All extracted DNA obtained for this study was stored at -20 °C until required for molecular analysis.

2.2 Nested PCR

A nested conventional PCR was performed amplifying a 366 bp fragment of the 18S rRNA gene of *D. fragilis* as previously described in the literature (Cacciò et al., 2012). Amplified PCR products were analysed by gel electrophoresis on pre-cast E-gel ® EX 2% (Life Technologies, Australia) as per manufacturer's instructions.

2.3 Real-time PCR

A previously published RT-PCR amplifying a 98 bp fragment of the 5.8S rRNA gene using a MGB-Taqman probe was performed as described in the literature (Verweij et al., 2007). Each PCR run was accompanied by a positive control consisting of *D. fragilis* DNA and a negative control consisting of molecular biology grade H_2O in replacement of a DNA template. All reactions were carried out on a Smart Cycler II (Cepheid).

2.4 Easyscreen™ PCR

Animal samples that were analysed using an EasyScreen™ Enteric Protozoan Detection Kit (Genetic Signatures, Australia) and were run in accordance to manufacturer's instructions. This multiplex PCR assay contained both an extraction control and an internal positive control to detect PCR inhibitors. Inhibited samples were diluted 1 in 5 with molecular biology grade H_2O and then retested.

2.5 Sensitivity

To determine the limit of detection for all PCR assays, cultured *D. fragilis* trophozoites previously isolated from a symptomatic patient were counted using KOVA ® Glasstic® Slides (ThermoFisher Scientific). A negative faecal sample was spiked with a solution containing a 550,000 *D. fragilis* cells per mL. This sample was then diluted in phosphate buffered saline (PBS) for a series of 1:10 dilutions. The samples having *D. fragilis* trophozoite concentrations ranging

from 5,500 to 0.55 cells were subjected to DNA extraction for assessment of assay sensitivity. Testing was performed in triplicate.

2.6 Specificity Controls

Specificity experiments were undertaken using DNA extracted from several trichomonads (Table 1). All assays were tested against these trichomonads to determine the suitability of the assays in relation to animal studies.

3 Results

3.1 Sensitivity Results

The sensitivity of all molecular assays was tested to determine the limit of detection. All three assays were able to detect 5 *D. fragilis* trophozoites. Reproducibility experiments conducted showed all assays consistently detected down to 5 trophozoites per ml of liquid stool.

3.2 Specificity Assay

To determine the specificity of each assay the real-time PCR and nested PCRs were tested against several trichomonads closely related to *D. fragilis* (Table 1). The nested PCR cross-reacted with several other trichomonads; *Tritrichomonas foetus*, *Pentatrichomonas hominis*, *Hypotrichomonas acosta*, *Trichomonas mobilensis* and *Histomonas meleagridis*. The Real-time PCR targeting the 5.8S rRNA gene was found to cross react with *T. foetus* only. The EasyScreen PCR only exhibited partial cross reactivity with *P. hominis* as the amplification curve produced was non-sigmoidal and had a late cycle crossing point (Figure 1). Subsequent melt curve analysis of the amplified product was able to distinguish between *D. fragilis* (Tm =64ºC) and *P. hominis* (Tm= 54ºC) (Figure 2). Cross reactivity was only seen at a concentration of approximately 5,000 trophozoites per ml. Lower concentrations of *P. hominis* failed to produce a product.

3.3 Prevalence of *D. fragilis* DNA in Animal samples

Given its excellent specificity, the EasyScreen assay was selected to further survey the animal samples (Table 3). A total of 420 samples were analysed for the presence of *D. fragilis* DNA.

Two (0.5%) samples from two different species, a dog and cat, were positive for *D. fragilis* (Figure 3).

The extraction control failed in 9/420 samples and 11/420 samples were found to contain inhibitors. Repeat testing of these samples following dilution failed to resolve the inhibition problem. These 20 (5%) samples were excluded from the final analysis.

