First Detection and Molecular Characterization of Dientamoeba fragilis in Cattle

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Research Article

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Abstract

*Dientamoeba fragilis* is a flagellated protozoan with amoeba-like morphology that inhabits the human gastrointestinal tract. It is endemic in a vast geography around the world, including developed countries. There are limited studies on non-human hosts of the parasite, and suitable hosts have not been clarified. The parasite has been detected in non-human primates, pigs, cats, dogs, and rats. There is no study in the literature investigating and detecting the presence of this parasite in cattle.

In the present study, stool samples taken from 128 different cattle and calves from 11 different farms between March 2017 and February 2020 were examined for the detection of *D. fragilis* via PCR. The isolates with the expected amplicon size were sequenced using the 18S ribosomal RNA region, and their genotypes were determined by BLAST analysis. Sequences were analyzed with the most similar and reference sequences in the literature, forming a phylogenetic tree.

We detected *D. fragilis* in 26 (20.31%) of the 128 stool samples. All PCR products selected for molecular analysis from positive samples had the same nucleotide sequence. As a result of BLAST analysis, all sequences were determined to belong to *D. fragilis* genotype 1.

This study determined for the first time that cattle are suitable hosts for *D. fragilis*. Furthermore, the parasite subtype we detected belongs to genotype 1, which is the most common type in humans, suggesting that the parasite may have a zoonotic character. Our result is important in terms of the epidemiology of the parasite, as the mode of transmission is controversial, and available data on its natural hosts are limited.

1 Introduction

*Dientamoeba fragilis* was first described as an apathogenic amoeba species in 1918. However, due to extensive morphological and genetic studies conducted in the following years, it was understood that the parasite was in fact a flagellate trichomonad. It is 5–15 µm in size, localized in the intestine, and detected in stool samples frequently as the trophozoite form (Silberman et al. 1996; Stark et al. 2016; Windsor and Johnson 1999). However, since the cyst form could not be detected for many years, the transmission route of *D. fragilis* was not clearly understood until the last decade. During this period, it was thought that the parasite was transmitted by trophozoites carried in nematodes eggs, such as *Enterobius vermicularis*, that can only settle in humans. In addition, due to the failure of different animal models, *D. fragilis* was thought to be identifiable only in humans (Stark et al. 2005).

Many studies from different countries provide information about the infection caused by *D. fragilis* in humans (Clemente et al. 2021; Sarzhanov et al. 2021; Sivcan et al. 2018; Stark et al. 2016; Yildiz et al. 2021). However, with the recent detection of the cyst form, it was thought that *D. fragilis* could also settle in non-human hosts, and studies aiming to detect the parasite in animals gained momentum (Cacciò et al. 2012; Chan et al. 2016; Munasinghe et al. 2013; Ogunniyi et al. 2014). Unfortunately, although a few
previous studies report the occurrence of *D. fragilis* in animals, there are almost no studies in the literature in which molecular methods were used for confirmation (Chan et al. 2016).

Two subtypes of *D. fragilis* have been detected in humans, genotype 1 and genotype 2. The most common type detected in humans is genotype 1. Genotyping studies using different gene regions such as 18S rRNA, actin, and elongation factor 1α gene report minor differences between the two subtypes (Stensvold et al. 2013). However, there is a paucity of data in the literature concerning the genetic diversity in animals, except for a few studies (Cacciò et al. 2012). In the limited number of studies reporting genotyping of *D. fragilis* isolates in animals, genotype 1 was detected more frequently, similar to the findings in humans (Cacciò et al. 2012).

The trophozoite form of the parasite, which is frequently detected, is highly vulnerable to environmental conditions. Of note, it is not possible to identify the parasite in fresh stool preparations without utilizing permanent staining. Moreover, the specificity and sensitivity of staining methods in diagnosing *D. fragilis* are very low compared to molecular methods. For these reasons, molecular methods such as PCR and real-time PCR (RT-PCR) are preferred for the detection of *D. fragilis* in recent years and are accepted as the gold standard (Stark et al. 2008a; Yıldız et al. 2021). Until now, *D. fragilis* has only been detected by molecular methods in cats, dogs, rodents, and pigs (Cacciò et al. 2012; Chan et al. 2016; Ogunniyi et al. 2014). There is no study investigating the presence of *D. fragilis* in cattle. Therefore, the present study aimed to investigate *D. fragilis* in cattle for the first time and genotype the detected parasites.

