Prevalence of *Cryptosporidium* spp. in Asymptomatic Food Workers

Asemptomatik Gıda Çalışanlarında *Cryptosporidium* spp.'nin Prevalansı

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ABSTRACT

Objective: *Cryptosporidium* spp. is obligatory intracellular parasite and causes intestinal infection. In intestine infections in the form of sporadic and epidemics, food and accordingly workers in food sector may play a role as the source of infection. In this study, it is aimed to reveal the existence of asymptomatic cryptosporidiosis.
Methods: In the study, stool samples of 393 workers -employed at various branches of food sector in the region of Van- are used. In order to detect *Cryptosporidium* spp. oocysts, Modified Ziehl Neelsen (MZN) Staining was used.
Results: In this study, asymptomatic cryptosporidiosis has been detected in 5 (1.27%) of 393 workers.
Conclusion: Epidemiological findings indicate that food workers can be source of cryptosporidiosis outbreak. Thus, searching for the existence of asymptomatic cryptosporidiosis food workers –which epidemiologically has potential significance- and taking the required measures in case of its determination are significant in respect of public health. *(Turkiye Parazitol Derg 2013; 37: 241-4)*

Key Words: Asymptomatic cryptosporidiosis, food worker, Modified Ziehl Neelsen.

Received: 20.11.2012 **Accepted:** 20.10.2013

ÖZET

Amaç: *Cryptosporidium* spp. zorunlu hücre içi parazit olup, bağırsak enfeksiyonuna neden olmaktadır. Sporadik ve salgınlar şeklinde neden olduğu barsak enfeksiyonlarında, gıdalar ve dolayısıyla gıda işçileri enfeksiyon kaynağı olarak rol oynayabilmektedir. Bu çalışmayla gıda işçilerinde asemptomatik kriptosporidiyozun varlığının ortaya konulması amaçlanmıştır.
Yöntemler: Çalışmada Van yöresinde gıda sektörünün çeşitli kollarında çalışan 393 işçiye ait dışkı örneği kullanıldı. *Cryptosporidium* spp. ookistlerinin tespiti için Modifiye Ziehl Neelsen (MZN) boyama yöntemi kullanıldı.
Bulgular: Çalışmada 393 gıda işçisinin 5'inde (%1,27) asemptomatik kriptosporidiyoz tespit edilmiştir.
Sonuç: Epidemiyolojik ve moleküler bulgular gıda işçilerinin kriptosporidiyoz salgınının kaynağı olabileceğini göstermektedir. Bu nedenle epidemiyolojik açıdan potansiyel önemi olan gıda işçilerinde asemptomatik kriptosporidiyozun varlığının araştırılması ve tespiti halinde gerekli tetbirlerin alınması, halk sağlığı açısından önemlidir. *(Turkiye Parazitol Derg 2013; 37: 241-4)*

Anahtar Sözcükler: Asemptomatik kriptosporidiyoz, gıda işçisi, Modifiye Ziehl Neelsen.

Geliş Tarihi: 20.11.2012 **Kabul Tarihi:** 20.10.2013

This study was presented in 2th National Veterinary Food Hygiene Congress 18-20 September 2006, İstanbul, Turkey.
Bu çalışma 2. Ulusal Veteriner Gıda Hijyeni Kongresi'nde sunulmuştur, 18-20 Eylül 2006, İstanbul, Türkiye.

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doi:10.5152/tpd.2013.2981

INTRODUCTION

Cryptosporidium spp. is a protozoan enteric parasite which can cause gastroenteritis in human and animals (1). *Cryptosporidium* spp. has both sexual and asexual development and a complex life cycle also covering autoinfectious cycles and it has ability to complete its development in a single host (2).

Cryptosporidium spp. can contaminate directly by individuals, as a result of contact with animal or through the contamination of water by asymptomatic or symptomatic animals and humans carrying *Cryptosporidium* oocysts. In human living under insufficient sanitation conditions exposure to *Cryptosporidium* oocysts is more frequent (3). This parasite can survive for weeks to months in the environment. Moreover its infective dose is low (4).

While some infections of cryptosporidiosis are acute and self-limiting, others are chronic. With pathogenic types, the period and severity of infection is relevant to the immune status of the infected individual. While slight, mild or severe illness occurs in immunocompetent individuals, severe chronic infection or death may occur in immunocompromised individuals (5). As this organism has the potential to cause extended urgent medical care and even death, cryptosporidiosis outbreak has importance in respect of public health (6). Persistent asymptomatic oocysts shedding can prolong following the clinical infection (7).

Cryptosporidium spp. is among the parasitic protozoons relevant to food production (8). Moreover *Cryptosporidium* spp is indicated among the five most important emerging food-borne zoonotic pathogens by some authors (9). Workers in food sector can play a role in the cryptosporidiosis outbreak (6).