4.0 Discussion

Several molecular tests, including real-time PCR and nested PCR have been used in clinical and epidemiological studies for the detection of *D. fragilis* from faecal samples, and molecular tests are considered the gold standard (Röser et al., 2013; Stark et al., 2014; Verweij et al., 2007). However, while these assays were evaluated on human clinical samples, no experiments were undertaken to assess the suitability of these for the testing of animal samples. Furthermore, it is imperative that the widest possible range of organisms is used to assess assay specificity. As such, this study assessed the specificity of each test

In this study, three molecular diagnostic assays were evaluated for their suitability when used for detection of *D. fragilis* in animal stool specimens. All three assays detected *D. fragilis* with a limit of detection of 5 trophozoites per mL of liquid stool. The real-time assay targeting the 5.8S rDNA developed by Verweij et al. (2007) cross reacted with *T. foetus* DNA. The nested PCR targetting the 18S rRNA gene demonstrated cross reactivity with *T. foetus, H. meleagridis, T. mobilensis, H. acosta* and *P. hominis*. *Tritrichomonas foetus* is closely related to *D. fragilis* and infects several hosts including cats, cattle and pigs . *Pentatrichomonas hominis* is another closely related flagellate that has been found in humans, cats, dogs, monkeys, laboratory-bred marmosets, pigs, water buffalo, cattle and goats (Inoue et al., 2015; Kamaruddin et al., 2014; Li et al., 2015; Michalczyk et al., 2015). Both *T. mobilensis* and *H. acosta* are not found in humans, however *T. mobilensis* has been reported in laboratory mice, squirrels and monkeys (Culberson et al., 1988; Kamaruddin et al., 2014), while *H. acosta* has been reported in several reptiles and amphibians including the indigo snake, rattle snake, gila monster, neotropical tree boa, calabar ground boa, rough-scaled sand boa, forest tree frog and monitor lizard (Ceza et al., 2015; Lee and Pierce, 1960).

The non-specific amplification observed in this study for two commonly used *D. fragilis* diagnostic PCR assays impedes their applicability for both and animal studies. Given that the EasyScreen assay was the only test available that could differentiate *D. fragilis* from other trichomonads, this assay was then used to test the 420 animal samples collected from various species. The assay detected *D. fragilis* in a cat and dog faecal sample. Due to the nature of the commercial assay used, sequencing of the amplicons was not possible. As part of the EasyScreen assay protocol the DNA undergoes a 3 base conversion via a patented sodium bisulfite conversion process that chemically alters all cytosine bases to thymine (Stark et al., 2014). This confounds sequence identification, particularly for short amplicons resulting from real-time PCR.

This is the first study to detect *D. fragilis* DNAin dog and cat stool specimens. Previously, only one study has explored *D. fragilis* infection in kittens (Knoll and Howell, 1945). Using oral and anal inoculation with *D. fragilis* trophozoites the researchers established a transient infection however on necroscopy no gross pathological changes were found and *D. fragilis* trophozoites were not detected in subsequent faecal samples (Knoll and Howell, 1945). To date only one other study has investigated the prevalence of *D. fragilis* in domestic animals and reported no *D. fragilis* from 40 samples from a range of companion animals (Stark et al., 2012). The overall prevalence of *D. fragilis* from animals tested in this study was 0.48%; this is in vast contrast to what has recently been reported from humans with prevalence's ranging upwards of 50% (Stark et al., 2016). No animal studies have been performed on dogs to date with the only established animal model for *D. fragilis* described in rodents, with chronic infections established both in mice and rats (Munasinghe et al., 2013).

Close relationships between humans and companion animals such as dogs and cats poses risks, as companion animals are potential sources of human infection with enteric zoonotic protozoa (Fletcher et al., 2012; Thompson and Smith, 2011). Parasites such as *Blastocystis* sp., *Giardia intestinalis* and *Cryptosporidium* sp. infect animals and are considered zoonosis (Ng et al., 2011; Parkar et al., 2007). Zoonotic transmission of *Blastocystis* sp. is supported by the presence of genetically identical *Blastocystis* subtypes in humans and animals. Furthermore, prevalence rates as high as 70.8% have been reported in dogs and 67.3% in cats (Duda et al., 1998). Studies have also genotyped a diverse range of *Blastocystis* subtypes (from animals which also

