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# Dientamoeba fragilis and Chronic Spontaneous Urticaria: A One Health-Based Zoonotic Perspective on Human-Parasite Interaction

## Dientamoeba fragilis y urticaria crónica espontánea: una perspectiva zoonótica basada en el principio de "Una Salud" sobre la interacción entre humanos y parásitos

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## ABSTRACT

*Dientamoeba fragilis* (*D. fragilis*) is one of the potential causes of Chronic Spontaneous Urticaria. The aim of the study is to investigate the association between urticaria and the presence of *Dientamoeba fragilis*, as well as to compare diagnostic methods. Additionally, this study aims to emphasize the zoonotic potential of *D. fragilis* within the One Health approach, which is based on the interactions between human health, animal health, and environmental factors. The study included patients with Chronic Spontaneous Urticaria (n: 90) and healthy individuals (n: 40). Direct microscopic examination was performed on stool samples using the native-Lugol method. DNA was isolated from stool samples, and Real-time Polymerase Chain Reaction was performed for detection. The incidence of *D. fragilis* was significantly higher in patients with Chronic Spontaneous Urticaria 17/90 (18.9%) compared to healthy controls 1/40 (2.5%) (p = 0.0261). In the direct microscopic examination of stool samples from Chronic Spontaneous Urticaria patients, *D. fragilis* was detected in 3 (2.3%) of the samples. *D. fragilis* was not detected in the stool samples of healthy volunteers by direct microscopy. However, the Real-time Polymerase Chain Reaction revealed *D. fragilis* DNA in one healthy sample. The data indicate that *D. fragilis*may impact on the chronic inflammatory skin disorders. Furthermore, the study highlights the value of molecular techniques, such as Real-time Polymerase Chain Reaction, for more accurate detection of zoonotic parasites.

**Keywords:** Chronic Spontaneous Urticaria; Dientamoeba fragilis; one Health; PCR; zoonozis.

## RESUMEN

*Dientamoeba fragilis* (*D. fragilis*), es un parásito zoonótico intestinal, y representa una de las posibles causas de la urticaria crónica espontánea. El objetivo de este estudio es investigar la asociación entre la urticaria y la presencia de *D. fragilis*, así como comparar métodos diagnósticos. Además, este estudio busca destacar el potencial zoonótico de *Dientamoeba fragilis* dentro del enfoque "Una Sola Salud", basado en la interacción entre la salud humana, la salud animal y los factores ambientales. El estudio incluyó a 90 pacientes humanos con urticaria crónica espontánea y 40 individuos sanos. Se realizó un examen microscópico directo de las muestras de heces mediante el método nativo con Lugol. Se aisló el ADN de las muestras de heces y se realizó la reacción en cadena de la polimerasa en tiempo real para su identificación. La incidencia de *D. fragilis* fue significativamente mayor en pacientes con urticaria crónica espontánea 17/90 (18,9%) en comparación con los controles sanos 1/40 (2,5%) (P= 0,0261). En el examen microscópico directo de muestras de heces de pacientes con urticaria crónica espontánea, se detectó *D. fragilis* en 3 (2,3%) de las muestras. No se detectó *D. fragilis* en las muestras de heces de voluntarios sanos mediante microscopía directa. Sin embargo, la reacción en cadena de la polimerasa en tiempo real reveló ADN de *D. fragilis* en una muestra sana. Los datos indican que *D. fragilis* puede tener un impacto en los trastornos inflamatorios crónicos de la piel. Además, el estudio destaca el valor de las técnicas moleculares, como la reacción en cadena de la polimerasa en tiempo real, para una detección más precisa de parásitos zoonóticos.

**Palabras clave:** Urticaria crónica espontánea; Dientamoeba fragilis; Una Sola Salud; PCR; zoonosis.

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## INTRODUCTION

Urticaria is a skin condition that affects both the skin and mucous membranes, causing symptoms such as congestion, redness (erythema), swelling (edema), and itchy plaques in the capillaries. This condition can significantly impact quality of life [1].

Urticaria is generally divided into two types: Acute Urticaria (AU) and Chronic Urticaria (CU). When the condition lasts for less than six weeks, it is classified as AU , if it persists more than six weeks, it is considered CU [2 , 3].

CU due to a known trigger is called Inducible Urticaria. However, when it occurs without an identifiable cause, it is referred to as CSU, which affects approximately 0.5 % to 5 % of the population [4 , 5 , 6].

*Dientamoeba fragilis* is a single-celled zoonotic organism and a common intestinal protozoan worldwide [7]. Known as a "flagellate-less" protozoan, it was first described approximately 100 years ago [8]. *D. fragilis* exhibits a pleomorphic trophozoite form, ranging in size from 4 µm to 20 µm [9].