## 2 Material And Methods

### 2.1 Stool samples

Stool samples were obtained from 128 cattle and calves from 11 different farms located in the Aydin province of Turkey. Aydin is a city located on the south-western Mediterranean coast of Turkey. The samples were collected between March 2017 and February 2020 and taken only once from each animal under veterinary control. The ages of the animals varied between four weeks and four years. Stool samples of cattle were taken from the floor immediately after defecation, and there was no direct contact with the animals, and therefore, ethics committee approval was not required. All samples were transported with cold chain shipment and reached our laboratory in perfect condition.

### 2.2 DNA extraction and PCR

*Dientamoeba fragilis* 18S rRNA gene was amplified with the following primers: DF400 (Forward: 5'-TATCGGAGGTGGTAATGACC-3') and DF1250 (Reverse: 5'-CATCTTCCTCCTGCTTAGACG-3') in a single PCR. Reaction's settings were: three minutes of denaturation held at 94°C, followed by 30 cycles (1 min at 94°C, 1.5 min at 57°C, 2 min at 72°C), and final elongation at 72°C for 10 minutes (Stark et al. 2005). PCR products were analyzed by 1.5% agarose gel electrophoresis and then visualized with a UV gel imaging system.

### 2.3 Sequencing and molecular characterization
Some positive PCR products were sent to Medsantek Co. (Istanbul, Turkey) for purification and sequence analysis. *D. fragilis* sequences obtained using the Basic local alignment search tool (BLAST) were compared with existing sequences in Genbank. As a result of the analysis, the subtypes of the sequences with total or highest similarity were determined. Genetic distances between isolates were examined according to the 18S rRNA gene region. With the obtained sequences, a phylogenetic tree based on genetic distance was drawn by using Neighbor-Joining method and bootstrap tests (1000 replicates) with use of the Molecular Evolutionary Genetics Analysis version 11.0 (MEGA) application. The evolutionary distances of the sequences were determined by the Maximum Composite Likelihood method (Felsenstein 1985; Tamura et al. 2004; Tamura et al. 2021).

### 3 Results

In 26 of 128 isolates examined in our study, amplification was observed at the expected size (~863bp), demonstrating *D. fragilis* positivity (Fig. 1).

Of the 26 positive PCR products, five were selected by simple random sampling (paying attention to the fact that each sample was obtained from different farms), and sequence analysis was performed. All five sequences analyzed were 100% similar, and the obtained sequence was registered in Genbank (Reference code: Dfc09, Genbank Acc. No. ON242172). There is a single base difference between our sequence and the genotype 1 reference sequence (Genbank Acc. No. AY730405.1). As a result of the BLAST analysis, the two sequences showed 99.75% similarity. In addition, the strain we obtained was determined to be 100% similar to the *D. fragilis* strain obtained from pigs in Italy (Genbank Acc. No. JQ677148.1) and the *D. fragilis* strain obtained from human feces in Iran (Genbank Acc. No. AB692772.1). There was a 95.04% similarity between the *D. fragilis* genotype 2 reference sequence (Genbank Acc. No. DFU37461) and ours. The alignment of the *D. fragilis* reference sequences with the sequences we detected using the MEGA program is shown in Fig. 2.

It was determined that all five *D. fragilis* strains sequenced in line with our data belonged to genotype 1. The reference sequences, the most similar sequences with ours, and the *D. fragilis* isolate we obtained were evaluated together to form a phylogenetic tree (Fig. 3).

### 4 Discussion

*Dientamoeba fragilis* is a protozoan that has been neglected in human and animal studies, even though it has been more than 100 years since its description. It is almost impossible to detect the parasite in direct microscopic examinations. Furthermore, detection in stained preparations is also not easy even when assessed by experienced laboratory workers. Therefore, molecular methods are currently accepted as the gold standard for diagnosing *D. fragilis* (McHardy et al. 2014; Röser et al. 2013; Verweij et al. 2007). In studies comparing the specificities and sensitivities of different diagnostic methods in detecting *D. fragilis*, molecular methods seem much more sensitive than direct microscopy, stained preparation examination, and culture methods (David É et al. 2015; Sarafraz et al. 2013; Yıldız et al. 2021). However,
molecular methods are still not widely used in diagnosing this parasite since *D. fragilis* is frequently overlooked and molecular methods have a high cost compared to microscopic examinations. Hence, the reported *D. fragilis* detection rates often do not reflect the real-world status.