occur in humans (namely ST1, 2, 4, 5 and 6) (Parkar et al., 2010; Parkar et al., 2007; Wang et al., 2013). *Giardia intestinalis*, assemblages A and B are potentially zoonotic, and considered transmissible between pets and pet owners, with pooled international prevalence rates of 15.2% in dogs and 12% in cats (Bouzid et al., 2015; Feng and Xiao, 2011). In Australia, one study reported *G. intestinalis* infections in 9.4% of dogs and 2% in cats (Palmer et al., 2008). In this same study, *Cryptosporidium* was reported in 0.6% of dog and 2.4% of cat samples surveyed (Palmer et al., 2008). The possibility of reverse zoonosis cannot be ruled out and the animals that tested positive for *D. fragilis* may have become infected from humans. Unfortunately, the presence or absence of symptoms in the animals examined in this study could not be determined as no clinical information was available at time of collection. Given that rodents can become chronically infected with *Dientamoeba*, we also cannot rule out the possibility that infection of these animals may have occurred via ingestion of an infected rodent.

Interestingly, there was no evidence for *D. fragilis* infection in the pig samples (0/136) surveyed in this study. Previous studies from Italy detected *D. fragilis* in swine with prevalence rates as high as 43.8% (Cacciò et al., 2012; Crotti et al., 2007). These studies utilised microscopy, nested PCR, real-time PCR and sequencing for detection and subsequent confirmation of *D. fragilis* infection in these pigs. However, the current study indicates that the assays used to detect *D. fragilis* in swine non-specifically amplified DNA from closely related trichomonads. Most notably, the PCR assays in question both cross reacted with *T. foetus*, a parasite commonly found in the intestines of pigs. This indicates that a degree of caution should be taken when using these assays, though this does not discredit the study by Cacciò et al., who confirmed the presence of *D. fragilis* from both humans and pigs by sequencing of the amplicons.

These findings have major implications for further study into the epidemiology of *D. fragilis* infection and in particular, when identifying animal hosts of *D. fragilis*. This study also highlights the conserved nature of the diagnostic targets used in several molecular diagnostic assays, which can potentially lead to misidentification of *D. fragilis* infections. Further evaluations are required to investigate the sensitivity and specificity of PCR assays available commercially and those described in the literature to determine the most appropriate assay to use in different circumstances. The role of other animal species, such as pigs, in the transmission of *D. fragilis* to

humans requires further substantiation as it was not confirmed, nor discredited, by this study. Indeed, more large scale animal studies are required to investigate the true role of animals in the lifecycle and transmission of *Dientamoeba*.

5 Acknowledgements

We acknowledge the Division of Microbiology at St Vincent's Hospital, Sydney for their help and support in completing this project. We would also like to acknowledge the support of the University of Technology, Sydney provided to Ph. D. student, D. Chan.

6 References

Armson, A., Yang, R., Thompson, J., Johnson, J., Reid, S., Ryan, U.M., 2009. *Giardia* genotypes in pigs in Western Australia: Prevalence and association with diarrhea. Experimental Parasitology 121, 381-383.

Barratt, J., Harkness, J., Marriott, D., Ellis, J., Stark, D., 2011a. The ambiguous life of *Dientamoeba fragilis*: the need to investigate current hypotheses on transmission. Parasitology 138, 557-572.

Barratt, J.L.N., Harkness, J., Marriott, D., Ellis, J.T., Stark, D., 2011b. A review of *Dientamoeba fragilis* carriage in humans: Several reasons why this organism should be considered in the diagnosis of gastrointestinal illness. Gut Microbes 2, 3-12.

Bouzid, M., Halai, K., Jeffreys, D., Hunter, P.R., 2015. The prevalence of *Giardia* infection in dogs and cats, a systematic review and meta-analysis of prevalence studies from stool samples. Veterinary parasitology 207, 181-202.

Cacciò, S.M., Sannella, A.R., Manuali, E., Tosini, F., Sensi, M., Crotti, D., Pozio, E., 2012. Pigs as Natural Hosts of Dientamoeba fragilis Genotypes Found in Humans. Emerging Infectious Diseases 18, 838-841.

Ceza, V., Panek, T., Smejkalova, P., Cepicka, I., 2015. Molecular and morphological diversity of the genus *Hypotrichomonas* (Parabasalia: Hypotrichomonadida), with descriptions of six new species. Eur J Protistol 51, 158-172.