The life cycle of *D. fragilis* has not been understood yet. It was previously thought that transmission occurred through trophozoites that were preserved within the eggs of helminths, such as *Enterobius vermicularis* and *Ascaris lumbricoides* [10].

Incidence rates are underestimated as a result of difficulties in accurate diagnosis [11]. A definitive diagnosis is performed by observation the parasite in stool samples that fixed, stained, and examined under a microscope [12]. Also, culture methods for *D. fragilis* include xenic culture might perform from stool sample [13].

The RT-PCR method confirmed specific detection of *D. fragilis* without interference from other similar Trichomonad organisms in human samples [14]. Treatments for *D. fragilis* infections include hydroxyquinoline, difetarsone, paromomycin, metronidazole, secnidazole, tetracycline, iodoquinol, and erythromycin [15].

Although the pathogenicity of *D. fragilis* is controversial, it can cause distinct symptoms, including abdominal pain and diarrhea [16]. Recent studies have also suggested an association between *D. fragilis* and urticaria [17 , 18]. Advances in molecular techniques have enhanced the understanding of its pathogenicity, and research has indicated a rise in positive cases among suspected infections [19].

The One Health approach, which considers environmental, animal, and human health, has become increasingly important in understanding infectious diseases, including parasitic infections. Many pathogens, especially zoonotic ones, are impacted by complex ecological and host-related factors and do not exist only within a single species [20].

Intestinal protozoa are implicated in non-gastrointestinal diseases such as CSU, a common inflammatory skin disorder with unknown etiology. Evidence suggests that chronic infections, immune dysfunction, and microbial interactions contribute to its development [21].

This study aims to investigate the presence of *D. fragilis* in patients with urticaria and examine its potential association with the disease. Additionally, it aims to compare the effectiveness of direct microscopic examination (DM) and PCR as diagnostic methods for *D. fragilis*. Furthermore, by exploring the association between *D. fragilis* and CSU, this study aims to emphasized a clinical association that has received limited attention parasitic infections within the One Health approach.

## MATERIALS AND METHODS

### Ethical statement

This study was approved as a research project by the Selcuk University Faculty of Medicine Local Ethics Committee with the decision dated April 1, 2020 (No: 2020/149).

### Selection of study groups, collection, and storage of samples

This study included 90 patients diagnosed with CSU applied the outpatient clinic of the Department of Dermatology and Venereal Diseases at Selçuk University Faculty of Medicine between June 2021 and December 2021. Patients were excluded from the study if they had suspected drug use, significant infections (viral, bacterial, or fungal) that could trigger urticaria within approximately one week, or had experienced intense stress. The control group consisted of 40 healthy adults who did not have any known acute or chronic diseases and had no history of CSU.

### Examination of stool samples by direct microscopy method

As part of the study, the presence of *Dientamoeba fragilis* was investigated in 130 stool samples using the DM method during routine parasitological examination. The stool samples were collected in fixative-free plastic containers and examined immediately upon arrival at the laboratory.

Stool samples were carefully mixed with 0.9 % NaCl (saline), and Lugol's iodine solution was applied to both edges of the slide. The samples were then examined under a light microscope at 20X and 40X magnification (Zeiss, Germany). Lugol staining facilitated the identification of *D. fragilis* by enhancing the visibility of its internal structures, allowing for detailed differentiation. After processing, the stool samples were stored at -80 °C for further analysis.

### Genomic DNA isolation

DNA isolation was performed using the ZymoBIOMICS™ DNA Miniprep Kit (Merck KGaA, Darmstadt, Germany). The extracted DNA samples were transferred into numbered Eppendorf tubes, labeled according to the corresponding volunteer, and stored at -20 °C.

### Real-time Polymerase Chain Reaction

In the RT-PCR analysis, DNA isolates obtained from the extraction process were used. The reaction mixture included the abm BlasTaq 2X qPCR MasterMix (Thermo Fisher Scientific, Germany) and Fluorogenic SYBR Green (Thermo Fisher Scientific, Germany). The amplification was performed in a 96-well plate using Roche LightCycler 96 (Basel, Switzerland) with appropriate primers.

**Statistical analysis**

R version 3.6.0 (The R Foundation for Statistical Computing, Vienna, Austria; [https://www.r-project.org] analysis was used. The Shapiro-Wilk test and Q-Q plot were used to assess the normality of the data, while the Levene test was used to evaluate the homogeneity of variances. Descriptive statistics for numerical data were presented as mean ± standard deviation (minimum – maximum), whereas categorical variables were summarized as numbers (n) and percentages (%). Statistical diagnostic measures were reported with a 95% confidence interval (CI). A 5 % significance level ($P < 0.05$) was considered for hypothesis testing.

