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DETECTION OF *CRYPTOSPORIDIUM* SPP. IN VARIOUS FOODS

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Summary

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Certain types of parasites are known as one of the main factors of foodborne diseases all over the World. They threaten human health so that Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) highlighted this group as emerging foodborne pathogens. *Cryptosporidium* spp. is one of the mentioned threats, causing a global health problem, called cryptosporidiosis, especially in developing countries like Turkey. This genus is characterised by self-limiting watery diarrhoea but also, infection may turn into chronic and life-threatening type if the individuals are immunocompromised. The present study was planned to determine the presence of *Cryptosporidium* spp. in various foods such as strawberry, lettuce, minced meat and sand mussel samples. Totally, 160 samples were collected from various sales points (n=40 for each group). First, these samples were analysed microscopically, than a trliable molecular technique, known as polymerase chain reaction (PCR) was used for confirmation of parasite's genetic material (DNA). *Cryptosporidium* spp. oocysts were detected in 4 (10%); 1 (2.5%); 2 (5%) and 5 (12.5%) samples of strawberry, lettuce, minced meat and sand mussel by microscopic examinations while 4 (10%); 3 (7.2%); 2 (5%) and 7 (17.5%) of samples were positive in molecular examinations, respectively. In this context, further studies should be conducted on larger number of samples from different locations to achieve more data about this parasite.

Key words: *Cryptosporidium* spp., lettuce, minced meat, sand mussel

INTRODUCTION

Foodborne parasites are substantial harmful agents of human infectious disease all over the world. Food and Agriculture Organisation of the United Nations and the World Health Organisation have highlighted this group as emerging foodborne pathogens. In this context, any kinds of parasite originated from food including *Cryptosporidium* spp. have been identified. *Cryptosporidium* spp. constitutes a source of global health problem, especially in developing countries such as Turkey. It is an obligate protozoan, belongs to the family of *Cryptosporididae*, which is a member of Apicomplexa phylum. Currently, eight *Cryptosporidium* spp. have been reported in humans causing cryptosporidiosis: *C. hominis, C. parvum, C. meleagridis, C. felis, C. canis, C. muris, C. suis* and *C. cervine*. In the case of cryptosporidiosis, incubation period lasts approximately 7 days (1–14 days).

Along the illness, there may be seen various symptoms such as diarrhoea, enterocolitis, cholangiopathy, abdominal cramps, vomiting, headache, fatigue and low-grade fever but on the other hand, infection can turn to chronicle and life-threatening type if the individuals are immunocompromised. *Cryptosporidium* spp. frequently exists in a form, called oocyst which is highly stubborn and robust in environmental conditions. General distribution of the parasite is through the faecal-oral route and the oocysts are shed with the faeces of a host. For parasite infestation, exposure to only a few oocysts is enough. After ingestion of eggs, asexual and sexual reproductions happen in the intestinal tract and oocysts infect epithelial cells of stomach and intestine. Two important routes of transmission of cryptosporidiosis are known. One of them is direct contact with infected persons (person-to-person or anthroponotic transmission) or animals (zoonotic transmission) and the other one is consumption of contaminated water or food (waterborne/foodborne transmission). In recent years, especially foodborne cases have increased. Foods can be contaminated by the mentioned parasite cysts, coming from animal faeces, dirty equipment, vectors or positive food handlers.

MATERIAL AND METHODS

This study was aimed to detect *Cryptosporidium* spp. in various foods such as strawberries, lettuces, minced meat and sand mussels. For this purpose, 160 samples were collected from different sales points (n=40 for each group) and transferred to laboratory cooled. Analyses were held within 2 hours. First, 25 g of all samples were weighted in filtered bags, diluted with 100 mL dH_2O and placed on vertical shaker and agitated for 30 min at room temperature. Supernatant was discarded to clean 50 mL conical tubes and centrifuged for 20 min at 2,000×g. Supernatants were aspired with 10 mL left with the pellet and homogenised. To the eluate, was added 0.1 mL volume of 0.1N HCl for 5 mins a room temperature and neutralised with 0.01 mL 1N NaOH.

Microscopy: 200 µL was taken from eluate and dyed using acid fast staining method and examined under light microscope at 100× magnification. Red oocysts on blue debris were accepted as positive.

PCR confirmation of Cryptosporidium spp.: 5 mL of eluate was used for PCR amplification. PCR primers (Table 1) are summariaed below. PCR products were screened using agarose gel electrophoresis and visualised by using a UV transilluminator.

Table 1. DNA primer sequences for *Cryptosporidium* genus-specific PCR amplification

Primer	Primer Specificity	Primer Sequence	Amplicon size (bp)
ExCry1 (forward)	*Cryptosporidium* spp.	5'GCCAGTAGTCATATGCTTGTCTC3	844
ExCry2 (reverse)	*Cryptosporidium* spp.	5'ACTGTTAAATAGAAATGCCCCC3'	844

RESULTS AND DISCUSSION

In the present study, *Cryptosporidium* spp. oocysts were detected in 4 (10%); 1 (2.5%); 2 (5%) and 5 (12.5%) samples of strawberry, lettuce, minced meat and sand mussel by microscopic examinations while 4 (10%); 3 (7.2%); 2 (5%) and 7 (17.5%) of samples were positive in molecular examinations, respectively.

Fruits and vegetables are among the major sources for the transport of *Cryptosporidium* spp. These foods are soil-grown and soil contaminated by manure is an important route for contamination. Besides, direct or indirect contamination of produce may occur at many points between the farm and consumer such as irrigation, washing water, equipment or handlers (Gamble, 2015). In this study, strawberry and lettuce samples were collected from various sales points randomly as the representative samples of fruits and vegetables. Four of strawberry (10%) and 1 lettuce (2.5%) samples analysed microscopically gave positive result for *Cryptosporidium* spp. whereas 4 of strawberry (10%) and 3 of lettuce (7.2%) samples examined by PCR were positive. Some recent researches have also centered on contamination of raw vegetables and fruits. However, some of them support our study with their results (Rzezutka *et al.*, 2010; Dixon *et al.*, 2013; Hong *et al.*, 2014). Another source of parasites is seafood, especially molluscan shellfish (mussels, oysters, cockles etc.). They generally live in surface or wastewater sources and have a filter system for feeding. So it is quite normal that they can keep oocysts in their structure. Therefore, they are used as a means of sanitary assessment of water quality. In the demonstrated study, 40 of mussel samples were examined and 5 (12.5%) of them were found positive in microscopic analyses whereas 7 (17.5%) of them were detected by PCR. Some reviewed surveys have showed results comparable to ours, e.g. Lowery *et al.* (2001), Srisuphanunt *et al.* (2009) and Mladineo *et al.* (2009). In the present study, from the total of 40 minced meat samples, only 2 (5%) samples were detected positive in microscopic analyses and PCR. Even though the results we got in this study were lower, it does not mean that these kinds of products pose no risk for public health. Several researches related to cryptosporidiosis have demonstrated that some foods of animal origin such as meat and meat products have a great potential (Millard *et al.*, 1994; Yoshida *et al.*, 2007).

CONCLUSION

Even though the results we got in this study are lower, it does not mean that tested products pose no risk for public health. According to us, further studies should be conducted on larger number of samples from different locations to achieve more data.

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