Crotti, D., Sensi, M., Crotti, S., Grelloni, V., Manuali, E., 2007. *Dientamoeba fragilis* in swine population: a preliminary investigation. Veterinary parasitology 145, 349-351.

Culberson, D.E., Scimeca, J.M., Gardner, W.A., Jr., Abee, C.R., 1988. Pathogenicity of *Tritrichomonas mobilensis*: subcutaneous inoculation in mice. J Parasitol 74, 774-780.

Duda, A., Stenzel, D.J., Boreham, P.F., 1998. Detection of *Blastocystis* sp. in domestic dogs and cats. Veterinary parasitology 76, 9-17.

Esch, K.J., Petersen, C.A., 2013. Transmission and Epidemiology of Zoonotic Protozoal Diseases of Companion Animals. Clinical Microbiology Reviews 26, 58-85.

Feng, Y., Xiao, L., 2011. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin Microbiol Rev 24, 110-140.

Fletcher, S.M., Stark, D., Harkness, J., Ellis, J., 2012. Enteric protozoa in the developed world: a public health perspective. Clin Microbiol Rev 25, 420-449.

Hegner, R., Chu, H.J., 1930. A comparative study of the intestinal protozoa of wild monkeys and man. American Journal of Epidemiology 12, 62-108.

Helenbrook, W.D., Wade, S.E., Shields, W.M., Stehman, S.V., Whipps, C.M., 2015. Gastrointestinal Parasites of Ecuadorian Mantled Howler Monkeys (*Alouatta palliata aequatorialis*) Based on Fecal Analysis. J Parasitol 101, 341-350.

Inoue, T., Hayashimoto, N., Yasuda, M., Sasaki, E., Itoh, T., 2015. *Pentatrichomonas hominis* in laboratory-bred common marmosets. Exp Anim 64, 363-368.

Kamaruddin, M., Tokoro, M., Rahman, M.M., Arayama, S., Hidayati, A.P., Syafruddin, D., Asih, P.B., Yoshikawa, H., Kawahara, E., 2014. Molecular characterization of various trichomonad species isolated from humans and related mammals in Indonesia. Korean J Parasitol 52, 471-478.

Knoll, E.W., Howell, K.M., 1945. Studies on *Dientamoeba fragilis*: its Incidence and possible pathogenicity. American Journal of Clinical Pathology 15, 178-183.

Knowles, R., DasGupta, B.M., 1936. Some observations on the intestinal protozoa of macaques. Indian Journal of Medical Research, 547-556.

Lee, J.J., Pierce, S., 1960. *Hypotrichomonas acosta* (Moskowitz) from Reptiles. 11. Physiology.*. The Journal of Protozoology 7, 402-408.

Li, W.C., Ying, M., Gong, P.T., Li, J.H., Yang, J., Li, H., Zhang, X.C., 2015. *Pentatrichomonas hominis*: prevalence and molecular characterization in humans, dogs, and monkeys in Northern China. Parasitol Res.

Michalczyk, M., Sokol, R., Socha, P., 2015. Detection of *Pentatrichomonas hominis* in dogs using real-time PCR. Pol J Vet Sci 18, 775-778.

Munasinghe, V.S., Vella, N.G., Ellis, J.T., Windsor, P.A., Stark, D., 2013. Cyst formation and faecal-oral transmission of *Dientamoeba fragilis*--the missing link in the life cycle of an emerging pathogen. International journal for parasitology 43, 879-883.

Myers, B.J., Kuntz, R.E., 1968. Intestinal protozoa of the baboon *Papio doguera Pucheran*, 1856. J Protozool 15, 363-365.

Ng, J., Yang, R., Whiffin, V., Cox, P., Ryan, U., 2011. Identification of zoonotic *Cryptosporidium* and *Giardia* genotypes infecting animals in Sydney's water catchments. Exp Parasitol 128, 138-144.

Noble, G.A., Noble, E.R., 1952. *Entamoebae* in Farm Mammals. The Journal of Parasitology 38, 571-595.

Ogunniyi, T., Balogun, H., Shasanya, B., 2014. Ectoparasites and Endoparasites of Peridomestic House-Rats in Ile-Ife, Nigeria and Implication on Human Health. Iranian Journal of Parasitology 9, 134-140.

