REVIEW ARTICLE

The ambiguous life of *Dientamoeba fragilis*: the need to investigate current hypotheses on transmission

JOEL L. N. BARRATT[1,2,3], JOHN HARKNESS[1,3], DEBORAH MARRIOTT[1,3], JOHN T. ELLIS[2,3] *and* DAMIEN STARK[1,2]*

[1] *Division of Microbiology, SydPath, St Vincent's Hospital, Darlinghurst, Australia*
[2] *University of Technology Sydney, i3 Institute, Broadway, Australia*
[3] *University of Technology Sydney, Department of Medical and Molecular Biosciences, Broadway, Australia*

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SUMMARY

Dientamoeba fragilis is an inhabitant of the human bowel and is associated with gastrointestinal illness. Despite its discovery over a century ago, the details of *Dientamoeba's* life cycle are unclear and its mode of transmission is unknown. Several theories exist which attempt to explain how *Dientamoeba* may be transmitted. One theory suggests that animals are responsible for the transmission of *Dientamoeba*. However, reports of *Dientamoeba* in animals are sporadic and most are not supported by molecular evidence. Another theory suggests that *Dientamoeba* may be transmitted via the ova of a helminth. Given that the closest relative of *Dientamoeba* is transmitted via the ova of a helminth, this theory seems plausible. It has also been suggested that *Dientamoeba* could be transmitted directly between humans. This theory also seems plausible given that other relatives of *Dientamoeba* are transmitted in this way. Despite numerous investigations, *Dientamoeba*'s mode of transmission remains unknown. This review discusses the strengths and weaknesses of theories relating to *Dientamoeba*'s mode of transmission and, by doing so, indicates where gaps in current knowledge exist. Where information is lacking, suggestions are made as to how future research could improve our knowledge on the life cycle of *Dientamoeba*.

Key words: *Dientamoeba fragilis*, animals, *Histomonas*, *Tritrichomonas*, transmission, *Enterobius vermicularis*.

INTRODUCTION

Dientamoeba fragilis is a trichomonad parasite of the human gastrointestinal tract that is associated with gastrointestinal disease (Stark *et al*. 2009*b*, 2010*b*). Despite its discovery over a century ago, the life cycle of *Dientamoeba* is not understood (Fig. 1). The only known stage in the life cycle of *Dientamoeba* is the trophozoite, which is extremely fragile once passed from the host. No environmentally resistant cyst stage has been identified. While it is possible that *Dientamoeba* trophozoites are transmitted directly from host to host, the fragile nature of the trophozoite stage has led some researchers to suggest that this mode of transmission is unlikely (Yang and Scholten, 1977). Consequently, several theories have emerged which attempt to explain how *Dientamoeba* trophozoites could survive outside their host for a sufficient period to allow their transmission. One possibility is that *Dientamoeba* is transmitted via the ova of a helminth. Another possibility is that a resistant cyst stage exists for *Dientamoeba* though remains undiscovered. Unfortunately, none of these theories has been sufficiently proven.

In the initial description of *Dientamoeba*, Jepps and Dobell (1918) commented on the fragile nature of the trophozoite stage though were unable to identify a cyst stage in the stools of infected human subjects. Subsequently, these authors theorized that *Dientamoeba* may produce cysts in an unidentified species of animal (Jepps and Dobell, 1918). While no cyst stage has been identified in humans or animals, several species of animal are reported to carry *Dientamoeba* (Knowles and DasGupta, 1936; Dobell, 1940; Noble and Noble, 1952; Myers and Kuntz, 1968; Crotti *et al*. 2007; Stark *et al*. 2008; Lankester *et al*. 2010).

Following the initial description of *Dientamoeba* (Jepps and Dobell, 1918) several authors described what appeared to be cysts, pseudocysts or cyst-like stages of *Dientamoeba* (Kofoid, 1923; Greenway, 1928; Wenrich, 1936; Knoll and Howell, 1945; Piekarski, 1948; Silard *et al*. 1979). However, these apparent cyst-like forms were found to be degenerate trophozoites or their true identity could not be confirmed (Johnson *et al*. 2004). Despite the relatively high incidence of *D. fragilis* infection reported in recent studies (Millet *et al*. 1983*b*; Girginkardesler *et al*. 2003; Bruijnesteijn van Coppenraet *et al*. 2009;

* Corresponding author: Department of Microbiology, St Vincent's Hospital, Victoria Street, Darlinghurst 2010, NSW, Australia. Tel: +61 2 8382 9196. Fax: +61 2 8382 2989. E-mail: dstark@stvincents.com.au

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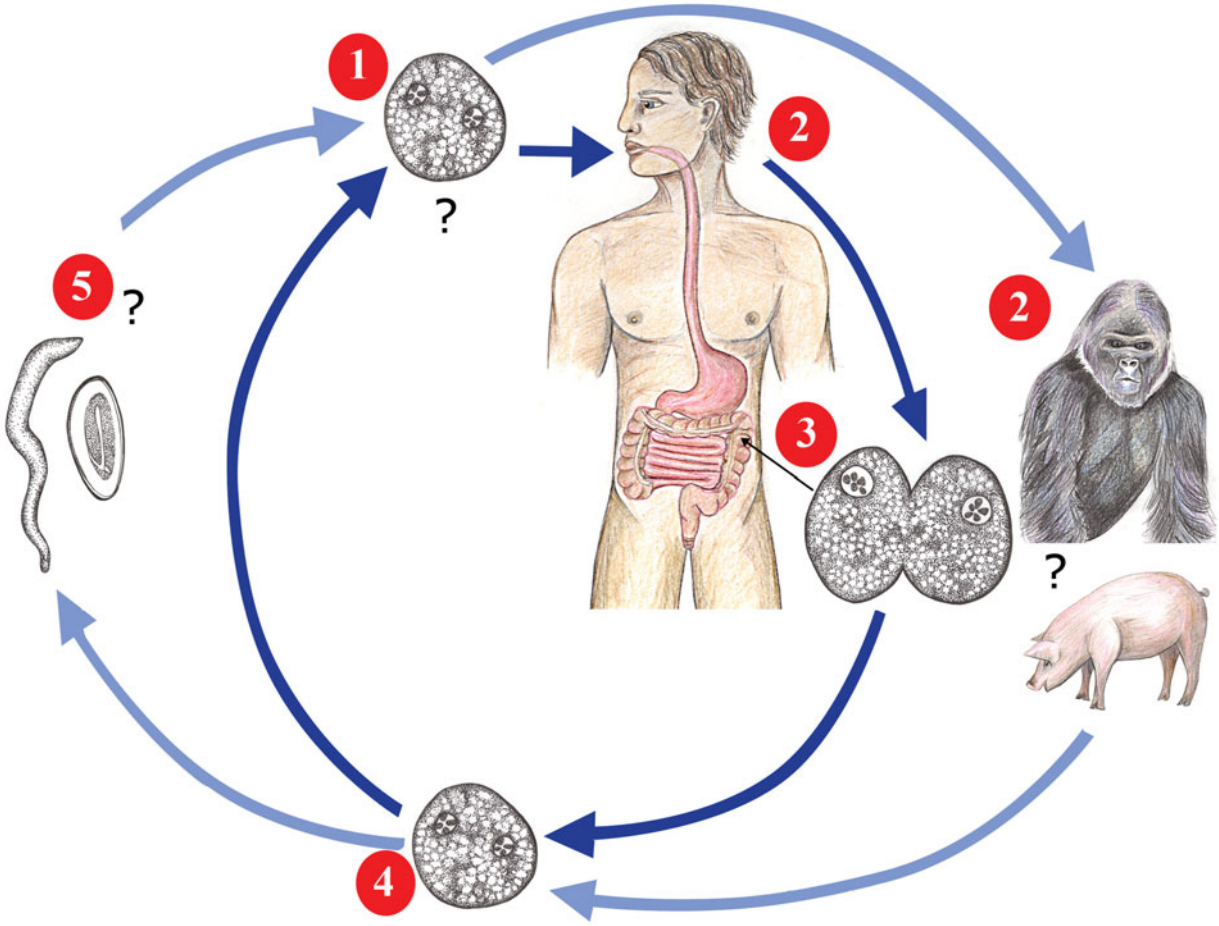

Fig. 1. The proposed life cycle of *Dientamoeba fragilis*. *Dientamoeba* trophozoites (or an undiscovered transmissible stage) are ingested from the external environment by a host species (1). Humans are thought to be the preferred host of *Dientamoeba*, though gorillas, pigs, sheep and other primate species may also be potential hosts (2). Once ingested, *Dientamoeba* travels to the large intestine where it multiplies by binary fission (3). *Dientamoeba* organisms are then passed into the environment in the faeces (4) where they contaminate food and/or water sources. *Dientamoeba* is then ingested by a new host, completing the cycle. Some authors propose that; due to the fragile nature of *Dientamoeba* trophozoites outside their host and the apparent lack of a cyst stage, it is unlikely that *Dientamoeba* can infect humans directly. It has been suggested that *Dientamoeba* may be transmitted in the ova of the helminth; *Enterobius vermicularis* (5) though the role of *Enterobius* in the life cycle of *Dientamoeba* is controversial.