Modified Ziehl-Neelsen staining method is a technique widely used for staining of oocysts in fecal smears (10, 11).

In this study, it is aimed to reveal the existence of asymptomatic cryptosporidiosis in food workers.

METHODS

In our study stool samples of 393 asymptomatic workers in food sector-employed within the province Van and its surrounding with an average age of 29.3- were used.

In order to detect *Cryptosporidium* spp. oocysts Modified Ziehl-Neelsen Staining method was used. With this aim a portion of stool samples were emulsified with formol-water containing 0.1% Tween 80. And then fecal smears were prepared. Fecal smears were covered with concentrated methanol and kept for 3 min. And then slides were covered with intense carbol-fuchsin and kept for 20 min. Slides rinsed with slight tap water were decolorized for 15-30 sec in methanol containing 1% HCl (v/v) and then were rinsed with tap water. It was kept for 30 sec in 0.4% malachite green as counterstain and rinsed with tap water. Slides dried by air were examined by 40x objective lens, and in case of the detection of oocysts the existence of oocysts were verified byx100 oil-immersion objective lens (10).

RESULTS

In our study stool samples of 393 workers -365 male and 28 female- employed at different business lines (restaurant, catering, bakery, canteen, cafe, butcher, school refectory, hospital refectory, food market, delicatessen) in food sector were investigated for the detection of *Cryptosporidium* spp. oocysts. As a result of the examination of stool samples *Cryptosporidium* spp. oocysts were detected in 5 (1.27%) samples of food workers one of whom was employed at hospital refectory, two at delicatessen and two at a butcher. The workers -in whom asymptomatic cryptosporidiosis was detected- were all male workers (Table 1).

DISCUSSION

Protozoon parasites can be transferred to food products through workers during the process of production (8). As *Cryptosporidium* does not multiply in food, food-borne cryptosporidiosis occurs via unhygienic food preparation, storage, preliminary preparation or food processing either through direct contamination by infected individuals during preparation or through fecal contamination of food (such as usage of contaminated water or biosolid, infected employee) (12).

Asymptomatic carriage can be significant and can cause person-to-person transmission (13). Chacin-Bonilla et al. (14) reported that they detected carriage at a rate of 60.6% in asymptomatic individuals among Indians of Venezuela. Park et al. (15)

Table 1. Prevalence of asymptomatic *Cryptosporidium* infection based on fecal oocyst detection in food workers by business lines

Business Lines	Gender (Male/Women)		Oocysts		Total	Number of positive specimens (% positive)
	M	W	M (+/-)	W (+/-)		
Restaurant	125	4	0/125	0/4	129	0
Catering	49	1	0/49	0/1	50	0
Bakery	45	3	0/45	0/3	48	0
Canteen, Cafe	43	0	0/43	0/0	43	0
Butcher	35	0	2/33	0/0	35	5.71
School Refectory	20	4	0/20	0/4	24	0
Food Market	17	8	0/17	0/8	25	0
Hospital Refectory	16	1	1/15	0/1	17	5.88
Delicatessen	15	7	2/13	0/7	22	9.09
Total	365	28	5/360	0/28	393	1.27

(+), *Criptosporidium* spp. oocyst positive; (-), *Criptosporidium* spp. oocyst negative.

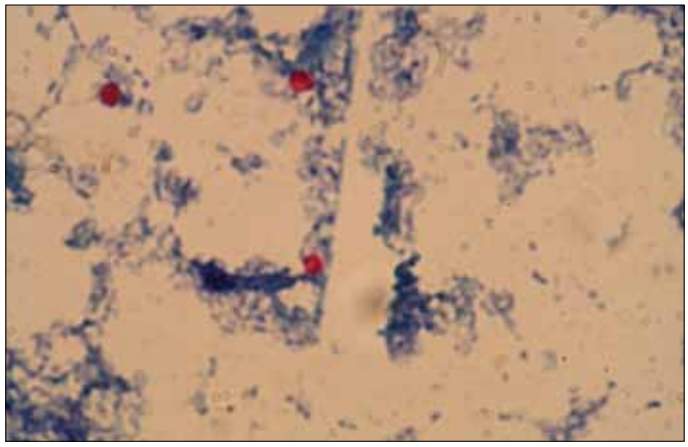

Figure 1. Modified Ziehl Neelsen stained slide showing three *Cryptosporidium* oocysts

in a study administered on individuals living in 25 different islands in Korea, detected *Cryptosporidium* spp. oocysts at a rate of 1.5%. Hörman et al. (16) informed that as a result of meta analysis studies, they estimated the rate of asymptomatic cryptosporidiosis prevalence in Scandinavian countries as 0.99%. In a study conducted in our country, positivity rate was detected as 3.1% in asymptomatic family members in the province of Mersin (17). However, in Sivas center, 6.2% *Cryptosporidium* antigen was detected in asymptomatic people (18).