Palmer, C.S., Thompson, R.C., Traub, R.J., Rees, R., Robertson, I.D., 2008. National study of the gastrointestinal parasites of dogs and cats in Australia. Veterinary parasitology 151, 181-190.

Parkar, U., Traub, R., Vitali, S., Elliot, A., Levecke, B., Robertson, I., Geurden, T., Steele, J., Drake, B., Thompson, R., 2010. Molecular characterization of Blastocystis isolates from zoo animals and their animal-keepers. Veterinary parasitology 169, 8 - 17.

Parkar, U., Traub, R.J., Kumar, S., Mungthin, M., Vitali, S., Leelayoova, S., Morris, K., Thompson, R.C.A., 2007. Direct characterization of *Blastocystis* from faeces by PCR and evidence of zoonotic potential. Parasitology 134, 359-367.

Roberts, T., Stark, D., Harkness, J., Ellis, J., 2013. Subtype distribution of *Blastocystis* isolates from a variety of animals from New South Wales, Australia. Veterinary parasitology 196, 85-89.

Röser, D., Simonsen, J., Nielsen, H.V., Stensvold, C.R., Mølbak, K., 2013. *Dientamoeba fragilis* in Denmark: epidemiological experience derived from four years of routine real-time PCR. Eur J Clin Microbiol Infect Dis 32, 1303-1310.

Ruaux, C.G., Stang, B.V., 2014. Prevalence of *Blastocystis* in shelter-resident and client-owned companion animals in the US Pacific Northwest. PLoS One 9, e107496.

Sarafraz, S., Farajnia, S., Jamali, J., Khodabakhsh, F., Khanipour, F., 2013. Detection of *Dientamoeba fragilis* among diarrheal patients referred to Tabriz health care centers by nested PCR. Tropical biomedicine 30, 113-118.

Smith, R.P., Chalmers, R.M., Elwin, K., Clifton-Hadley, F.A., Mueller-Doblies, D., Watkins, J., Paiba, G.A., Giles, M., 2009. Investigation of the Role of Companion Animals in the Zoonotic Transmission of Cryptosporidiosis. Zoonoses and Public Health 56, 24-33.

Stark, D., Al-Qassab, S.E., Barratt, J.L.N., Stanley, K., Roberts, T., Marriott, D., Harkness, J., Ellis, J.T., 2011. Evaluation of Multiplex Tandem Real-Time PCR for Detection of *Cryptosporidium* spp., *Dientamoeba fragilis*, *Entamoeba histolytica*, and *Giardia intestinalis* in Clinical Stool Samples. Journal of Clinical Microbiology 49, 257-262.

Stark, D., Barratt, J., Chan, D., Ellis, J.T., 2016. Dientamoeba fragilis, the Neglected Trichomonad of the Human Bowel. Clin Microbiol Rev 29, 553-580.

Stark, D., Barratt, J., Roberts, T., Marriott, D., Harkness, J., Ellis, J., 2010. A Review of the Clinical Presentation of Dientamoebiasis. The American Journal of Tropical Medicine and Hygiene 82, 614-619.

Stark, D., Phillips, O., Peckett, D., Munro, U., Marriott, D., Harkness, J., Ellis, J., 2008. Gorillas are a host for *Dientamoeba fragilis*: an update on the life cycle and host distribution. Veterinary parasitology 151, 21-26.

Stark, D., Roberts, T., Ellis, J.T., Marriott, D., Harkness, J., 2014. Evaluation of the EasyScreen™ Enteric Parasite Detection Kit for the detection of *Blastocystis* spp., *Cryptosporidium* spp., *Dientamoeba fragilis*, *Entamoeba* complex, and *Giardia intestinalis* from clinical stool samples. Diagnostic Microbiology and Infectious Disease 78, 149-152.

Stark, D., Roberts, T., Marriott, D., Harkness, J., Ellis, J.T., 2012. Detection and transmission of *Dientamoeba fragilis* from environmental and household samples. Am J Trop Med Hyg 86, 233-236.

Thompson, R.C.A., Smith, A., 2011. Zoonotic enteric protozoa. Veterinary parasitology 182, 70-78.