Schuster and Jackson, 2009), a cyst stage has not been reported. It is now generally accepted that *D. fragilis* does not have a cyst stage (Johnson *et al*. 2004).

Dobell (1940) was the first to postulate that *Dientamoeba* may be transmitted in the ova of a helminth. This theory was based on *Dientamoeba*'s similarity to *Histomonas meleagridis* which is transmitted in the ova of the poultry helminth *Heterakis gallinarum*. While several authors provide support for this theory (Burrows and Swerdlow, 1956; Ockert, 1972*a*, *b*, 1975; Ockert and Schmidt, 1976; Yang and Scholten, 1977; Girginkardesler *et al*. 2008), other researchers report no association between helminths and *Dientamoeba* (Vandenberg *et al*. 2006; Stark *et al*. 2010*b*). As such, the role of helminths in the transmission of *Dientamoeba* remains a matter of debate.

According to phylogenetic studies *Dientamoeba*, *H. meleagridis* and *Parahistomonas wenrichi* share a recent common ancestor with members of the genus *Tritrichomonas* (Gerbod *et al*. 2001, 2002; Ohkuma *et al*. 2005) (Fig. 2). The life cycles of *Histomonas*, *Parahistomonas* and *Tritrichomonas* spp. are generally well characterized and it is postulated that the lives of these species' could provide some clues as to how *Dientamoeba* is transmitted. However, the lives of *Histomonas*, *Parahistomonas* and *Tritrichomonas* are quite different. *Histomonas* and *Parahistomonas* are gastrointestinal parasites of poultry (Levine, 1985; McDougald, 2005) while members of the genus *Tritrichomonas* include a sexually transmitted pathogen of cattle (Felleisen *et al*. 1998), the aetiological agent of a feline diarrhoeal disease (Levy *et al*. 2003; Corbeil *et al*. 2008), and parasites of the porcine (Tachezy *et al*. 2002), simian (Culberson *et al*. 1986), reptilian and amphibian (Borges *et al*. 2004) gut. Despite the apparent differences between the life cycles of these organisms, similarities do exist which

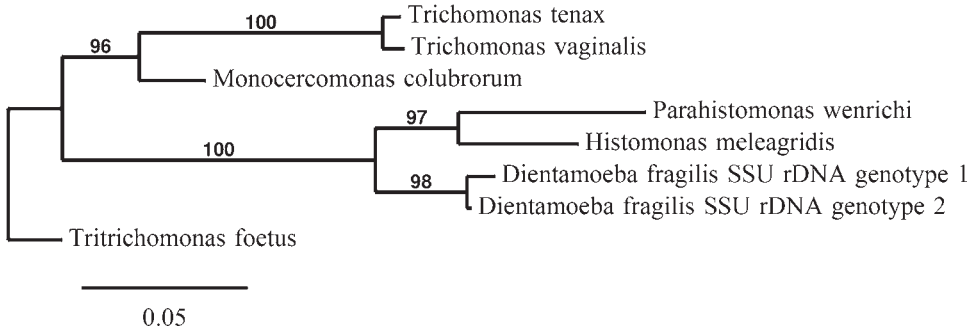

Fig. 2. Phylogenetic tree showing the relative phylogenetic positions of *Dientamoeba fragilis* genotype 1 (GenBank Accession: AY730405.1), *Dientamoeba fragilis* genotype 2 (U37461.1), *Histomonas meleagridis* (AJ920323.1), *Parahistomonas wenrichi* (EU647889.1) *Tritrichomonas foetus* (M81842.1), *Monocercomonas colubrorum* (AY319278.1), *Trichomonas vaginalis* (AY338475.1) and *Trichomonas tenax* (U37711.1) based on Small Subunit Ribosomal DNA (SSU rDNA) sequences. Support values for branches are shown as a percentage. The length of the distance scale bar is equivalent to a sequence difference of 5%. This tree was constructed using the software available on the website; www.phylogeny.fr/

may provide some insights into the life cycle of *Dientamoeba*.

This manuscript critically reviews the theories relating to *Dientamoeba*'s transmission by discussing the strengths and weaknesses of each. Where gaps in current knowledge exist, suggestions are made on how future research could improve our understanding on the life cycle of *Dientamoeba*. Also, the life cycles of *Histomonas, Parahistomonas* and *T. foetus* are explored to identify similarities which may aid in the further characterization of *Dientamoeba*'s life cycle.

DIENTAMOEBA'S MODE OF TRANSMISSION IS UNKNOWN

Based on the absence of a cyst stage and the fragility of *Dientamoeba* trophozoites once passed from their host, some researchers suggest that direct faecal oral transmission of *Dientamoeba* is unlikely (Yang and Scholten, 1977). *Dientamoeba*'s mode of transmission presents a problem for parasitologists. Despite years of research all efforts to elucidate the details of *Dientamoeba*'s life cycle have been mostly unsuccessful.

Attempts to infect humans with cultured *D. fragilis* trophozoites via the oral route failed (Dobell, 1940), suggesting that they do not survive the acidic conditions of the stomach. Furthermore, *Dientamoeba* trophozoites are reported to survive from 6 to 48 h after being passed from the host, which is too short a period to make transmission efficient (Kean and Malloch, 1966; Stark *et al*. 2010*b*). To complicate matters further, *Dientamoeba* trophozoites are said to burst when placed in boiled pond water (Wenrich, 1944) or tap water (Butler, 1996). This suggests that water sources contaminated with human faeces are unlikely to be a source of *Dientamoeba* infection. Furthermore, *Dientamoeba* trophozoites do not grow at ambient room temperature (Brug, 1938; Barratt *et al*. 2010), indicating that *Dientamoeba* is not a free-living organism which infects humans opportunistically.

THE ROLE OF ANIMALS IN *DIENTAMOEBA*'S LIFE CYCLE

Animal hosts play an important role in the transmission of many enteric protozoa that infect humans (Schlundt *et al*. 2004; Smith *et al*. 2007; Pozio, 2008). Animal reservoirs are also a potential source of many human parasitic infections (Yoshikawa *et al*. 2003; Inpankaew *et al*. 2007; Robertson, 2009; Traub *et al*. 2009). As such, it is possible that animals are involved in the transmission of *Dientamoeba*. As few studies have explored this possibility, the role of animals remains uncertain. In most cases the finding of *Dientamoeba* in animals was incidental.

Knowles and Das Gupta (1936) detected *Dientamoeba* in the stools of captive macaques (1/30) using an iron haematoxylin staining technique. According to these authors, the organism was encountered in 'scanty numbers' and was of 'typical appearance' (Knowles and DasGupta, 1936). Hegner and Chu (1930) reported *Dientamoeba* infections in 2/44 wild monkeys from the Philippines. Myers and Kuntz (1968) detected *D. fragilis* in <1% of captive baboons and <2% of those trapped in the wild. Microscopic examination of stool samples was the method employed though the specific staining technique was not described (Myers and Kuntz, 1968). Stark *et al*. (2008) identified *Dientamoeba* in the stools of 3 western lowland gorillas using an iron haemotoxylin staining technique and confirmed these results by PCR. More recently, Lankester *et al*. (2010) described a case of irritable bowel-like disease in a western lowland gorilla. The illness described by Lankester (2010) was later attributed to *Dientamoeba*

by identification of trophozoites in faecal smears stained with a Field's stain.

Noble and Noble (1952) observed *D. fragilis* trophozoites in stained smears (haematoxylin and/or Giemsa stains) made from the stools of sheep though make no mention of the prevalence. In contrast, Stark *et al*. (2008) reported *Dientamoeba* infections in 0/50 sheep using an iron haematoxylin technique. Crotti *et al*. (2007) detected *Dientamoeba* trophozoites in the stools of 53/121 farmed pigs using a Giemsa staining technique. In contrast, Noble and Noble (1952) examined stools from 30 pigs and made no mention of *D. fragilis* in these specimens. Similarly, Stark *et al*. (2008) found no evidence of *D. fragilis* in the stools of 135 swine. Interestingly, one study described contact with rabbits as a risk factor for *Dientamoeba* infection (Stensvold *et al*. 2009). However, Stark *et al*. (2008) examined the stools of 20 rabbits and did not detect *D. fragilis*.

