Detection of *Cryptosporidium* and *Giardia* in foods of plant origin in North-Western Greece

Hercules Sakkas, Vangelis Economou, Petros Bozidis, Panagiota Gousia, Chrissanthy Papadopoulou and Panagiotis Karanis

ABSTRACT

Giardia and *Cryptosporidium* are recognized as leading causes of waterborne and foodborne diarrhoeal disease with worldwide distribution. The study aimed to determine the protozoan contamination of various foods of plant origin. A total of 72 samples from 27 different varieties of fresh vegetables and fruits were collected from supermarkets and open markets in North-Western Greece and were examined using conventional diagnostic methods. Two out of 72 (2.8%) samples were found positive for *Cryptosporidium* oocysts, while no sample was found to be positive for *Giardia* cysts. The results show the presence of protozoan contamination in foods of plant origin, which may constitute a potential health hazard.

Key words | *Cryptosporidium*, foodborne, *Giardia*, plant-based foods, protozoa

Hercules Sakkas
Petros Bozidis
Panagiota Gousia
Chrissanthy Papadopoulou
Microbiology Department, Faculty of Medicine, School of Health Sciences,
University of Ioannina,
45110 Ioannina,
Greece

Vangelis Economou
Laboratory of Hygiene of Foods of Animal Origin – Veterinary Public Health, School of Veterinary Medicine, Faculty of Health Sciences,
Aristotle University of Thessaloniki,
54124 Thessaloniki,
Greece

Panagiotis Karanis (corresponding author)
Medical Faculty and University Hospital Cologne,
University of Cologne,
Cologne,
Germany
and
Department of Basic and Clinical Sciences, Medical School,
University of Nicosia,
2408 Nicosia,
Cyprus
E-mail: *karanis.p@unic.ac.cy*

HIGHLIGHTS

- Determination of *Cryptosporidium* and *Giardia* in various foods of plant origin.
- Fresh vegetables and fruits from supermarkets and open markets.
- 2.8% samples were found positive for *Cryptosporidium* oocysts.
- The protozoan may constitute a public health risk through contamination of fresh vegetables.

INTRODUCTION

Foodborne diseases occur worldwide and outbreaks caused by contaminated food have drawn the attention of the scientific community, consumers and the mass media during the past few decades. Parasitic protozoa usually cause waterborne and foodborne outbreaks of asymptomatic to self-limited gastroenteritis; however, foodborne parasites, although neglected, can result in a high burden of disease, while human parasitoses can have prolonged and serious consequences especially in immunocompromised individuals (Karanis *et al.* 2007). The spectrum of foodborne parasites includes a variety of protozoa, cestodes, nematodes, and trematodes, which have been ranked considering the number and the distribution of foodborne infections globally, the severity of morbidity and case mortality rates, the potential for increased burden, the trade relevance and the socio-economic impact (Robertson *et al.* 2013).

Among protozoa, *Cryptosporidium* and *Giardia* are recognized as leading causes of waterborne and foodborne diarrhoeal diseases with worldwide distribution, since many outbreaks caused by both protozoan parasites have been reported during the last four decades (Karanis *et al.* 2002; Efstratiou *et al.* 2017). Interestingly, *Cryptosporidium* and *Giardia* were the causative agents in 60.3% and 35.2% of the worldwide waterborne parasitic protozoan outbreaks that occurred during the last decade (Baldursson & Karanis

doi: 10.2166/wh.2020.037

2011). Infections with both protozoa are most often acquired via ingestion of infectious (oo)cysts from faecally contaminated water or food, via direct or indirect contact with animals (zoonotic transmission) and via person to person contact (anthroponotic transmission) (Ahmed & Karanis 2018; Salma *et al.* 2020). Most often, outbreaks arise from the food service and catering industries (Ahmed & Karanis 2018) involving food handlers insufficiently trained in hygiene procedures, who are providing services to elderly or immunocompromised individuals (Girotto *et al.* 2013).