In other studies conducted in our country regarding asymptomatic children, Otağ et al. (19) informed that they detected oocysts as of 5.5% and Akyön et al. (20) reported as 3.5% in children having diarrhea and that they could not determine oocyst in control group.

In Thailand it was reported that *Cryptosporidium* oocyst was detected as 0.3-12% in asymptomatic children (21). Oocyst was detected in asymptomatic children as 2.3% by Palit et al. (22), 24.17% by Okafor and Okunji (23), 3% by Reinthaler et al. (24), 6.4% by Pettoello-Mantovani et al. (25), 4.7% Al Braiken et al. (26) and 31.6% by Esteban et al. (27). While Freites et al. (28) informed that they detected oocyst as 11.8% in workers at food sector in Venezuela, Adam et al. (29) informed that they did not detect *Cryptosporidium* oocyst in the stools of asymptomatic workers employed in food sector and asymptomatic children.

The rate detected in our study (1.27%) was found to be in good agreement with the rate (1.5%) obtained by the studies of Park et al. (15). A difference from the positivity rates obtained by other studies was detected. This difference may arise from different methods employed (5, 30), geographical (9), seasonal (9,31-34) or age related differences.

Food workers employed in food production and catering services can play a role as a means in contaminating the products (35).

Pönka et al. (36) -in their first reports regarding an food-borne outbreak in Finland due to *C. parvum*- reported that stools samples were obtained from 12 of 72 individuals with diarrhea who dined at the same canteen and got sick, and that *C. parvum* was detected in four of these samples. The same researchers informed that they suspected of salad mixture as the source of outbreak and that the workers employed in food sector should be aware of the requirements for the appropriately prepared vegetables as to prevent contamination.

Quiroz et al. (6) as a result of the DNA analysis of *Cryptosporidium* isolates obtained from outbreak, reported that the possible source of the outbreak was an ill foodhandler. Moreover foodborne cryptosporidiosis outbreak due to fresh apple juice, non pasteurized milk, chicken salad, uncooked onion, fresh vegetable and fruit prepared by an infected cook was reported (37). While an effective drug for treatment and prophylaxis was not available until recently (5), nitazoxanide was licensed for the treatment of all age groups in the USA (12).

CONCLUSION

In the control of infection, the infection control measures are the basis, however, it has been informed that nitazoxanide can be used to control the outbreaks since the antimicrobial treatment may have potential contribution. Moreover the requirement for extensive studies regarding the parasitological and clinical effectiveness of nitazoxanide has been indicated (38). Schlundt et al. (9) emphasized the significance of scientific programs intended to decrease pathogens constantly at relevant points of food production chain. Presently, there is much debate with regard to the management of *Cryptosporidium*-positive food handlers within the food processing sector. Within this scope, for the prevention of contamination due to food workers, the screening stool specimens of personnel is suggested (35). Moreover it is also suggested that the food workers thoroughly wash their hands before handling food items and utensils and not contact cooked food, uncooked vegetables and fruits with bare hands that the kitchen surfaces be thoroughly washed between each preparation and that staff with gastroenteritis not be employed during their illnesses (39).

The existence of asymptomatic cryptosporidiosis in food workers who pose a potential epidemiological significance in respect for cryptosporidiosis-is revealed in this study.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

Peer-review: Externally peer-reviewed.

Ethics Commitee Approval: The study was conducted in 2006 and an ethics commitee approval was not received.

Informed Consent: The study was conducted in 2006 and an informed consent was not obtained.

Author Contributions: Concept - H.K.; Design - H.K.; Supervision - H.K., Y.G.; Funding - H.K .; Materials - H.K.; Data Collection and/or Processing - H.K.,Y.G.; Analysis and/or Interpretation - H.K.,Y.G.; Literature Review - H.K., A.D.A.; Writing - H.K., A.D.A.; Critical Review - Y.G., M.B., M.G.K.,K.E.; Other - M.B., M.G.K.,K.E.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Hakem değerlendirmesi: Dış bağımsız.

Etik Komite Onayı: Çalışmamız 2006 tarihinde gerçekleştirilmiş olup etik komite onayı alınmamıştır.

Hasta Onamı: Çalışmamız 2006 tarihinde gerçekleştirilmiş olup hasta onamı alınmamıştır.

Yazar Katkıları: Fikir - H.K.; Tasarım - H.K.; Denetleme - H.K., Y.G.; Kaynaklar - H.K.; Malzemeler - H.K.; Veri toplanması ve/veya işlemesi - H.K., Y.G.; Analiz ve/veya yorum - H.K., Y.G.; Literatür taraması - H.K., A.D.A.; Yazıyı yazan - H.K., A.D.A.; Eleştirel İnceleme - Y.G., M.B., M.G.K., K.E; Diğer - M.B., M.G.K., K.E.

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