Attempts to induce experimental infections in a range of animals have been unsuccessful (Mollari and Anzulovic, 1938; Dobell, 1940; Wenrich, 1944; Knoll and Howell, 1945; Kean and Malloch, 1966). Mollari and Anzulovic (1938) failed in their attempt to infect kittens with *Dientamoeba*. Dobell (1940) tried to infect 6 chicks by rectal inoculation of cultured *Dientamoeba* trophozoites. A transient infection was achieved in 1 chick though the infection was spontaneously cleared after 1 week. At the end of this experiment, the chick was sacrificed and examination of the caeca and liver revealed no pathological changes (Dobell, 1940). This author also tried to infect himself and 2 macaques orally with cultured *Dientamoeba* trophozoites though without success. Efforts made to infect 1 of these macaques with cultured *Dientamoeba* trophozoites via rectal injection also failed (Dobell, 1940).

Wenrich (1944) tried to infect laboratory rats with cultured *Dientamoeba* trophozoites orally and via rectal injection, also without success. Knoll and Howell (1945) were unable to infect kittens with cultured *Dientamoeba* trophozoites via rectal injection and the oral route. According to Knoll and Howell (1945), no *Dientamoeba* trophozoites were recovered at autopsy and no gross pathological changes in the gastrointestinal tract were noted. Knoll and Howell (1945) also examined the entrails of 12 laboratory rats obtained from an unrelated study and found no trace of *Dientamoeba* infection. Attempts were also made by Kean and Malloch (1966) to infect laboratory rats. Apparently, preliminary observations showed that *Dientamoeba* does 'attach to the caecal mucosa and cause damage to the underlying cells' and, 'oedema of the mucosa [was] evident, but actual ulceration [had] not yet been produced' (Kean and Malloch, 1966). However, no later reference was made pertaining to these experiments (Kean and Malloch, 1966). Studies that report the finding of *Dientamoeba* in animals are summarized in Table 1.

THE ROLE OF HELMINTHS IN THE TRANSMISSION OF *DIENTAMOEBA*

It was originally postulated by Dobell (1940) that *D. fragilis* could be transmitted via the ova of a nematode such as *Trichuris trichuria* or *Ascaris lubricoides*. This was based on *Dientamoeba*'s similarity to *Histomonas* and a noted association between *Dientamoeba* and helminth infections (Dobell, 1940). Burrows and Swerdlow (1956) were the first to propose that *Enterobius vermicularis* (pinworm) was the probable vector of *Dientamoeba* and described what appeared to be *Dientamoeba* trophozoites in the ova of *E. vermicularis*. Several years later, Ockert and coworkers published a series of reports which supported the opinion that *D. fragilis* was transmitted in the ova of *E. vermicularis* (Ockert, 1972*a*, *b*, 1975; Ockert and Schmidt, 1976). Ockert claimed that he had infected himself and 2 other subjects with *Dientamoeba* using pinworm eggs derived from a boy who was infected with both pinworm and *Dientamoeba* (Ockert, 1972*b*; Ockert, 1975). Isoelectric studies performed by Ockert and Schmidt (1976) showed that the nuclei and cytoplasm of amoeboid bodies which occurred in *Enterobius* ova and trophozoites of *Dientamoeba* from culture had almost identical isoelectric points (Ockert and Schmidt, 1976; Johnson *et al*. 2004). In a later study, Yang and Scholten (1977) noted a strong association between *D. fragilis* infections and infections with *E. vermicularis* in a large survey examining 43 000 individuals. Girginkardesler *et al*. (2008) recently reported a relationship between the incidence of *Dientamoeba* and *Enterobius* infections. Interestingly, Sukanahaketu (1977) also identified *Dientamoeba*-like structures within the ova of *A. lumbricoides* isolated from the stools of subjects with mixed infections of *Dientamoeba* and *Ascaris*.

In contrast, several studies found no relationship between *Dientamoeba* and *E. vermicularis* (Kean and Malloch, 1966; Walker *et al*. 1985; Oxner *et al*. 1987; Cuffari *et al*. 1998; Menghi *et al*. 2005; Stark *et al*. 2006, 2009*a*, 2010*b*). In the study by Kean and Malloch (1966), only 2/100 patients with pure *Dientamoeba* infections had a history of pinworm infection. A study performed in the Sydney suburb of French's Forest found that only 2/125 subjects had *E. vermicularis* ova in their stools while 21/125 were infected with *Dientamoeba*. The authors noted, however, that the prevalence of *E. vermicularis* in this group could have been under-represented as only stools were examined and *E. vermicularis* ova are rarely observed in stools (Walker *et al*. 1985). In a study performed at Christchurch Hospital Microbiology department (New Zealand) (Oxner *et al*. 1987) the incidence of *D. fragilis* was 41/1350 (3%). At the same time, the incidence of helminth infections was only 1/1350 (Oxner *et al*. 1987). In another study, DNA extracted from *E. vermicularis* ova derived

Table 1. Studies that report the finding of *Dientamoeba* in animals

Reference	Animal species examined (no. of animals)	*Dientamoeba* detected? Yes/No (% prevalence)	Technique employed*
Hegner and Chu (1930)	Wild monkeys from the Philippines – *Macacus philipinensis* (44)	Yes (4·5%)	Iron-haematoxylin staining technique
Knowles and DasGupta (1936)	Captive macaques (31)	Yes (3·2%)	Heidenhains' iron haematoxylin technique
Noble and Noble (1952)	Bovine (34), Goat (28), Pig (30).	No	Usually a Heidenhain's haematoxylin stain though sometimes a Harris' haematoxylin stain, Giemsa stain and/or Lugol's iodine stain
Noble and Noble (1952)	Sheep (25)	Yes (exact incidence not disclosed)	Usually a Heidenhain's haematoxylin stain though sometimes a Harris' haematoxylin stain, Giemsa stain and/or Lugol's iodine stain
Noble and Noble (1952)	White laboratory rats (12)	No	Rats were sacrificed and direct smears and cultures were made from the contents and walls of the caecum and large intestine
Noble and Noble (1952)	An undisclosed number of dogs, kittens and laboratory rats	No	Techniques employed not disclosed
Myers and Kuntz (1968)	Baboon – *Papio doguera* (49)	Yes (2%)	MIFC concentration technique – The staining technique used was not disclosed
Crotti *et al*. (2007)	Swine (121)	Yes (43·8%)	Giemsa-stained smears
Stark *et al*. (2008)	Bovine (50), horse (25), goat (25), swine (135), sheep (50), chimpanzee (19), De Brazza's monkey (2), Francois' leaf monkey (2), orang-utan (4), Red faced spider monkey (8), several bird species including several Australian native species, chickens and ducks (78), bush rat (2), domestic mouse (25), black rat (25), dog (50), cat (50), large flying fox (6), guinea pig (20), rabbit (20), fat tailed dunnart (1).	No	Modified iron haematoxylin stain
Stark *et al*. (2008)	Western lowland gorilla (10)	Yes (30%)	Modified iron haematoxylin stain/PCR
Lankester *et al*. (2010)	Western lowland gorilla (1)	Yes (N/A)	Field's stained faecal smears

* The diagnostic technique employed is important to note due to differences in sensitivity and specificity. Usually, molecular techniques such as PCR are more sensitive and specific than light microscopy (Stark *et al*. 2010*a*).

from people infected with *D. fragilis* failed to produce a PCR product using *Dientamoeba* specific primers (Menghi *et al*. 2005). Stark *et al*. (2009*a*) found no current pinworm infection in *D. fragilis*-infected patients in 2 unrelated families from Sydney, Australia. Stark *et al*. (2010*b*) also found no co-infections with *Dientamoeba* and any helminth in a group of 19 patients infected with *Dientamoeba*.

THE LIFE CYCLES OF *HISTOMONAS*, *PARAHISTOMONAS* AND *TRITRICHOMONAS*

According to phylogenetic studies, the closest relatives of *Dientamoeba* include *Histomonas, Parahistomonas* and members of the genus *Tritrichomonas* (Gerbod *et al*. 2001, 2002; Ohkuma *et al*. 2005; Mantini *et al*. 2009). A simple phylogenetic tree constructed for the purposes of this discussion summarizes these relationships (Fig. 2). While the lives of these related trichomonads seem quite different, some similarities do exist which appear to be inherent in members of this group. It is postulated that these similarities may provide some information on the life cycle of *Dientamoeba*.