The faecal–oral route of transmission, along with the low infectious dose and the remarkable resistance to both environmental and water treatment procedures, poses a major risk factor influencing *Cryptosporidium* and *Giardia* contamination of foods, including vegetables and fruits (Smith *et al.* 2007; Li *et al.* 2020). Regarding household dietary habits, eating raw or undercooked fresh vegetables to maintain their taste and nutrients, may also increase the risk of foodborne transmission of both protozoa (Slifko *et al.* 2000). This study aimed to determine the protozoan contamination of various foods of plant origin and its importance for public health.

METHODS

A total of 72 samples from 27 different varieties of fresh vegetables and fruits were collected from supermarkets ($n = 31$) and open markets ($n = 41$) in Ioannina, the largest city in the Epirus Prefecture (North-Western Greece), which is built on the bank of Lake Pamvotis. Samples included: apples ($n = 3$), beetroots ($n = 3$), cabbage ($n = 3$), carrots ($n = 3$), celery ($n = 3$), cucumbers ($n = 3$), cucurbits ($n = 3$), dandelions ($n = 3$), eggplants ($n = 3$), endives ($n = 3$), grapefruits ($n = 2$), lettuce ($n = 3$), melons ($n = 2$), mint ($n = 2$), nectarines ($n = 2$), oranges ($n = 2$), parsley ($n = 3$), peaches ($n = 2$), pears ($n = 2$), pepper plants ($n = 3$), plums ($n = 2$), potatoes ($n = 3$), spinach ($n = 3$), spring onions ($n = 3$), strawberries ($n = 3$), tomatoes ($n = 3$) and watermelons ($n = 2$).

All samples were examined within less than 12 h using conventional diagnostic methods, as previously described (Ahmed & Karanis 2018; Hernandez-Arango *et al.* 2019). A thorough washing procedure with drinkable tap water (underground chlorinated water), which was previously examined for *Cryptosporidium* and *Giardia* infestation, was used to remove the (oo)cysts from the foods' surface. The water from the first washing was collected in sterile beakers and concentration was developed through centrifugation by the formalin-ether sedimentation procedure. The concentrated samples were examined with modified Kinyoun's acid fast staining (Thermo Fisher Scientific Remel Products, Lenexa, KS, USA) for *Cryptosporidium* oocysts and Trichrome stain (Thermo Fisher Scientific Remel Products) for *Giardia* cysts.

RESULTS

Two out of 72 (2.8%) samples examined were found positive for *Cryptosporidium* oocysts: strawberries collected from a supermarket and dandelions obtained from an open market. No sample was found to be positive for *Giardia* cysts (Table 1). The sample of strawberries was very heavily contaminated with bacteria, too. The drinkable tap water was found negative for both *Cryptosporidium* and *Giardia* contamination.

DISCUSSION

In contrast to foodborne diseases caused by biological and chemical hazards, the respective parasitic diseases generally do not receive extensive attention and awareness. They are even underestimated because they often cause asymptomatic or subclinical infection. Such infections caused by *Cryptosporidium* and/or *Giardia* may not lead the affected individuals to seek out health care services, thus going unreported. Also, protozoan identification is not performed routinely in laboratories. Today, foodborne outbreaks have significant economic consequences beyond the costs of healthcare, while they have a considerable impact on consumer confidence to existing food production practices and the end products (Tauxe 2002; Chomel 2008; Newell *et al.* 2010; Ryan *et al.* 2018). The observed emergence of parasitic foodborne diseases is attributed to the unprecedented globalization of fresh produce, the animal trade and human population migration and travel around the world, cultural

Table 1 | Detection of *Cryptosporidium* and *Giardia* in various fruits and vegetables