Almost all molecular data regarding the detection rates and characteristics of *D. fragilis* are obtained from scientific studies in humans (Bruijnesteijn van Coppenraet et al. 2009; Menéndez et al. 2019; Stark et al. 2014). Studies in animals are exceedingly rare even in comparison to human studies which are also insufficient. Thus, the zoonotic features of the parasite have not been clarified, and epidemiological data contain serious deficiencies. In addition, available data on the natural hosts of the parasite are minimal. Especially after the initial imaging of the cyst form of the parasite in 2013, a consensus emerged that the transmission route of *D. fragilis* was fecal-oral. This issue has made it more important to investigate the possible natural hosts and zoonotic characteristics of the parasite (Chan et al. 2016; Munasinghe et al. 2013). In this context, although there are few studies investigating *D. fragilis* in animals, this issue has become one of the topics that have interested researchers in recent years. In the first quarter of the 21st century, several studies were conducted that examined the role of different animals in the transmission of the parasite, possible natural hosts, and the elucidation of the zoonotic features of *D. fragilis*. In pioneering studies, various wild bird species and some domestic animals were examined, but the presence of *D. fragilis* was not detected (Ogunniyi et al. 2014; Stark et al. 2008b). However, as the scopes of the studies have been expanded, successful results are obtained. In a large-scale study conducted in Australia, 420 fecal samples were collected from 37 different animal species including cats, dogs, horses, lions, tigers and zebras, and all samples were examined for the presence of *D. fragilis*. In this comprehensive research, in terms of animal species, the method has also been strengthened by using three different diagnostic methods (conventional PCR, Real-time PCR and Nested PCR methods). As a result, *D. fragilis* DNA was detected in only 2 of 420 samples (a cat and a dog). There are no cattle among the 37 animal species included in the study (Chan et al. 2016).

In a study examining gastrointestinal parasites in the howler monkey species (*Alouatta palliata aequatorialis*), 96 stool samples were examined, and *D. fragilis* was detected in 3 samples (Helenbrook et al. 2015). In another study investigating the ectoparasites and endoparasites of rats in Nigeria, 50 house rats were examined for *D. fragilis*, and positivity was found in 2 of the animals (Ogunniyi et al. 2014).

A study similar to ours (due to the examination of livestock) reported the genotypes of *D. fragilis* detected. In a total of 152 pig feces samples, the presence of *D. fragilis* using microscopy and molecular methods was investigated. A total of 71 samples were positive for *D. fragilis*, and it was reported that pigs could be one of the natural hosts of *D. fragilis*. In addition, one of their sequences (Genbank Acc. No. JQ677148.1) showed 100% similarity with ours (Genbank Acc. No. ON242172), and it was also reported as genotype 1 (Cacciò et al. 2012). However, our study differs from others in terms of detecting the presence of *D. fragilis* in cattle for the first time and determining the genotypes of the isolates. In addition, the fact that the sequence we obtained is the same as genotype 1, which is the most frequently detected subtype in humans, supports the idea that the parasite may exhibit zoonotic transmission from cattle. It has also been thought that cattle may be one of the natural hosts for *D. fragilis*. In geographies where animal
husbandry is common, diseases seen in these animals cause economic losses and threaten public health.

5 Conclusion

It has been demonstrated for the first time in our study that cattle, one of the essential farm animals, is a suitable host for *D. fragilis*. This data is crucial to clarify the epidemiology of the parasite, as cattle are one of the most widely raised livestock worldwide and human interaction / exposure through this route is unquestionable. In addition, our data suggests that cattle may represent an important factor of the zoonotic transmission dynamics of the parasite, leading to a possible risk to public health. Therefore, to investigate the natural host range of *D. fragilis* and to better understand the possible zoonotic transmission mechanism, we believe that larger-scale studies with other farm animals and different domestic animals should be conducted.

Declarations

**Funding:** Not applicable.

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**Authors contributions:** İ.Y. wrote the main manuscript text, İ.Y prepared figures 2-3 and Z.E.A prepared figure 1, Z.E.A contributed to study design. All authors read and approved the final manuscript.

**Ethics approval:** Stool samples of cattle were taken from the floor immediately after natural defecation, and there was no direct contact with the animals, and therefore, ethics committee approval was not required.

**Consent to participate:** Not applicable.

**Consent for publication:** All of the material is owned by the authors and/or no permissions are required.

References


Figures

Figure 1

Image of *D. fragilis* PCR products at 863bp after running on 1.5% agarose gel (M: marker, P: positive control, N: negative control).
Figure 2

Alignment of *Dientamoeba fragilis* Genotype 1, Genotype 2 and Dfc09 sequences with MEGA.

![DNA and protein sequence alignment]

Figure 3

Evolutionary distances of ON242172 (Adu Dfc09, sequence we obtained), JQ67160, AB692772, JQ677148, AY30405 (Genotype 1) and U37461 (Genotype 2) sequences.