Tritrichomonas foetus

Members of the genus *Tritrichomonas* infect a broad range of animals including reptiles, mammals and birds. The most important member of the genus *Tritrichomonas* is *Tritrichomonas foetus* due to its economic significance in the cattle-raising industries. As such, *T. foetus* will be discussed here as a representative of the genus *Tritrichomonas*.

Tritrichomonas foetus has a broad host range though is best known as a sexually transmitted pathogen of cattle (Felleisen *et al*. 1998). However, based on recent reports the host range of *T. foetus* has expanded to include other animals.

Until recently, *Pentatrichomonas hominis* was considered to be the cause of a diarrhoeal disease in cats (Gookin *et al*. 1999; Romatowski, 2000; Levy *et al*. 2003). However, later reports utilizing DNA sequence analysis, DNA restriction analysis and electron microscopy confirmed that the aetiological agent was actually *T. foetus* (Levy *et al*. 2003; Tolbert and Gookin, 2009). However, experimental infections in cows have demonstrated that *T. foetus* isolates derived from cats induce a similar yet slightly different disease in cows when compared to *T. foetus* isolates derived from cattle (Stockdale *et al*. 2007). Similarly, *T. foetus* isolates derived from cattle may be used to infect cats experimentally, though infectivity is reduced compared to isolates derived from cats (Stockdale *et al*. 2008). As such, these organisms probably represent different subtypes of the same species.

Based on molecular, biochemical and morphological evidence, *Tritrichomonas suis* was also found to be identical to *T. foetus* (Tachezy *et al*. 2002; Lun *et al*. 2005). Therefore *T. foetus* is now considered an inhabitant of the porcine gut and snout (Levine, 1985; Tachezy *et al*. 2002). However, Cobo *et al*. (2001) were unable to induce colonization of the genito-urinary tract of 9 heifers with *T. suis* via vaginal inoculation. As with the *T. foetus* isolates derived from cats, it is possible that the organism known as *T. suis* is actually a different subtype of *T. foetus* which has adapted to specifically infect swine. Interestingly, phylogenetic studies based on ribosomal RNA genes suggest that *Tritrichomonas mobilensis* which was originally described in the Bolivian squirrel monkey (Culberson *et al*. 1986), is also synonymous with *T. foetus* and *T. suis* (Felleisen, 1997; Kleina *et al*. 2004).

Tritrichomonas foetus has also been isolated from the faeces of dogs with diarrhoea (Gookin *et al*. 2005). According to Levine (1985), *T. foetus*-like organisms have also been found in the genito-urinary tract and aborted foetuses of pigs, horses and roe deer. These reports could have important ramifications to the epidemiology and control of trichomoniasis. This is because interspecies transmission of *T. foetus* may become a future problem. The finding of a *T. foetus*-type organism in non-human primates could also have implications for human health. However, given the failure to establish a *T. suis* infection in cattle (Cobo *et al*. 2001) and the limited infectivity of cattle *T. foetus* isolates in cats (Stockdale *et al*. 2008), it is more likely that different strains or subtypes of *T. foetus* exist, each with a fairly restricted host range. Given the number of animals reported to carry *T. foetus*, it is possible that other animals may be identified as hosts of *T. foetus* in the future.

The life cycle of *T. foetus* is thought to involve 2 forms; a tear-shaped trophozoite form and a recently described pseudocyst form (Pereira-Neves and Benchimol, 2009). The *T. foetus* trophozoite is 10–25 μm long and possesses 3 posterior flagella, 1 anterior flagellum and an undulating membrane (Levine, 1985). Trophozoites multiply asexually by binary fission (Levine, 1985).

Pseudocysts usually appear in response to unfavourable conditions though a small percentage of pseudocysts exist under normal conditions (Pereira-Neves *et al*. 2003). Pseudocysts occur when *T. foetus* trophozoites round up and internalize their flagella in response to various stimuli (Granger *et al*. 2000; Pereira-Neves *et al*. 2003; Mariante *et al*. 2004). This form lacks a protective cyst wall and does not represent a true cyst form (Granger *et al*. 2000). No true cyst stage exists (Levine, 1985).

In cattle, *T. foetus* is known as a cause of infertility and abortion (Felleisen *et al*. 1998) and infections are usually transferred during coitus. Infections may also be transferred to cows during gynaecological examinations or artificial insemination (Rae and Crews, 2006; Mardones *et al*. 2008). In bulls, infections are usually chronic and asymptomatic

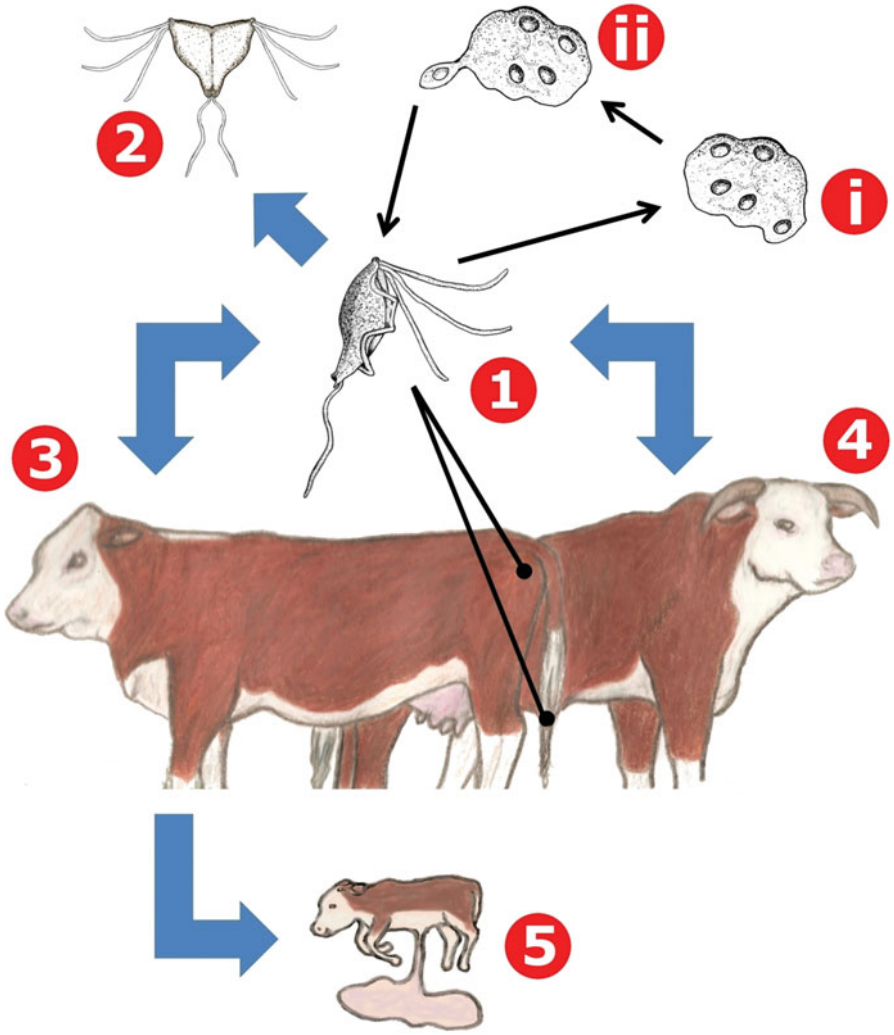

Fig. 3. The life cycle of *Tritrichomonas foetus* in cattle. Trophozoites of *Tritrichomonas* are transmitted between cows and bulls during coitus (1) and remain in the genito-urinary tract where they multiply by longitudinal binary fission (2). Under stress conditions trophozoites will internalize their flagella and replication of the nuclei and other cellular structures will occur, resulting in a multinucleated pseudocyst form (i). When conditions become desirable once more, mononucleate trophozoites will bud from the pseudocyst (ii). In bulls (4), infections are usually chronic and asymptomatic and often persist for the life of the animal. Infected cows (3) will initially experience vaginitis which may or may not resolve spontaneously. In some cases, endometritis can occur resulting in complete sterility. *Tritrichomonas* infections may also result in foetal loss during pregnancy (5).

(Mardones *et al*. 2008) and spontaneous recoveries are rare (Levine, 1985). There is no legal treatment for bovine trichomoniasis in several countries and as a result infected bulls are often slaughtered (Cobo *et al*. 2004, 2007; Agnew *et al*. 2008). Infected cows will experience vaginitis which may or may not resolve spontaneously. Infections which exist during pregnancy will often result in foetal loss. In some cases endometritis as a result of *T. foetus* infection can result in complete sterility (Levine, 1985) (Fig. 3).