Samples	*Cryptosporidium*	*Giardia*
Apples ($n = 3$)	n.d.	n.d.
Beetroots ($n = 3$)	n.d.	n.d.
Cabbage ($n = 3$)	n.d.	n.d.
Carrots ($n = 3$)	n.d.	n.d.
Celery ($n = 3$)	n.d.	n.d.
Cucumbers ($n = 3$)	n.d.	n.d.
Cucurbits ($n = 3$)	n.d.	n.d.
Dandelions ($n = 3$)	1/3	n.d.
Eggplants ($n = 3$)	n.d.	n.d.
Endives ($n = 3$)	n.d.	n.d.
Grapefruits ($n = 2$)	n.d.	n.d.
Lettuce ($n = 3$)	n.d.	n.d.
Melons ($n = 2$)	n.d.	n.d.
Mint ($n = 2$)	n.d.	n.d.
Nectarines ($n = 2$)	n.d.	n.d.
Oranges ($n = 2$)	n.d.	n.d.
Parsley ($n = 3$)	n.d.	n.d.
Peaches ($n = 2$)	n.d.	n.d.
Pears ($n = 2$)	n.d.	n.d.
Pepper plants ($n = 3$)	n.d.	n.d.
Plums ($n = 2$)	n.d.	n.d.
Potatoes ($n = 3$)	n.d.	n.d.
Spinach ($n = 3$)	n.d.	n.d.
Spring onions ($n = 3$)	n.d.	n.d.
Strawberries ($n = 3$)	1/3	n.d.
Tomatoes ($n = 3$)	n.d.	n.d.
Watermelons ($n = 2$)	n.d.	n.d.
Total ($n = 72$)	**2/72 (2.8%)**	**0/72 (0%)**

n.d., not detected.

integration, especially concerning food habits, and climate changes (Chomel 2008; Broglia & Kapel 2011; Taghipour *et al.* 2019). Nevertheless, it was the observed increase in foodborne diseases worldwide which has eventually drawn concern to food-transmitted parasites. It has been reported that humans may act as hosts for approximately 300 and 70 species of helminths and protozoa, respectively, while a total number of 100 species are considered to be foodborne pathogens (Broglia & Kapel 2011).

The vegetable and fruit samples examined in our study could be contaminated with animal, bird or human faeces, either directly or indirectly, from contaminated water used for rinsing upon collection at the farm, from soil or sewage, during transport or by contaminated food handlers (Newell *et al.* 2010; Ahmed & Karanis 2018). In our study, the most probable cause of contamination of the locally produced fresh foods was reckoned to be the use of lake water from nearby Lake Pamvotis for watering or rinsing of the fresh produce. However, there are several animal breeding farms located within the proximity of the Pamvotis Lake ecosystem, which is surrounded by an urban and rural environment. The farms' effluents constitute a potential source of considerable microbial burden for the ecosystem, which is used for irrigation and leisure activities and may contribute to human infections with various pathogens including *Cryptosporidium*. In a previous study of ours for *Cryptosporidium* and *Giardia* infestation in Lake Pamvotis, both parasites were found in considerable numbers (Karanis *et al.* 2002). However, in the present study, the results indicate that *Cryptosporidium* occurrence in the examined vegetable and fruit samples was quite low (2.8%), while none of the samples was found positive for *Giardia* contamination. It must be pointed out, that our previous study on lake water was carried out before the Ioannina Municipality sewage treatment plant was operational and this observation indicates the importance of sewage treatment plants in public health issues.

Studies from different geographical areas and periods, which usually pose many procedural problems that arise from the heterogeneity of these parameters, have yielded variable results, which can be attributed to the diversity of collected samples and methodological approach and the substantial impact of several risk factors as well. Ortega *et al.* (1997) found 14.5% of the vegetables collected from a Peruvian endemic region and examined by direct microscopy, acid-fast staining (AFS) and immunofluorescent assays were positive for *Cryptosporidium* cysts. Different results were obtained from a study conducted in an African region where *Giardia* was observed in 9.6% of the vegetable and fruits collected from an open market (Bekele & Shumbej 2019). In another study, 7.4% of lettuce and 3.7% of cabbage samples, collected from supermarkets and street vendors, were positive for *Giardia* cysts by polymerase chain reaction (PCR) (Hernandez-Arango *et al.* 2019).