Verweij, J.J., Mulder, B., Poell, B., van Middelkoop, D., Brienen, E.A., van Lieshout, L., 2007. Real-time PCR for the detection of *Dientamoeba fragilis* in fecal samples. Molecular and cellular probes 21, 400-404.

Wang, W., Cuttell, L., Bielefeldt-Ohmann, H., Inpankaew, T., Owen, H., Traub, R., 2013. Diversity of *Blastocystis* subtypes in dogs in different geographical settings. Parasites & Vectors 6, 215.

Figure Captions

Figure 1: Real-time analysis of *D. fragilis* compared with *P. hominis* using the EasyScreen PCR. , (A) *D. fragilis* DNA, (B) *P. hominis* DNA (ATCC: 30000), negative control

Figure 2: Melt curve analysis of *D. fragilis* compared with *P. hominis* using the EasyScreen PCR. *D. fragilis* (A) compared to *P. hominis* (B), *P. hominis* had a melting peak at 54ºC (C), compared to *D. fragilis* at 64ºC (D)

Figure 3: Positive amplification curves for *D. fragilis* from the cat and dog sample: Amplification curve from cat (A), dog (B), positive control (C) and negative control (D).

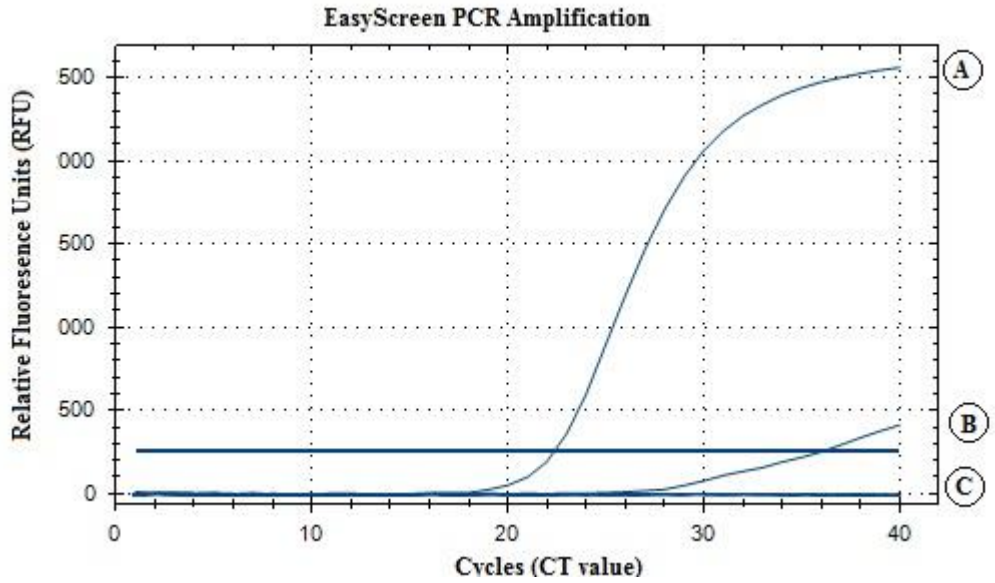

Fig. 1

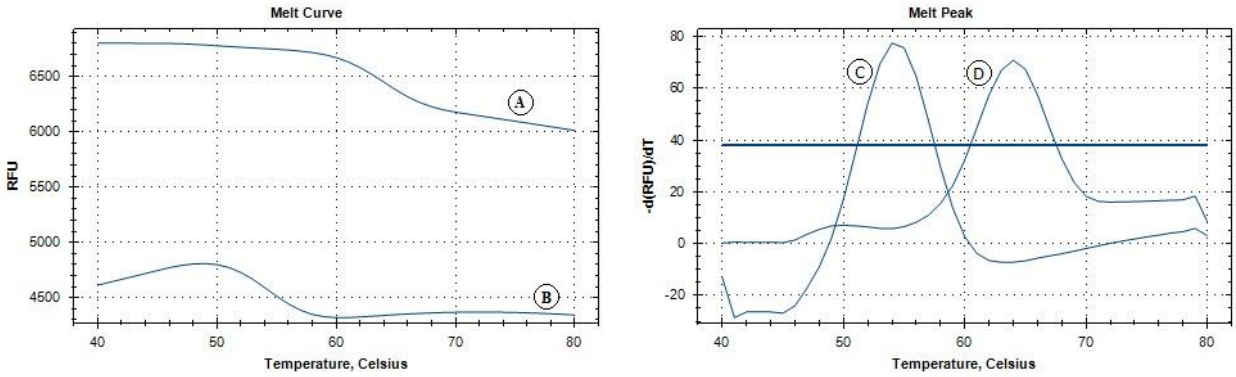