Diagnosis of bovine trichomoniasis is often complicated by the presence of non-pathogenic *T. foetus*-like organisms (namely, *Pentatrichomonas hominis* and *Tetratrichomonas spp*.) in the genito-urinary tract of cattle (Cobo *et al*. 2004, 2007; Dufernez *et al*. 2007; Agnew *et al*. 2008; Huby-Chilton *et al*. 2009). It is postulated that the presence of these non-pathogenic organisms is most likely due to sodomy practiced amongst young bulls (BonDurant *et al*. 1999; Cobo *et al*. 2003, 2004). As such, it is recommended that PCR and culture techniques are employed in conjunction with light microscopy for an accurate diagnosis of bovine trichomoniasis (Campero *et al*. 2003; Hayes *et al*. 2003; Cobo *et al*. 2004).

In cats, *T. foetus* infection is acquired via ingestion of trophozoites from material contaminated with faeces. Trophozoites then travel to the intestines where they remain, inducing chronic diarrhoea (Holliday *et al*. 2009; Stockdale *et al*. 2009; Tolbert and Gookin, 2009). *Tritrichomonas* infections in cats show no preference in terms of breed or sex (Stockdale *et al*. 2009). Trophozoites are reported to remain culturally viable in cat faeces for up to 6 h after defecation (Hale *et al*. 2009) which allows

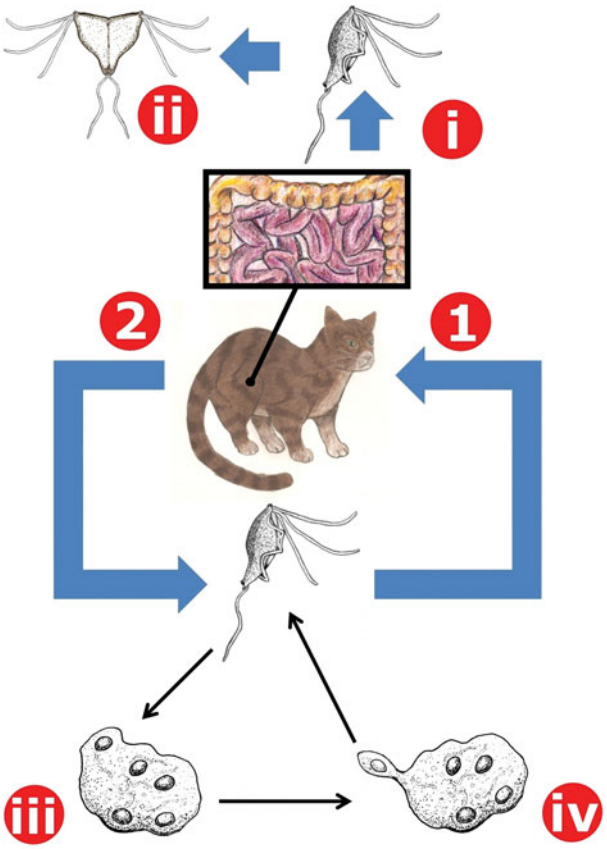

Fig. 4. The life cycle of *Tritrichomonas foetus* in cats. *Tritrichomonas* trophozoites are ingested by a feline host (1) and travel to its colon (i) where they multiply by longitudinal binary fission (ii). The presence of *Tritrichomonas* trophozoites in the colon induces chronic diarrhoea. Trophozoites are then passed in the faeces to the external environment (2) where they contaminate food and water sources of other potential hosts. Trophozoites of *T. foetus* are reported to survive for up to 6 h after being passed from the host. Under stress conditions trophozoites will internalize their flagella and replication of the nuclei and other cellular structures occurs, resulting in a multinucleated pseudocyst form (iii). When conditions become desirable once more, mononucleate trophozoites will bud from the pseudocyst (iv).

only a short period for re-infection to take place (Fig. 4).

Histomonas meleagridis *and* Parahistomonas wenrichi

Histomonas meleagridis is the closest known relative of *D. fragilis* and is the only species within the genus *Histomonas*. *Histomonas* infects a broad range of gallinaceous birds including chickens, pheasants, quails, guinea fowl and peafowl, although it is most renowned for its ability to decimate commercial turkey flocks resulting in huge economic losses (McDougald, 2005; Bleyen *et al*. 2009; Leberl *et al*. 2009). Compared to other gallinaceous production birds, turkeys are the most susceptible to histomoniasis (McDougald, 2005; Powell *et al*. 2009). The disease caused by *Histomonas* in turkeys is often referred to as 'blackhead disease' (McDougald, 2005).

Levine (1985) described 4 distinct stages within the *Histomonas* life cycle; a non-flagellated 'invasive stage', a non-flagellated 'vegetative stage', a non-flagellated 'resistant stage' and a flagellated 'caecal stage'. For the purposes of this manuscript, these 4 stages will be condensed into 2 stages; the flagellated caecal stage and the amoeboid tissue stage which comprises the 3 non-flagellated stages described by Levine (1985). The spherical non-flagellated stage is between 8 and 21 μm in diameter. The caecal stage is spherical, between 5 and 30 μm in diameter and has a single flagellum. The early invasive stage and the caecal stage both possess active pseudopodia and multiply by binary fission (Levine, 1985; Mielewczik *et al*. 2008; Munsch *et al*. 2009*a*).

Transmission of *H. meleagridis* is known to occur via 2 routes. Firstly and most simply, the flagellated caecal stage can be transmitted directly by the faecal-oral route (Levine, 1985; Hu and McDougald, 2003; McDougald and Fuller, 2005; Liebhart and Hess, 2009) which is thought to occur mostly in turkeys (McDougald, 2005). The second route of infection involves the helminth *H. gallinarum*.

In the event of a co-infection between *Histomonas* and *H. gallinarum*, *Histomonas* takes advantage of *H. gallinarum* to improve its survival in the external environment. In the caeca, *Histomonas* trophozoites are ingested by *H. gallinarum*. *Histomonas* then travels to the reproductive organs of the helminth. In the female worm, *Histomonas* enters the ovaries and eventually penetrates the undeveloped oocytes. The helminth ova containing *Histomonas* become embryonated, and are shed by the female worm into the caeca where they are eventually passed in the hosts faeces (Lee, 1969*b*; Ruff *et al*. 1970). *Heterakis* ova harbouring *Histomonas* are able to survive in the soil for up to 2 years (Levine, 1985) (Fig. 5). The protozoa are liberated when the helminth ova are ingested by an appropriate host and hatch, releasing both immature worms and the protozoa. In the soil, *Heterakis* ova containing *Histomonas* may also be ingested by the common earthworm and still remain infective for both *Histomonas* and *Heterakis* (Lund *et al*. 1966; Kemp and Franson, 1975). A gallinaceous bird can become infected with *Histomonas* by eating an earthworm that has ingested *Heterakis* ova containing *Histomonas* (Fig. 5). The earthworm is thought to play an important role in the long-term survival of *Histomonas* in the soil (Levine, 1985). Several studies exist which describe the relationship between *Histomonas* and *H. gallinarum* in greater detail (Lund and Burtner, 1957; Kendall, 1959; Gibbs, 1962; Lee, 1969*a*; Ruff *et al*. 1970; Lee, 1971; Lund and Chute, 1973). While it is generally

