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Makale yazarın başvurusu üzerine yayından kaldırılmıştır…

'CUMHURİYET TIP DERGİSİ EDİTÖRLÜĞÜNE

Derginizin Aralık 2018 (Volüme 40, Number 4) sayısında yayınlanan 10.7197/223.vi.470466 doi numaralı "The prevalence of Cyclospora cayatenensis in patients with gastrointestinal system complaints" başlıklı yazımızda pozitif örnekler başka bir çalışma için FDA (U.S. Food and Drug Administration)'ya gönderilmiş ancak pozitif örnek sayısı bizim makalemizle paralellik göstermemiştir. Makalemiz şu ana kadar herhangi bir atıf almamıştır. Bu durum göz önüne alınarak yazımızın yayından kaldırılmasını arz ederim.04.07.2019'

The prevalence of *Cyclospora cayatenensis* in patients with gastrointestinal system complaints

Gastrointestinal sistem şikayeti bulunan hastalarda *Cyclospora cayatenensis* yaygınlığı

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SUMMARY

Objective: In our study, the prevalence of *Cyclospora cayatenensis* was investigated molecularly in patients with gastrointestinal complaints.
Method: Seventy patients with gastrointestinal system complaints were included in the study. Nested PCR was applied to all samples taken from patients.
Results: In this study, 70 stool samples taken from the patients with gastrointestinal system complaints and sent to the parasitology laboratory of Ordu University Faculty of Medicine were examined molecularly for *Cyclospora cayatenensis* and of the 70 samples, 25 (35.7%) were found to be positive.
Conclusions: The high rates determined from the patient samples and the environmental samples with the studies suggest that this parasite may be the cause of an epidemic in the future. Especially diarrhea patients, water and food samples should be evaluated in terms of coccidian protozoa like *Cyclospora*.
Keywords: *Cyclospora cayatenensis*, Prevalance, PCR

ÖZET

Amaç: Çalışmamızda gastrointestinal sistem şikayeti bulunan hastalarda *Cyclospora cayatenensis* yaygınlığı moleküler olarak araştırılmıştır.
Yöntem: Gastrointestinal sistem şikayeti bulunan 70 hasta çalışmaya alındı. Hastalardan alınan bütün örneklere nested PCR uygulandı.
Bulgular: Çalışmada gastrointestinal sistem şikayeti bulunan hastalardan alınan ve Ordu Üniverstesi Tıp Fakültesi Parazitoloji Laboratuvarına gönderilen 70 adet gaita numunesi *Cyclospora cayatenensis* açısından moleküler olarak incelendi ve bu 70 örnekten 25 (%35,7) tanesi pozitif bulundu.
Sonuç: Yapılan araştırmalarla hasta örnekleri ve çevresel öreklerden tespit edilen yüksek oranlar, bu parazitin ileride bir salgının etkeni olabileceğini düşündürmektedir. Özellikle diyareli hastalar, su ve gıda örnekleri *Cyclospora* gibi coccidian protozoonlar açısından da değerlendirilmelidir.
Anahtar sözcükler: *Cyclospora cayatenensis*, Prevalans, PZR

INTRODUCTION

Nowadays, frequent use of immunosuppressive drugs with various reasons, antineoplastic chemotherapy, as a result of the progressive spread of immunosuppressive diseases all over the World increased the importance of opportunistic parasitic diseases in immunosuppressive cases. One of these diseases is cyclosporidiosis caused by *Cyclospora cayatenensis* (*C. cayatenensis*) which has increased importance in recent years [1]. Symptoms appear seven days after oocysts are taken orally through food or water in cyclosporidiosis infection. These symptoms manifest as severe diarrhea, nausea, fatigue, weight loss, gas and bloating, [2] while the severity of symptoms varies depending on the age of the host, the immune system and the number of oocysts taken orally [1]. Cyclosporidiosis is a common disease in developed countries such as America and Canada and developing countries. In 2017, there were a total of 1065 cases reported from 40 states in the U.S [3]. In Turkey, cyclosporidiosis has been ignored for years and some cases have been reported in recent years [4-7].

The diagnosis of *Cyclospora* infection is usually made by microscopic examination of oocysts stained with acid resistant stains in stool, duodenum aspiration fluid or biopsy samples. *Cyclospora* oocysts are difficult to diagnose because of the small number of feces. For this reason, the concentration methods should be used [1].

In our study, 70 stool samples taken from patients with gastrointestinal system complaints and sent to Ordu University Medical Faculty Parasitology Laboratory were examined molecularly for *Cyclospora*. The aim of this study was to determine the prevalence of *Cyclospora* which was ignored until recently.