## RESULTS AND DISCUSSION

The study participants ranged in age from 19 to 85 years (41.1 ± 16.85). The group included 57 males (43.8 %) and 73 females (56.2 %), with 90 CSU patients and 40 healthy individuals. Age and gender distributions were similar between the groups (P= 0.753 and P= 0.713, respectively).

In the direct microscopic examination from 90 CSU patients, *D. fragilis* was detected in 3 cases (2.3 %). However, *D. fragilis* was not found in any of the stool samples from 40 healthy volunteers.

According to RT-PCR results, *D. fragilis* DNA was detected in 17 (18.9 %) of the stool samples from the patient group. In contrast, among the stool samples of healthy volunteers, *D. fragilis* DNA was identified in only one case (2.5 %) (P= 0.0261).

The diagnostic performance of direct microscopy, using RT-PCR as the gold standard, is presented in TABLE I. The consistency between PCR and direct microscopy results was found to be 25.6 % (κ = 0.256), indicating a low level of concordance between the two methods.

**TABLE I**
**Comparison of the performance of PCR and Direct Microscopy methods in the diagnosis of D.fragilis**

| | PCR results | | |
|---|---|---|---|
| | **Negative** | **Positive** | **Total** |
| Direct Microscopy results | | | |
| Negative | 112 (TN) | 15 (FN) | 127 (97.7%) |
| Positive | 0 (FP) | 3 (TP) | 3 (2.3%) |
| Total | (86.2 %) | (13.8 %) | |
| Statistical Diagnostic Measures | | | |
| Sensitivity (95 % CI) | 16.7 (3.6 – 41.4) | | |
| Specificity (95 % CI) | 100 (96.8 – 100) | | |
| PPV (95 % CI) | 100 (29.2 – 100) | | |
| NPV (95 % CI) | 88.2 (81.3 – 93.2) | | |
| Accuracy (95 % CI) | 97.7 (93.4 – 99.5) | | |
| *K* | 0.256 | | |

**TN:** true negative, **FN:** false negative, **FP:** false positive, **TP:** true positive, 95 % **CI:** 95 % confidence interval, Sensitivity: sensitivity, Specificity: specificity, **PPV:** positive predictive value, **NPV:** negative predictive value, Accuracy: accuracy, **κ:** Value of Kappa test statistic.

Stool samples were also examined microscopically for intestinal parasites other than *D. fragilis*. *Blastocystis* spp. was the most frequently detected intestinal parasite in both groups, identified in 8 cases (8.8 %) among CSU patients and 1 cases (2.5 %) in the healthy control group. Additionally, *Entamoeba* spp. was detected in 2 CSU patients (2.2 %) and 1 healthy individual (2.5 %) (TABLE II).

**TABLE II**
**Intestinal parasites detected by direct microscopy**

| | Direct Microscopy | | |
|---|---|---|---|
| | ***D. fragilis*** | ***Blastocystis* spp.** | ***Entamoeba* spp.** |
| | n / % | n / % | n / % |
| Control Group (n:40) | 0/ 0,0 | 1 / 2.5 | 1 / 2.5 |
| Patient Group (n:90) | 3 / 3.3 | 8/ 8.8 | 2 / 2.2 |

*Dientamoeba fragilis* can cause distinct disease manifestations, such as abdominal pain and diarrhea [22 ,23]. Advancements in molecular diagnostic methods have allowed for a more precise examination of its pathogenicity. Studies have shown that the detection rate of *D. fragilis* has increased in suspected cases, further supporting its potential role as a pathogen. *D. fragilis* is among the parasites suspected to contribute to urticaria [24]. A meta-analysis conducted in Germany examined the possible relationship between *D. fragilis* and CSU. The analysis found that the incidence of intestinal parasites, including *D. fragilis*, in CSU patients can reach 75 % [25]. *D. fragilis* DNA was detected in 17 (18.9 %) of the stool samples from CSU patients in this study. Additionally, parasites such as *Blastocystis* spp. and *Entamoeba* spp. were detected from CSU patients.