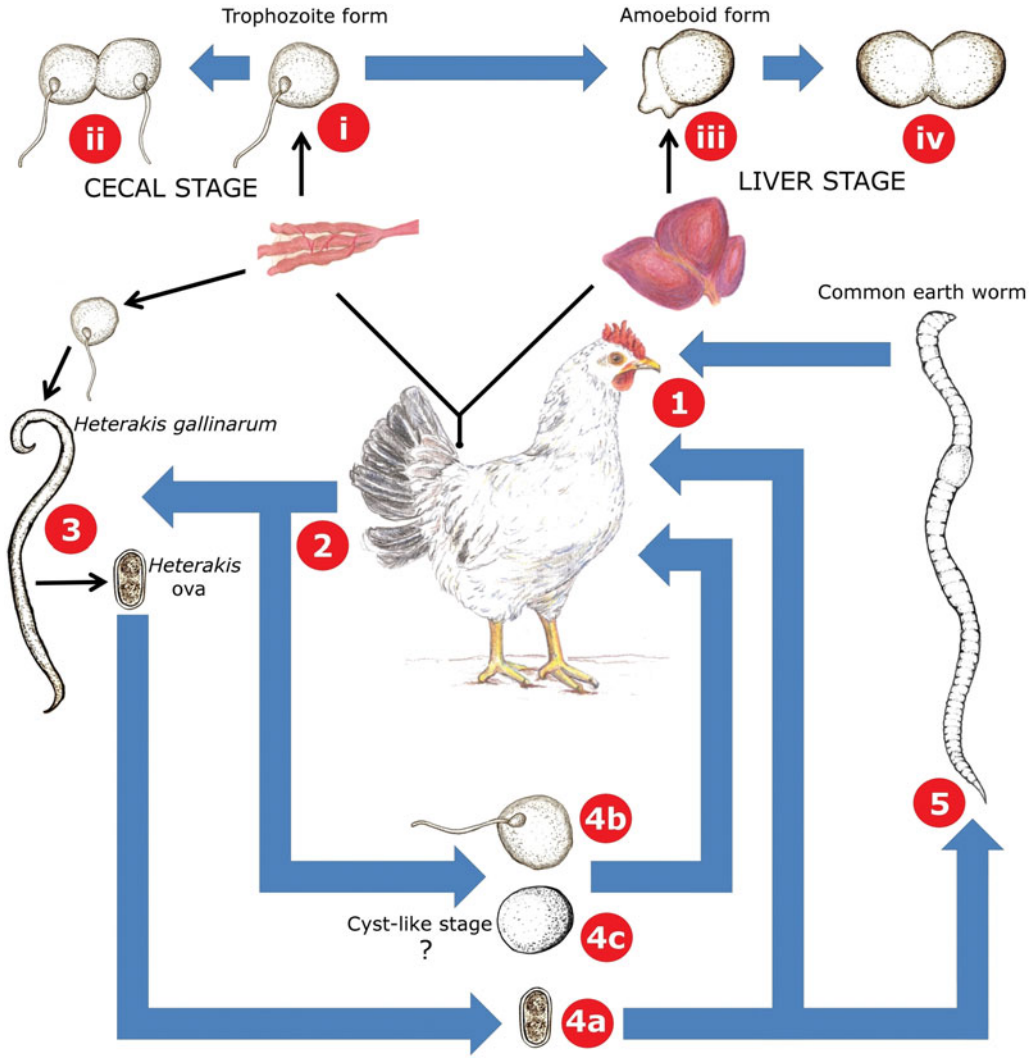

Fig. 5. The life cycle of *Histomonas meleagridis*. *Histomonas* trophozoites are ingested from the external environment in various forms by a gallinaceous bird (1). The flagellated trophozoite form of *Histomonas* travels to the caeca (i) where it multiplies by longitudinal binary fission (ii). Infections with *Histomonas* usually result in lesions on the caecal wall accompanied by a yellowish diarrhoea. Eventually, *Histomonas* trophozoites penetrate the caecal mucosa and travel to the liver, where they take on an amoeboid form (iii). The amoeboid form also multiplies by binary fission (iv). The damage caused to liver tissues during the invasive liver stage is often so severe that death will ensue. *Histomonas* organisms in various forms are then passed in the host's faeces (2) and contaminate food and water sources of other gallinaceous birds. In the event of a *Heterakis*/*Histomonas* coinfection, *Histomonas* trophozoites are ingested by a female *Heterakis* worm and invade its ovaries. Once in the *Heterakis* ovaries, *Histomonas* can then penetrate the developing *Heterakis* ova (3). These ova are then shed into the host's caeca by the female worm and are eventually passed in the host's faeces. *Histomonas* can remain viable outside the host within these ova for up to 2 years (4a). Alternatively, free flagellated trophozoites of *Histomonas* which were shed in the faeces may be directly ingested by a new host resulting in a *Histomonas* infection (4b). Recently, cyst-like structures of *Histomonas* have been described (4c) which could represent a newly discovered transmissible stage. However, the infectivity of these cyst-like structures is yet to be demonstrated. In the soil, *Heterakis* ova containing *Histomonas* organisms (4a) may also be ingested by the common earth worm (5) which may then be consumed by a gallinaceous bird, resulting in a *Histomonas* infection. Earthworms are believed to play a significant role in the survival of *Histomonas* organisms in the soil.

accepted that a cyst stage does not exist for *Histomonas*, some researchers report the formation of cyst-like structures *in vitro* which may represent another transmissible stage of *Histomonas* (Munsch *et al*. 2009*a*) (Fig. 5).

After an incubation period of 15–21 days, infected birds become weak and drowsy in appearance (Levine, 1985). This is accompanied by the appearance of a yellowish diarrhoea and ulcerative lesions in the caeca and liver (Huber *et al*. 2006). Other tissues such as the kidneys and lungs may also be involved (Levine, 1985). In turkey flocks, the mortality rate approaches 100% in some cases, while in chicken flocks the mortality rate approaches 10–20% though with high morbidity (McDougald, 2005). Interestingly, experiments involving gnotobiotic birds indicate that in the absence of certain bacteria *Histomonas* loses its pathogenicity (McDougald, 2005).

Parahistomonas wenrichi [synonym: *Histomonas wenrichi* (Mantini *et al*. 2009)] is similar to *Histomonas* in terms of its life cycle and biology. Like *Histomonas, Parahistomonas* is spherical though is approximately 1·5 times larger than *Histomonas* (Levine, 1985). *Parahistomonas* possesses 4 flagella as opposed to *Histomonas*' single flagellum though its movement and feeding are mostly dependent on its pseudopodia. Unlike *Histomonas, Parahistomonas* is non-pathogenic (Lund, 1963). *Parahistomonas*' preferred host range also includes gallinaceous birds such as turkeys, chickens and pheasants (Lund, 1963; Levine, 1985). *Parahistomonas* infections can also be transmitted in the ova of *H. gallinarum* (Lund, 1968, 1971).

Pseudocyst forms and cyst-like structures in Trichomonads

Under stress conditions several trichomonads enter a pseudocyst stage which is characterized by 'rounding up' of trophozoites and internalization of flagella (Lipman *et al*. 1999; Granger *et al*. 2000; Boggild *et al*. 2002; Ribeiro *et al*. 2002; Pereira-Neves *et al*. 2003; Borges *et al*. 2004; Mariante *et al*. 2004; Hussein and Atwa, 2008; Pereira-Neves and Benchimol, 2009). During this process, duplication of the nuclei and other cellular structures also occurs resulting in a multinucleated giant cell. When conditions become favourable again, flagellated trophozoites begin to bud from the multinucleated cell (Pereira-Neves and Benchimol, 2009). Pseudocysts of trichomonads are generally described as compact, spherical, multinucleated forms which lack flagella and do not have a true cyst wall (Pereira-Neves *et al*. 2003).

Pseudocysts were once thought to be degenerative forms though are now known to represent a true stage in the life cycle of some trichomonads. This is because mitosis occurs in the pseudocyst and the process of pseudocyst formation is reversible (Pereira-Neves *et al*. 2003). Pseudocysts from various trichomonads are also infective to their respective hosts (Friedhoff *et al*. 1991; Lipman *et al*. 1999; Pereira-Neves *et al*. 2003; Pereira-Neves and Benchimol, 2009). Moreover, pseudocysts of *T. foetus* are able to adhere to vaginal epithelial cells more effectively than the trophozoite stage (Mariante *et al*. 2004; Pereira-Neves and Benchimol, 2009). Usually, a small portion of the normal cell population will exist as pseudocysts (Pereira-Neves *et al*. 2003).

Various stress conditions are known to trigger pseudocyst formation. In cultures of *Monocercomonas*, the largest number of pseudocyts were produced when cultures were incubated for 4–5 days at pH levels between 5 and 6 (Borges *et al*. 2007). Fewer pseudocysts were produced by nutrient depletion and incubation at 20 °C compared to 37 °C (Borges *et al*. 2007). Pseudocyst formation can also be induced in *Tritrichomonas* by the cooling of cultures to just below 16 °C (Granger *et al*. 2000). The addition of certain drugs to growth media can also trigger pseudocyst formation (Pereira-Neves and Benchimol, 2009). Mariante *et al*. (2004) found that incubation of cultures with the drug colchicine, incubation with dimethyl sulfoxide and submitting trophozoites to cycles of temperature oscillation will also induce pseudocyst formation.