Fig. 2

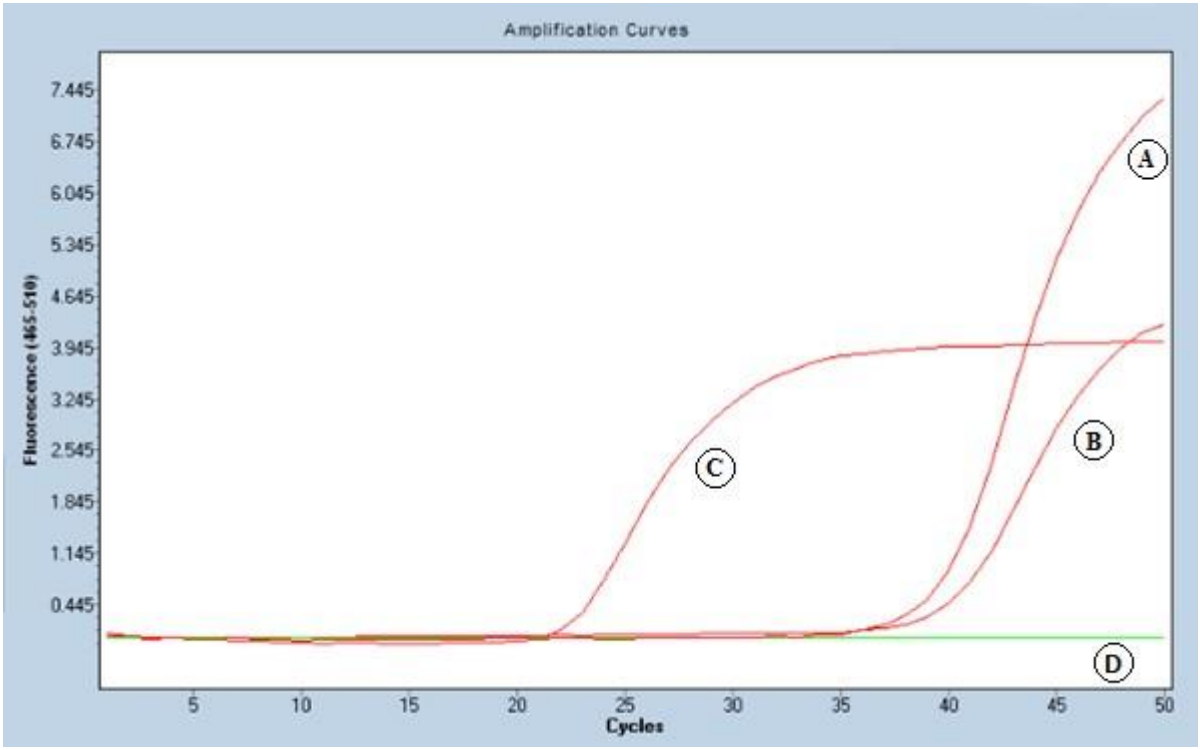

Fig. 3

Table 1: Specificity against other trichomonads

Trichomonad species	**Source of DNA**	**Nested PCR - Caccio et al (2012)**	**Real Time PCR - Verweij et al (2006)**	**EasyScreen - Genetic Signatures**
Dientamoeba fragilis (Isolate P)	SydPath, St Vincent's Hospital	Positive	Positive	Positive
Tritrichomonas foetus	University of Sydney	Positive	Positive	Negative
Pentatrichomonas hominis (ATCC ® PRA-151)	American Type Culture Collection	Positive	Negative	Negative
Trichomonas vaginalis (PNG-21)	University of Technology, Sydney	Negative	Negative	Negative
Tritrichomonas muris	University of Sydney	Negative	Negative	Negative
Hypotrichomonas acosta	University of Sydney	Positive	Negative	Negative
Tritrichomonas mobilensis	University of Sydney	Positive	Negative	Negative
Histomonas meleagridis	University of Technology, Sydney	Positive	Negative	Negative
Chilomastix mesnili	SydPath, St Vincent's Hospital	Negative	Negative	Negative

Table 2: Animal species surveyed within the study and number of *D. fragilis* positive samples for each species.