MATERIAL AND METHODS

Ethical approval

This study was approved by the decision of the Clinical Trials Ethics Committee of Ordu University dated 03.24.2015 and numbered 2015/03.

Collection of the stool samples

In this study, 70 stool samples taken from patients with gastrointestinal system complaints and sent to Ordu University Faculty of Medicine Parasitology Laboratory were examined. Sheather's floatation method was applied to all samples before DNA isolation was performed.

Polymerase change reaction (PCR)

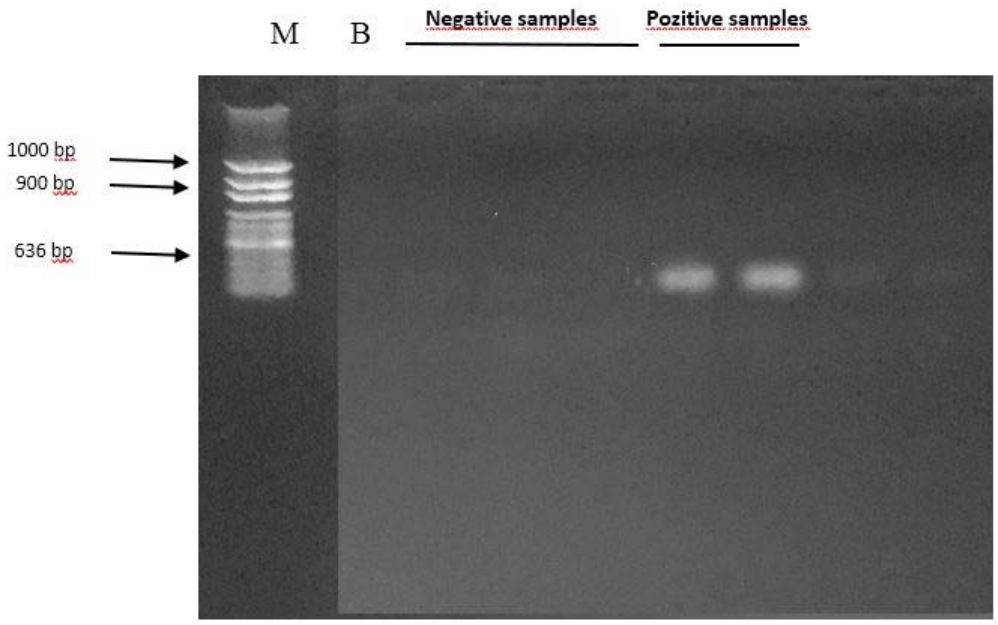
DNA extractions were performed with Qiagen DNA isolation kit (51306), and the PCR procedure was performed as follows.

Nested-PCR

In the study, two-stage nested PCR protocol, which is used frequently in routine, was used for the identification of *C. cayatenensis*.

The reaction mixture was prepared according to the Premix (Bioneeer, K-2111) kit protocol.

Accordingly, 14 µl of PCR water, 1 µl of each primer, 4 µl of sample DNA was added into 96-well ready-to-use Premix, and a total volume of 20 µl was obtained. R2B and F1E primers were used in the first stage of Nested PCR (Table 1). Conduct the first-round PCR were prepared with 10 min at 95 °C, 30 s at 95 °C, 45 s at 53 °C, 1 min at 72 °C, 5 min at 72 °C, and PCR completed in 35 cycles. The PCR product was run on a 1% agarose gel and 636 bp positive bands were detected (Figure 1).

