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Molecular epidemiology of *Dientamoeba fragilis*

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ABSTRACT

The flagellated protozoan *Dientamoeba fragilis* is one of the most commonly diagnosed parasite of the human gut, with a global distribution. Nevertheless, essential aspects of its biology remain incompletely understood or controversial, most notably life cycle, host range, transmission routes and the ability to cause disease. Molecular epidemiologic studies are also scarce, and limited by the lack of informative genotyping tools. To date, two *D. fragilis* genotypes (1 and 2) are recognized, with a strong predominance of genotype 1 in both humans and few animal hosts. Recent studies have shown that a very low level of genetic variability characterizes parasite isolates collected in various geographic areas and from both symptomatic and asymptomatic cases. This has raised the hypothesis *D. fragilis* may be a clonal organism. The recent availability of transcriptome data should greatly assist the development of markers useful to understand genetic diversity of *D. fragilis* at the population level.

1. Introduction

Dientamoeba fragilis was first described almost a century ago and initially classified as an enteric amoeba of uncertain pathogenicity (Jepps and Dobell, 1918). Subsequent morphological, immunological and molecular studies have shown that the parasite is phylogenetically related to flagellated trichomonads. The currently taxonomy places *D. fragilis* in the Kingdom Excavata, Subkingdom Metamonada, Phylum Parabasalia, Class Tritrichomonadidae, Order Trichomonadida, Family Dientamoebidae, Genus *Dientamoeba*, and species *Dientamoeba fragilis*. A detailed account of the different studies that have contributed to the establishment of the current taxonomic placement of the parasite has been recently published (Stark et al., 2016).

Clinicians and diagnostic microbiologists have ignored the parasite for long, likely because of the scarce clinical relevance that was attributed to it (Johnson et al., 2004). While evidence supporting the pathogenicity of *D. fragilis* has been accumulated over the years, the notion that *D. fragilis* is truly a pathogen is still debated.

This review will briefly cover relevant aspects of epidemiology and then provides an account of the currently available approaches to characterize *D. fragilis* at the molecular level, and implication in terms of population genetics.

2. Epidemiology

2.1. Prevalence and geographic distribution in humans

Human infection with *D. fragilis* have been reported in many countries from all continents, although most studies are from industrialized countries and less is known from developing areas of the world (Barratt et al., 2011; Stark et al., 2016). Based on different diagnostic procedures, the prevalence ranges from as low as 0.2% to as high as 82%, and, contrary to what observed for other intestinal protozoa, is generally higher in developed countries. However, it is still difficult to conclude that such differences are genuine, as diagnostic procedures have different sensitivity and specificity, and difference in study design may also account for the large variation observed. Nevertheless, when only molecular epidemiologic studies are taken into account, it appears that high prevalence (> 20%) are observed in several regions of the world, including countries from Europe, Middle East, and South America (Table 1).

2.2. Age and gender distribution in humans

Infection with *D. fragilis* shows a marked age distribution in some studies but not in others, and therefore a unilateral trend has not emerged. A higher prevalence is often observed in children (e.g., Fletcher et al., 2014) and is considered the results of poorly developed hygiene habits and susceptibility to enteric infections. A second peak is seen in females of parental age (Röser et al., 2013a), who, in general, seem to carry *D. fragilis* more often than men (Barratt et al., 2011). Therefore, the concept that child-parent interaction influences the age and gender distribution of the parasite appears to be supported by the available data.

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Table 1
Prevalence of *D. fragilis* infection in humans, as determined by molecular methods.

Country	Year	Origin of samples	N. of samples	Prevalence (%)	Method	Reference
Netherlands	2009	Patients with GI complaints	397	32	qPCR	Bruijnesteijn van Coppenraet et al. (2009)
Australia	2010	Patients with GI complaints	750	5	qPCR	Stark et al. (2011)
Australia	2010	Patients	472	6	PCR	Stark et al. (2011)
Italy	2010	Patients	491	21	qPCR	Calderaro et al. (2010)
Pakistan	2010	Patients with IBS-associated diarrhea	171	4	PCR	Yakoob et al. (2010)
Netherlands	2011	Paediatric patients	739	38	qPCR	Verweij and van Lieshout (2011)
Denmark	2013	Patients with *C. difficile* infection	259	3	qPCR	Soes et al. (2014)
Denmark	2013	Patients without *C. difficile* infection	455	14	qPCR	Soes et al. (2014)
Iran	2013	Patients	1000	2	PCR	Sarafraz et al. (2013)
Denmark	2013	Patients	9945	43	qPCR	Röser et al. (2013a)
Denmark	2013	Patients with IBD	100	14	qPCR	Petersen et al. (2013)
Denmark	2013	Healthy controls	96	15	qPCR	Petersen et al. (2013)
Netherlands	2013	Children with GI complaints	163	62	qPCR	Maas et al. (2014)
Italy	2014	Patients	491	30.3	qpCR	Calderaro et al. (2014)
Sweden	2015	School A children, staff and parents	299	60	qPCR	Ögren et al., 2015
Sweden	2015	School B children, staff and parents	89	60	qPCR	Ögren et al., 2015
Portugal	2015	Children with acute gastrointestinal disease	176	6.3	qPCR	Júlio et al. (2015)
Brazil	2015	Asymptomatic individuals	88	21.6	PCR	David et al. (2015)
Netherlands	2016	Children with chronic abdominal pain	132	43.2	qPCR	de Jong et al. (2014)
Netherlands	2016	Healthy controls	77	50.6	qPCR	de Jong et al. (2014)
Netherlands	2016	Children with GI symptoms	107	55.1	qPCR	Holtman et al. (2017)
Netherlands	2016	Healthy controls	44	30.3	qPCR	Holtman et al. (2017)
Lebanon	2016	School children	249	60.6	qPCR	Osman et al. (2016)
Vietnam	2016	Patients with GI symptoms	180	2.1	qPCR	Ögren et al., 2016
Vietnam	2016	Healthy controls	88	2.3	qPCR	Ögren et al., 2016
Venezuela	2017	Rural community	228	35.5	qPCR	Incani et al. (2017)