Apart from the sample size, our study also has methodological limitations. Although there is no 'gold standard per se' specifically designated for protozoan detection from foods of plant origin, there is an ISO standard method in place (18744:2016) for the detection of *Cryptosporidium* and *Giardia* in leafy vegetables and berry fruits. The ISO method, which is also applicable to other plant foodstuffs, and most of the used assays often demonstrate variable recovery efficiencies (Amoros *et al.* 2010). It has been reported that simple washing does not completely remove *Cryptosporidium* oocysts from the surface of the vegetables since they demonstrate high resistance to washing and are very well attached to the peel (Ortega *et al.* 1997). In such cases, the elution process should be used. However, the effectiveness of several different elution buffers and elution methods on oocysts recovery may be doubtful (Ahmed & Karanis 2018). Besides, immunofluorescence and PCR assays have been reported as more sensitive procedures than conventional microscopy, which has been reported as the 'gold standard' method for the detection of enteric parasites (Iqbal *et al.* 2015). In our study, we used AFS, a method which remains one of the most widely used assays in many resource-limited settings, since it is rapid, cost-effective, easy to perform, and the only requirement for a routine laboratory is just an ordinary light microscope (Ahmed & Karanis 2018). Nevertheless, considering the methodological limitations, the detection results with the employed AFS method may be an underestimation of the actual *Cryptosporidium* and *Giardia* contamination of fresh produce.

CONCLUSIONS

In conclusion, the results of the present study show that the protozoan contamination of foods of plant origin may constitute a potential health hazard if these kinds of foods, which are normally consumed unprocessed and raw, are not properly handled and hygienically processed at domestic, street vendor, restaurant or mass production level. Our findings underline the role of the polluted aquatic environment, in our case of the lake water, in the protozoan contamination of fresh produce and its impact on public health. Since there are limited data about the contamination of fruits and vegetables with protozoa, more research and surveillance studies are needed to evaluate the health impact of *Cryptosporidium* and *Giardia* contaminated fresh produce among different population groups (immunosuppressed, very young, very old, vegans, vegetarians, omnivorous) in different countries with different socioeconomic, sanitation and education levels.

REFERENCES

Ahmed, S. & Karanis, P. 2018 An overview of methods/techniques for the detection of *Cryptosporidium* in food samples. *Parasitology Research* **117**, 629–653.

Amoros, I., Alonso, J. L. & Cuesta, G. 2010 *Cryptosporidium* oocysts and *Giardia* cysts on salad products irrigated with contaminated water. *Journal of Food Protection* **73**, 1138–1140.

Baldursson, S. & Karanis, P. 2011 Waterborne transmission of protozoan parasites: review of worldwide outbreaks – an update 2004–2010. *Water Research* **45**, 6603–6614.

Bekele, F. & Shumbej, T. 2019 Fruit and vegetable contamination with medically important helminthes and protozoans in Tarcha town, Dawuro zone, South West Ethiopia. *Research and Reports in Tropical Medicine* **10**, 19–23.

Broglia, A. & Kapel, C. 2011 Changing dietary habits in a changing world: emerging drivers for the transmission of foodborne parasitic zoonoses. *Veterinary Parasitology* **182**, 2–13.

Chomel, B. B. 2008 Control and prevention of emerging parasitic zoonoses. *International Journal of Parasitology* **38**, 1211–1217.

Efstratiou, A., Ongerth, J. E. & Karanis, P. 2017 Waterborne transmission of protozoan parasites: review of worldwide outbreaks – an update 2011–2016. *Water Research* **114**, 14–22.