Several flagellates are also capable of producing true cysts including members of the genera *Retortomonas, Chilomastix* and *Enteromonas* (Levine, 1985) as well as *Trichomitus batachorum, Trichomitus sanguisugae* and *Monocercomonas tipulae* (Brugerolle, 1973; Pereira-Neves *et al*. 2003). Interestingly, Mielewczik *et al*. (2008) also observed cyst-like structures in the faeces of chickens infected with *Histomonas*. However, these structures could not be attributed to *H. meleagridis* definitively, as all methods of purification failed (Mielewczik *et al*. 2008). A number of later studies also report the finding of cyst-like stages in cultures of *Histomonas* (Munsch *et al*. 2009*a*, *b*; Zaragatzki *et al*. 2010). According to Zaragatzki *et al*. (2010), formation of these structures can be induced by cultivating *Histomonas* trophozoites at pH values between 7 and 8. However, the infectivity of these structures is yet to be demonstrated.

FURTHER RESEARCH IS REQUIRED

With respect to animals

Given the close relationship between humans and primates, it is not surprising that most reports in Table 1 describe the finding of *Dientamoeba* in monkeys and apes. The evidence for *D. fragilis* infections in gorillas is the most recent and is also well supported. Stark *et al*. (2008) provided molecular evidence to support their finding of *D. fragilis* in the stools of western lowland gorillas. The recent report by Lankester *et al*. (2010) describing an irritable bowel-like illness in a western lowland gorilla also provides support for the findings of Stark *et al*. (2008). While monkeys and apes may be among the preferred hosts of *Dientamoeba*, transmission of *Dientamoeba* between humans and other primates is not a suitable model in parts of the British Isles (Schuster and Jackson, 2009), the USA (Millet *et al*. 1983*a*) and the Netherlands (van Gool and Dankert, 1996). This is because *Dientamoeba* infections are quite common in these places, although human contact with monkeys and apes is virtually non-existent. In these regions, if an animal reservoir is ever identified it is more likely to be a pet or livestock animal, as these are more commonly integrated into those societies.

Dientamoeba has also been reported in the stools of sheep (Noble and Noble, 1952) and swine (Crotti *et al*. 2007). In regions where human contact with apes is low, these animals are more plausible as reservoirs of *Dientamoeba* infection. However, Stark

et al. (2008) found no evidence of *Dientamoeba* in the stools of 50 sheep and 135 swine. According to Stark *et al*. (2008), a number of reasons could have attributed to these non-concordant reports. Stark *et al*. (2008) suggested that the Giemsa stain employed by Crotti *et al*. (2007) may not have been ideal for visualization of the nuclear structure of *D. fragilis*. Johnson *et al*. (2004) noted that the fragmented nuclear structure of *D. fragilis* enables one to distinguish it from organisms such as *Endolimax nana* which can appear quite similar to *Dientamoeba* in stained preparations. In the study by Noble and Noble (1952), the staining technique which detected *Dientamoeba* was not disclosed and no image of *Dientamoeba* was provided. Stark *et al*. (2008) did note, however, that differences in farming practices such as caged farming as opposed to free-range style farming or the use of anti-protozoal compounds could have attributed to these conflicting reports. The screening of wild or feral animals for the presence of *Dientamoeba* may be informative as differences in farming practices do not apply and these animals are unlikely to have been treated with anti-protozoal compounds.

In light of these conflicting reports, the role of swine and sheep in the life cycle of *Dientamoeba* is uncertain. However, their potential role in the life cycle of *Dientamoeba* cannot be dismissed. In the study by Stark *et al*. (2008), a two-step screening approach was employed where stained faecal smears were prepared and those found to contain *Dientamoeba* were then confirmed with PCR. It is well documented that PCR is more sensitive than light microscopy (Stark *et al*. 2010*a*). The intermittent shedding of *Dientamoeba* trophozoites in humans is also well documented (Stark *et al*. 2010*b*). It has been shown that the results of molecular tests are less likely to be influenced by the phenomenon of intermittent shedding (Stark *et al*. 2010*a*). As such, the study performed by Stark *et al*. (2008) could have been improved by testing specimens with PCR or real-time PCR prior to microscopic analysis. The use of molecular tests as the first step in the screening process would have reduced the chances of obtaining false negatives that occur as a result of low parasite loads, intermittent shedding and human error.

While the use of PCR by Stark *et al*. (2008) provides strong evidence that gorillas are a true host for *Dientamoeba*, DNA sequence data derived from the SSU rDNA of these gorilla isolates would have been ideal. Similarly, the study by Crotti *et al*. (2007) could have been greatly improved had their results been supported by molecular evidence in the form of a PCR product and preferably, some DNA sequence data. In order to improve future studies, it is extremely important that researchers utilize molecular techniques to substantiate all findings. Researchers should also obtain sequence data to ensure that their PCR products are specific for *Dientamoeba*. Sequence data would also be useful for genotyping purposes.

Regarding the experimental infections in humans and animals, these reports are also non-concordant. For instance, Dobell's attempt to infect macaques by rectal inoculation failed (Dobell, 1940). However, 2 reports describe the finding of *Dientamoeba* in the stools of macaques (Hegner and Chu, 1930; Knowles and DasGupta, 1936). There are several plausible explanations for Dobell's failed attempt to infect macaques. One possibility is that the organisms described in these reports (Hegner and Chu, 1930; Knowles and DasGupta, 1936) were misidentified and macaques are not a true host for *Dientamoeba*. Another plausible explanation is that Dobell's cultured isolates had been attenuated over time and lost their ability to infect new hosts. However, Dobell's success in infecting 1 of 6 chicks by rectal inoculation is surprising when considering that infections could not be achieved in macaques using the same technique (Dobell, 1940). However, we do not know whether these experiments were carried out at the same time using the same isolate of *Dientamoeba*. As mentioned previously, Kean and Malloch (1966) also reported some success in their attempt to experimentally infect laboratory rats with *Dientamoeba* although these experiments are only briefly discussed and appear to be incomplete. Other than the reports by Kean and Malloch (1966) and Dobell (1940), all additional attempts to experimentally infect animals with *Dientamoeba* have failed.

Taken as a whole, these conflicting reports are difficult to interpret. However, it seems that animal experiments like those performed by Dobell (1940) and Kean and Malloch (1966) must be repeated. The development of a simian model of dientamoebiasis could represent a breakthrough in *Dientamoeba* research. This would not only allow researchers to explore *Dientamoeba*'s mode of transmission in a controlled manner, but could also be used to determine whether *Dientamoeba* satisfies Koch's postulates as a cause of gastrointestinal illness.

Given Dobell's success in inducing a transient infection in a chick (Dobell, 1940), the role of poultry in the transmission of *Dientamoeba* is also worth exploring further. Interestingly, the optimum temperature for growth of *Dientamoeba in vitro* is 41–42 °C rather than 37 °C (Dobell, 1940; Barratt *et al*. 2010). Therefore, the optimum growth temperature for *Dientamoeba* is closer to the body temperature of birds rather than humans. Given that *Dientamoeba*'s closest relative is a poultry pathogen; it is plausible that poultry could be involved in *Dientamoeba*'s transmission.

With respect to helminths

While Ockert claimed to have infected himself with *Dientamoeba* using the ova of *E. vermicularis*, this is difficult to substantiate without the aid of molecular or electron-microscopic evidence. Ideally,

if structures resembling *Dientamoeba* trophozoites are observed in the ova of a helminth, electron microscopic images of these *Dientamoeba*-like bodies should be taken and compared to those produced by Camp *et al*. (1974) and Silard *et al*. (1984). Such evidence would provide strong support for Ockert's claims. Furthermore, without molecular evidence it is difficult to ascertain whether Ockert's *Dientamoeba* was of the same genotype as the *Dientamoeba* in the child from which he had infected himself. Given Ockert's frequent handling and processing of stool specimens containing *Dientamoeba*, it is possible that he infected himself from another source. The most compelling support for the lack of an association between *Enterobius* and *Dientamoeba* is the molecular evidence provided by Menghi *et al*. (2005) who failed to amplify a *Dientamoeba*-specific PCR product from *Enterobius* ova derived from a patient who was also infected with *Dientamoeba*.

While associations may have been observed between *Enterobius* and *Dientamoeba*, similar associations between *Dientamoeba* and other enteric parasites have also been reported (Johnson *et al*. 2004). Ayadi and Bahri (1999) noted, that *Dientamoeba* infections were most often associated with *Blastocystis*. Stark *et al*. (2005) also found that *Dientamoeba* infections were most often associated with *Blastocystis*. Ozcakir *et al*. (2007) noted that *Blastocystis* was more frequently detected alongside *D. fragilis* compared to any other enteric protozoa. Stensvold *et al*. (2009) found that 34·8% ($n=32$) of patients with *Blastocystis* infection were also infected with *D. fragilis*. As such, it is possible that the associations between *Dientamoeba* and *Enterobius* described by Yang and Scholten (1977) and Girginkardesler *et al*. (2008) may represent nothing more than a shared mode of transmission between these organisms.