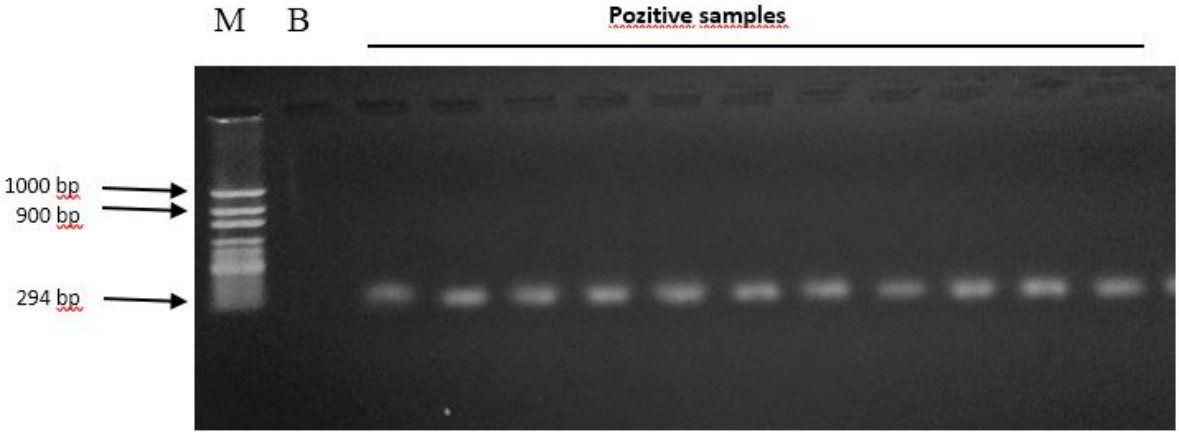


Şekil 1: Nested PCR firs-round image. M: Marker, B: Blank

In the second round of the nested PCR, the PCR product obtained in the first round was used instead of DNA. R4B and F3E were used at this round (Table 1). Conduct the second-round PCR were prepared with 10 min at 95 °C, 30 s at 95 °C, 45 s at 53 °C, 1 min at 72 °C, 5 min at 72 °C, and PCR completed in 35 cycles. The PCR product was run on a 1% agarose gel and 294 bp positive bands were detected (Figure 2).

Table 1: The PCR procedure was applied using primer pairs

Gene	Primer sequences 5'-3'	Amplicon size (bp)
F1E	TAC CCA ATG AAA ACA GTT T	636
R2B	CAG GAG AAG CCA AGG TAG G	
F3E	CCT TCC GCG CTT CGC TGC GT	294
R4B	CGT CTT CAA ACC CCC TAC TG	



Şekil 2: Nested PCR second-round image. M: Marker, B: Blank

RESULTS

In this study, 70 stool samples taken from patients with gastrointestinal system complaints and sent to Ordu University Medical Faculty parasitology laboratory were examined molecularly and *C. cayatenensis* was detected in 25 (35.7%) of stool samples.

DISCUSSION

When the *C. cayatenensis* life cycle is investigated, it is seen that oocysts excreted from the host with fresh feces are not infective. These oocysts are excreted with host feces and become infective in appropriate environmental conditions. Oocysts of *Cyclospora* spp. may remain long-term infective in foods, water and outdoor environment. It is reported that the transmission and spread of this infection can be caused by contaminated water and food. While this parasite is known to be a major public health problem in developed countries like USA, this situation has been ignored in Turkey for years. When the studies conducted in recent years, it is seen that there are still insufficient number of studies. In a study investigated in Turkey, stool samples from 326 patients with gastrointestinal complaints were examined phenotypically and *Cyclospora* oocysts were found in 23 (7%) patients. As a result of the study, it was concluded that *Cyclospora* parasite may be considered as a cause of disease in patients with gastrointestinal complaints after travel [8]. Another research was evaluated retrospectively. According to the results obtained from the study of 5073 patients who applied to the Parasitology Laboratory of Ege University Faculty of Medicine, 187 (16.43%) patients were diagnosed as *Cyclospora* phenotypically [9]. In recent years, parasitic contamination has been reported in studies investigated with environmental water samples in Turkey. In a study investigated with environmental water samples of Samsun province, 228 water samples were examined and 56 (24,5%) samples were determined *Cyclospora* spp [10]. Another study was investigated with 300 environmental water samples. Of these, 225 were streams and 75 of them consisted of sea water. Phenotypic examinations of the samples determined *Cyclospora* spp. oocysts in 112 (49.7%) samples [11]. In our study, patients with gastrointestinal complaints were evaluated molecularly and *C. cayatenensis* was determined in 25 (35.7%) of 70 patients. Our detection is particularly consistent with the high rates of *Cyclospora* found in environmental waters of the close region [10, 11]. In the literature search, we did not find any research on the contamination in foods of *Cyclospora* in Turkey. The fact that *Cyclospora* oocysts are found in the stool very little, and the experienced people are not in the hospital labs cause this parasite not to be detected. In a study, 225 children with diarrhea were examined. The samples were examined phenotypically by three independent researchers. The researchers were composed of microbiology and parasitology experts. Only one researcher in all of the samples identified *Cyclospora* spp. in one patient sample. As a result of research, different results could be obtained according to the experience and education level of the evaluator. Therefore, it

should be done by persons with adequate training and experience in these types of examinations like direct microscopy [12].

A majority of cases were reported as sporadic cases in Turkey. However, the high rates determined from the patient samples and the environmental samples with the studies suggest that this parasite may be the cause of an epidemic in the future. Especially diarrhea patients, water and food samples should be evaluated in terms of coccidian protozoa like *Cyclospora* spp.

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