Host	Scientific name	Sample number (n)	Positive (n)	Source
Horse	*Equus ferus caballus*	1	0	(Roberts et al., 2013)
Guinea Pig	*Cavia porcellus*	2	0	(Roberts et al., 2013)
Chicken	*Gallus gallus domesticus*	23	0	(Roberts et al., 2013)
Rabbit	*Oryctolagus cuniculus*	1	0	(Roberts et al., 2013)
Guinea fowl	*Numida meleagris*	2	0	(Roberts et al., 2013)
Cat	*Felis catus*	43	1	(Roberts et al., 2013)
Dog	*Canis lupus familiaris*	56	1	(Roberts et al., 2013)
Possum	*Trichosurus vulpecula*	1	0	(Roberts et al., 2013)
Monkey	*Macaca* sp.	1	0	(Roberts et al., 2013)
Frog	*Litoria ewingii*	1	0	(Roberts et al., 2013)
Pig	*Sus scrofa domesticus*	156	0	(Roberts et al., 2013) (Armson et al., 2009)
Eastern Wallaroo	*Macropus robustus*	3	0	(Roberts et al., 2013)
Swamp Wallaby	*Wallabia bicolor*	1	0	(Roberts et al., 2013)

Asian Elephant	*Elephas maximus*	3	0	(Roberts et al., 2013)
Tiger	*Panthera tigris*	10	0	(Roberts et al., 2013)
Lion	*Panthera leo*	10	0	(Roberts et al., 2013)
Ostrich	*Struthio camel*us	6	0	(Roberts et al., 2013)
Chimpanzee	*Pan t*r*oglodytes*	10	0	(Roberts et al., 2013)
Orang Utan	*Pongo abelii*	1	0	(Roberts et al., 2013)
Gorilla	*Gorilla gorilla*	8	0	(Roberts et al., 2013)
Snow Leopard	*Panthera u*nc*ia*	6	0	(Roberts et al., 2013)
Meerkat	*Suricata suri*c*atta*	10	0	(Roberts et al., 2013)
Kodiak Bear	*Ursus arctos m*idd*endorffi*	5	0	(Roberts et al., 2013)
Francois Langur	*Trachypithecus franco*is	6	0	(Roberts et al., 2013)
Giraffe	*Giraf*fa *camelopardalis*	5	0	(Roberts et al., 2013)
Zebra	*Equus* b*urchellii*	4	0	(Roberts et al., 2013)
Cassowary	*Casuarius casuarius*	9	0	(Roberts et al., 2013)
Brazillian Tapir	*Tapirus* t*errestris*	3	0	(Roberts et al., 2013)

Southern Hairy Nosed Wombat	*Lasiorhinus latifrons*	3	0	(Roberts et al., 2013)
Common Wombat	V*ombatus ursinus*	2	0	(Roberts et al., 2013)
Western Grey Kangaroo	*Macropus fuliginosus*	2	0	(Roberts et al., 2013)
Eastern Grey Kangaroo	M*acropus giganteus*	4	0	(Roberts et al., 2013)
Red Kangaroo	*Macropus rufus*	4	0	(Roberts et al., 2013)
Short beaked echidna	*Tachyglossus Aculeatus*	1	0	(Roberts et al., 2013)
Long beaked echidna	*Zaglossus bartoni*	2	0	(Roberts et al., 2013)
Koala	*Phascolarctos cinereus*	10	0	(Roberts et al., 2013)
Tasmanian Devil	*Sarcophilus harrisii*	5	0	(Roberts et al., 2013)
Total number of samples		420	2	
Total number of samples excluded from data		20		
Total samples surveyed in this study (Total number of samples – total number of samples excluded from data)		400	2	