The report by Sukanahaketu (1977) provides support for the existence of a relationship between *Dientamoeba* and *Ascaris*. While the images presented by Sukanahaketu (1977) are interesting, the finding of *Dientamoeba*-like structures in the ova of *Ascaris* is not supported by the occurrence of *Dientamoeba* infections in non-tropical, western countries where *Ascaris* infections are very uncommon (Walker *et al*. 1985; Stensvold *et al*. 2007; Schuster and Jackson, 2009). Therefore, the transmission of *Dientamoeba* by means of an *Ascaris* vector is not a suitable model for *Dientamoeba* transmission in these regions.

Given the molecular evidence described by Menghi *et al*. (2005) and the lack of an association between pinworm and *Dientamoeba* observed in several studies, the role of *Enterobius* in the life cycle of *Dientamoeba* remains controversial. Despite this, Ockert's hypotheses regarding the role of *Enterobius* in the transmission of *Dientamoeba* should not be disregarded. Johnson *et al*. (2004) noted that spontaneous remissions of *Enterobius* infection do occur and it is often not clear in some reports whether patients were tested correctly for the presence of *Enterobius*. Clearly, if future researchers observe *Dientamoeba*-like bodies in the ova of a helminth, support for these findings in the form of molecular evidence and electron microscopic images is essential.

With respect to Dientamoeba*'s relatives*

Given the existence of pseudocysts in *T. foetus* and the recent evidence for cyst-like structures in *Histomonas*, it is plausible that similar structures could exist for *Dientamoeba*. Pseudocyst formation as observed in some trichomonads is easily noted by the invagination of the flagella. Unfortunately, such an event cannot be observed in *Dientamoeba* because it completely lacks flagella. Furthermore, another feature of pseudocysts in trichomonads is that they are multinucleated (Pereira-Neves *et al*. 2003; Borges *et al*. 2007). *Dientamoeba* trophozoites are often multinucleated (Johnson *et al*. 2004) which would also make it difficult to identify pseudocyst forms in *Dientamoeba* if they did exist.

To explore the possible existence of a pseudocyst stage in *Dientamoeba*, the experiments performed by Borges *et al*. (2007), Pereira-Neves *et al*. (2009), and Mariante *et al*. (2004) should be repeated for *Dientamoeba*. Staining *Dientamoeba* cells with a specific nuclear stain such as DAPI (Noel *et al*. 2003; Al-Adhami *et al*. 2007; Taniwaki *et al*. 2007) would be helpful in these experiments to identify if any changes occur with respect to the number of nuclei in the cells. It is possible that the *Dientamoeba* cells occasionally reported to contain 4 or more nuclei (Johnson *et al*. 2004) could represent a pseudocyst form.

As previously mentioned, several flagellates are also capable of producing true cysts (Brugerolle, 1973; Levine, 1985; Pereira-Neves *et al*. 2003). Therefore, the possibility that a cyst stage could exist for *Dientamoeba* should not be dismissed. Given the recent discovery of the cyst stage of *Blastocystis* in 1991 (Stenzel and Boreham, 1991) it is not impossible that a cyst stage for *Dientamoeba* is yet to be discovered. Moreover, given the recent reports of a cyst-like stage for *Histomonas* (Mielewczik *et al*. 2008; Munsch *et al*. 2009*a*; Zaragatzki *et al*. 2010), the existence of a similar stage for *Dientamoeba* is plausible. Should a cyst-like stage be identified for *Dientamoeba*, the findings must be substantiated through the use of electron microscopic comparisons of these structures to the cyst stages of other trichomonads. Molecular support for such a substantial claim is also important. Finally, the infectivity of these structures must also be demonstrated.

When examining the life cycles of *Histomonas* and *Tritrichomonas*, one major similarity becomes apparent. That is that these organisms can be transmitted

directly between their preferred hosts. As such, the trophozoite stage of *Dientamoeba* may still be the only stage in its life cycle. It was noted previously that *T. foetus* does not possess a cyst stage and remains culturally viable for up to 6 h after being excreted in the faeces of cats (Hale *et al*. 2009). Also, direct bird-to-bird transmission of the trophozoite stage of *Histomonas* in the absence of a *Heterakis* vector has been well documented (Levine, 1985; Hu and McDougald, 2003; McDougald and Fuller, 2005; Liebhart and Hess, 2009). Given that *Dientamoeba* trophozoites are reported to remain viable for up to 8 times longer than those of *T. foetus* (Kean and Malloch, 1966; Stark *et al*. 2010*a*), it is not unreasonable to suggest that *Dientamoeba* trophozoites may be transmitted directly from human to human. Nevertheless, if trophozoites are the transmissible form, it is uncertain as to why Dobell (1940) was unable to infect himself and 2 macaques orally with cultured trophozoites. This could be the result of attenuation of the trophozoites after long-term culture though, without information on the passage number and source of Dobell's cultures it is difficult to speculate. The development of an animal model for dientamoebiasis would greatly assist in addressing these problems.

CONCLUDING REMARKS

Dientamoeba fragilis is an inhabitant of the human gastrointestinal tract for which the mode of transmission is unknown. As no cyst stage has been identified for *Dientamoeba* (at the time of writing), the trophozoite form is generally accepted as the only stage in its life cycle. However, the fragile nature of *Dientamoeba* trophozoites once passed from their host implies that direct human-to-human transmission of the trophozoite form seems unlikely. Numerous investigations have been carried out in an attempt to better understand *Dientamoeba*'s life cycle. Despite these efforts our knowledge of this organism has progressed very little. Clearly, more research is required.

While several reports attempt to address the lack of knowledge on this organism, most are lacking in one crucial component; that is evidence in the form of molecular and/or electron microscopic data. It is imperative that claims relating to the life cycle and transmission of *Dientamoeba*, are substantiated using these techniques for several reasons. As discussed previously, light microscopic analysis is less sensitive and less specific than PCR. Moreover, light microscopy introduces a greater element of human error when compared to molecular techniques. Sequencing of PCR products allows researchers to accurately determine the presence and/or identity of an organism almost beyond a doubt. Electron microscopy is a powerful tool which allows detailed observations to be made on the intracellular architecture of cells which cannot be matched by light microscopy. It has become apparent that if modern techniques such as PCR, DNA sequencing and electron microscopy are not employed to substantiate findings related to the life cycle of *Dientamoeba*, it is unlikely that they will be accepted by the broader scientific community.

Unfortunately, our lack of an animal model for this organism is also a short coming which must be addressed. The lack of an animal model for *Dientamoeba* infection hampers our ability to study its biology, mode of transmission and mechanisms of pathogenicity in a well-controlled manner. Such a model may also help in the fulfilment of Koch's postulates for *Dientamoeba*.

Dientamoeba has recently emerged as a significant cause of gastrointestinal illness in humans (Girginkardesler *et al*. 2003; Johnson *et al*. 2004; Lagace-Wiens *et al*. 2006; Crotti and D'Annibale, 2007; Stark *et al*. 2009*a*, 2010*b*). As such, the lack of research being performed on this organism limits our capacity to introduce appropriate control methods. As the importance of this neglected parasite becomes increasingly recognized, the need for more research on this organism becomes more apparent.

The life cycles of *Dientamoeba*'s closest relatives are generally well characterized and it is postulated that these organisms could provide some clues in relation to *Dientamoeba*'s mode of transmission. Based on the life cycles of *Histomonas* and *Tritrichomonas*, it is plausible that helminths and/or animals could play a role in the transmission of *Dientamoeba*. Moreover, the recent reports of pseudocysts and cyst-like structures in some trichomonads imply that similar structures could exist for *Dientamoeba*. Direct human-to-human transmission of *Dientamoeba* is also plausible given that *Histomonas* and *Tritrichomonas* can be transmitted between their respective hosts in this way. As none of these theories has been sufficiently proven or disproven, none can be dismissed at this stage.

Unfortunately, our lack of knowledge on the life cycle of *Dientamoeba* makes prevention and control of infections extremely difficult. Moreover, the fact that Koch's postulates have not yet been fulfilled for this organism means that some still consider its pathogenicity as a matter of question. Ultimately, it is essential that more research is carried out on *Dientamoeba* to better understand the life cycle and biology of this neglected albeit important organism.

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