Girotto, K. G., Grama, D. F., da Cunha, M. J. R., Faria, E. S. M., Limongi, J. E., Pinto, P. M. C. & Cury, M. C. 2013 Prevalence and risk factors for intestinal protozoa infection in elderly residents at long term residency institutions in Southeastern Brazil. *Revista do Instituto de Medicina Tropical de São Paulo* **55**, 19–24.

Hernandez-Arango, N., Pinto, V., Munoz-Sanchez, D. & Lora-Suarez, F. 2019 Detection of *Giardia* spp. with formalin/ether concentration in *Brassica oleracea* (cabbage) and *Lactuca sativa* (lettuce). *Heliyon* **5**, e02377.

Iqbal, A., Labib, M., Muharemagic, D., Sattar, S., Dixon, B. R. & Berezovski, M. V. 2015 Detection of *Cryptosporidium parvum* oocysts on fresh produce using DNA aptamers. *PLoS ONE* **10**, e0137455.

Karanis, P., Papadopoulou, C., Kimura, A., Economou, E., Kourenti, C. & Sakkas, H. 2002 *Cryptosporidium* and *Giardia* in natural, drinking and recreational water of Northwestern Greece. *Acta Hydrochimica et Hydrobiologica* **30**, 49–58.

Karanis, P., Kourenti, C. & Smith, H. 2007 Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. *Journal of Water and Health* **5**, 1–38.

Li, X., Zhang, X., Jian, Y., Wang, G., Ma, L., Schou, C. & Karanis, P. 2020 Detection of *Cryptosporidium* oocysts and *Giardia* cysts in vegetables from street markets in the Qinghai Tibetan Plateau Area (QTPA) of China. *Parasitology Research* **119** (6), 1847–1855. doi.10.1007/s00436-020-06661-z

Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F., van der Giessen, J. & Kruse, F. 2010 Food-borne diseases – The challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology* **139**, S3–S15.

Ortega, Y. R., Roxas, C. R., Gilman, R. H., Miller, N. J., Cabrera, L., Taquiri, C. & Sterling, C. R. 1997 Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemic region in Peru. *The American Journal of Tropical Medicine and Hygiene* **57**, 683–686.

Robertson, L., van der Giessen, J., Batz, M., Kojima, M. & Cahill, S. 2013 Have foodborne parasites finally become a global concern? *Trends in Parasitology* **29**, 101–103.

Ryan, U., Hijjawi, N. & Xiao, L. 2018 Foodborne cryptosporidiosis. *International Journal for Parasitology* **48**, 1–12.

Salma, B., Sandie, E. B., Rajae, H., Antoine, H., Pierre, F., Loïc, F., Isabelle, V. & Jamaleddine, H. 2020 Detection methods and prevalence of transmission stages of *Toxoplasma gondii*, *Giardia duodenalis* and *Cryptosporidium* spp. in fresh vegetables: a review. *Parasitology* **147**, 516–532.

Slifko, T. R., Smith, H. V. & Rose, J. B. 2000 Emerging parasite zoonoses associated with water and food. *International Journal for Parasitology* **30**, 1379–1393.

Smith, H. V., Caccio, S. M., Cook, N., Nichols, R. A. B. & Tait, A. 2007 *Cryptosporidium* and *Giardia* as foodborne zoonoses. *Veterinary Parasitology* **149**, 29–40.

Taghipour, A., Javanmard, E., Haghighi, A., Mirjalali, H. & Zali, M. R. 2019 The occurrence of *Cryptosporidium* sp., and eggs of soil-transmitted helminths in market vegetables in the north of Iran. *Gastroenterology Hepatology From Bed to Bench* **12**, 364–369.

Tauxe, R. V. 2002 Emerging foodborne pathogens. *International Journal of Food Microbiology* **78**, 31–41.

First received 31 January 2020; accepted in revised form 6 May 2020. Available online 10 June 2020