2.3. Non-human hosts

Beside humans, very little is known about the natural host range of this parasite, and data remain sparse. Early studies were based on microscopy as the only diagnostic procedure, often without reporting the staining technique and photographs, making data difficult to compare. Therefore, more emphasis is given here to recent studies, which combine microscopy and molecular detection. The current data (Table 2) show that only a few animal species appear to shed *D. fragilis* with their feces. This includes non-human primates (gorilla; Stark et al., 2008; Lankester et al., 2010), livestock (pigs; Cacciò et al., 2012) and pets (dog and cat; Chan et al., 2016). The fact that the parasite circulates in livestock and pets suggests a potential for zoonotic transmission, which, in the case of pigs, is further supported by the presence of genotype 1 (Cacciò et al., 2012). However, the high prevalence observed in pigs in Italy (Crotti et al., 2007; Cacciò et al., 2012) was not confirmed in another study in Australia (Chan et al., 2016). Likewise, the single dog and cat samples positive for *D. fragilis* were tested by a commercial real-time test that did not allow sequence analysis of the products (Chan et al., 2016). Clearly, further investigations are required to understand the role of animals in the lifecycle and transmission of *D. fragilis*.

3. Transmission

The lifecycle of the parasite has been, and still is, unclear. The trophozoite, the vegetative form that thrives in the gut, has been for long the only described stage. Recently, however, a cyst stage has been described (Munasinghe et al., 2013; Stark et al., 2014). Two main routes of transmission have been considered: the first suggests the involvement of a helminth vector (*Enterobius* or *Ascaris*), whereas the second points to a typical fecal-oral route. Here below, we provide a short account of the evidence supporting these mechanisms, which are not mutually exclusive.

Table 2
Molecular detection of *D. fragilis* in non-human hosts.

Host	N of sample tested	N of positive samples	Detection method	Reference
Dog	56	1	qPCR	Chan et al. (2016)
Cat	43	1	qPCR	Chan et al. (2016)
Pig	38	24	qPCR, PCR and sequencing	Cacciò et al. (2012)
Gorilla	10	3	PCR	Stark et al. (2008)

3.1. Transmission via pinworms

The vector potentially involved is *Enterobius vermicularis*. There are a number of observations in support of this hypothesis. First, ingestion of nematode eggs from a carrier co-infected with *D. fragilis* resulted in infection of a volunteer (Ockert, 1972). Second, DNA of *D. fragilis* has been detected in DNA extracted from *E. vermicularis* eggs of human origin from adhesive tape samples, swabs, or female adult worms (Ögren et al., 2013; Röser et al., 2013b). Third, there is an epidemiological association between *Enterobius* and *Dientamoeba:* in fact, the parasites show a similar age distribution, and coinfection occurs at a higher than expected level (Clark et al., 2014). Finally, but importantly, there is a parallelism with a closely related organism, *Histomonas meleagridis*, a parasite of poultry. This parasite is transmitted with the eggs of the nematode *Heterakis gallinae* (Hess et al., 2015), and interaction between the two organisms leading to transmission of *Histomonas* have been described.

The arguments in favor of the helminth hypothesis can be criticized. In fact, detection of *D. fragilis* DNA in nematode eggs does not demonstrate the presence of live organisms. Likewise, epidemiologic associations may be blurred by the high frequency of polyparasitism, particularly among children. Finally, since *Histomonas* can spread between turkeys and from turkeys to chickens in the absence of the nematode (Armstrong and McDougald, 2011), nematode eggs are not indispensable for successful transmission.

3.2. Transmission via cyst

The existence of a classical fecal-oral route has gained support from