*Dientamoeba fragilis* and *Enterobius vermicularis* were detected in 49 patients presenting with nausea, vomiting, diarrhea, abdominal pain, urticaria, anal itching, and weight loss in Spain. These patients were treated with metronidazole, and symptoms completely resolved in 43 cases. However, in 6 patients, symptoms persisted, and follow-up stool examinations after four weeks still tested positive for *D. fragilis*. A second treatment with paromomycin was administered to these patients, after which all recovered, and no *D. fragilis* was detected in their stool samples [26]. In this study, the success rate of direct stool microscopy was found to be lower than that of molecular methods. Direct stool microscopy may not be sufficient for diagnosis in clinically symptomatic patients.

Vezir *et al*. [1] investigated the prevalence of intestinal parasites in CSU patients, including 76 children and 38 adults in Turkey. In the pediatric group, stool sample analysis revealed *Blastocystis* spp. in 18.4 % (n=14), *D. fragilis* in 2.6 % (n=2), and *Giardia lamblia* in 1.3 % (n=1). In the adult group, *Blastocystis* spp. was the most frequently detected parasite, found in 18.4 % (n=7) of samples. Following anti-parasitic treatment, urticaria symptoms significantly improved in 57.1 % of pediatric patients and 60% of adult patients. In this study, a total of 8 *Blastocystis* spp. were detected in 90 adult CSU patients, which is consistent with the findings. These findings suggest that *Blastocystis* spp. and *D. fragilis* may contribute to chronic urticaria, significantly affecting patients' quality of life [1].

*D. fragilis* was detected in 59 (12.04 %) of 490 samples using RT-PCR analysis. Among patients with *D. fragilis* infection, diarrhea was significantly more common (16.3 %; P = 0.001). The diarrhea rate was higher in *D. fragilis* positive patients (84.09 %) compared to *D. fragilis* negative patients (P = 0.0005), showing a statistically significant association. According to the study results, the incidence of *D. fragilis* was significantly higher in patients with CSU (18.9 %) compared to controls (2.5 %) (P = 0.0261) [27]. *D. fragilis* DNA was detected in 17 (18.9 %) of the stool samples from the patient group. *D. fragilis* DNA was identified in only one case of healthy volunteers (2.5 %) which is consistent with the findings.

Thirty-one stool samples taken from patients with GIS disease in Australia and previously determined to be *D. fragilis* positive by microscopy were studied with the "Conventional PCR" method, targeting the SSU rRNA gene region. According to the results, *D. fragilis* was found positive in 29 of 31 samples. In Italy, researchers developed a new RT-PCR method for *D. fragilis* by targeting the SSU rRNA gene region. With this new method, false positives by cross-reacting with other Trichomonads other than *D. fragilis*, seen in previous RT-PCR methods, are eliminated. It has also been determined that this new method is more sensitive than "Microscopy" and "Conventional PCR" methods [6].

In Australia, 31 stool samples from patients with gastrointestinal system (GIS) disease, previously identified as *D. fragilis* positive through microscopy, were analyzed using the Conventional PCR method, targeting the SSU rRNA gene region. The results showed that *D. fragilis* was detected in 29 out of 31 samples [28].

A total of 472 stool samples were analyzed to compare the effectiveness of Multiplex PCR (MT-PCR), RT-PCR, and Microscopy methods. The study found that RT-PCR and MT-PCR demonstrated 100% sensitivity and specificity. In contrast, the microscopy method showed a specificity of 90 % but significantly lower sensitivity (40-50 %) [29]. These findings confirm that molecular methods provide higher sensitivity and specificity compared to microscopy, making them more reliable for detecting *D. fragilis*.

Stool samples from patients suspected to be *D. fragilis* positive in Netherlands were analyzed using the RT-PCR method. The results showed that 43 % of the samples tested positive for *D. fragilis* DNA. In comparison, positivity rates detected by microscopy ranged between 8 % and 19.8 %. These findings demonstrate that RT-PCR provides more accurate and reliable detection of *D. fragilis* compared to microscopy [30]. In this study, *D. fragilis* DNA was detected in 17 out of 90 CSU patient samples using PCR, whereas only 3 cases were identified through direct microscopy. Based on these results, the agreement between PCR and direct microscopy was found to be 25.6 % ($\kappa = 0.256$). In conclusion, RT-PCR exhibited higher sensitivity and specificity in detecting *D. fragilis* compared to direct microscopy, confirming its superiority as a diagnostic tool [31].

## CONCLUSION

The results of the study suggest a potential association between *D. fragilis* infection and Chronic Spontaneous Urticaria, emphasizing the importance of considering intestinal parasitic infections in the differential diagnosis of chronic inflammatory skin conditions.

Furthermore, the results highlight the importance of using molecular diagnostic techniques, such as Real-time Polymerase Chain Reaction, together with conventional microscopic methods for detection.

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**Conflict of Interest**

The authors declare no conflicts of interest.

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