



Kent Academic Repository

Suleiman, Aisha Jamo, Mavrides, Daphne E., Maxamhud, Sadiya, Gentekaki, Eleni and Tsaousis, Anastasios D. (2024) *Presence of Cryptosporidium parvum in pre-washed vegetables from different supermarkets in South East England: A pilot study.* Parasitology Research, 123 . ISSN 0932-0113.

Downloaded from

<https://kar.kent.ac.uk/106150/> The University of Kent's Academic Repository KAR

The version of record is available from

<https://doi.org/10.1007/s00436-024-08250-w>

This document version

Publisher pdf

DOI for this version

Licence for this version

CC BY (Attribution)

Additional information

Versions of research works

Versions of Record

If this version is the version of record, it is the same as the published version available on the publisher's web site. Cite as the published version.

Author Accepted Manuscripts

If this document is identified as the Author Accepted Manuscript it is the version after peer review but before type setting, copy editing or publisher branding. Cite as Surname, Initial. (Year) 'Title of article'. To be published in **Title of Journal**, Volume and issue numbers [peer-reviewed accepted version]. Available at: DOI or URL (Accessed: date).

Enquiries

If you have questions about this document contact ResearchSupport@kent.ac.uk. Please include the URL of the record in KAR. If you believe that your, or a third party's rights have been compromised through this document please see our [Take Down policy](https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies) (available from <https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies>).



Presence of *Cryptosporidium parvum* in pre-washed vegetables from different supermarkets in South East England: A pilot study

Aisha Jamo Suleiman¹ · Daphne E. Mavrides^{1,2,3} · Sadiya Maxamhud¹ · Eleni Gentekaki^{1,2} · Anastasios D. Tsaousis¹

Received: 15 March 2024 / Accepted: 24 May 2024
© The Author(s) 2024

Abstract

Cryptosporidium is an important water-borne and food-borne parasite with a high burden of disease. This organism has been shown to contaminate various leafy vegetables; however, studies assessing the presence of *Cryptosporidium* spp in pre-washed and ready-to-eat vegetables are limited. Routine surveillance in the UK revealed a nationwide exceedance of human cases of *Cryptosporidium*. Therefore, this study aims to assess the presence of this parasite in pre-washed vegetables from supermarkets in the UK. A total of 36 samples were purchased from four different supermarkets. A nested PCR targeting the SSU rRNA was carried out on 24 samples, 58% were PCR-positive for *Cryptosporidium*. Sanger sequencing confirmed that, of these sequences, 4/24 (17%) produced significant similarities to *Cryptosporidium parvum*. This study provides evidence for the presence of *C. parvum* in pre-washed and ready-to-eat vegetables. Future work to identify the point of contamination is required.

Keywords Cryptosporidium · Vegetables · Small subunit ribosomal RNA · Epidemiology · Food · Public health

Introduction

Cryptosporidium is an intracellular extracytoplasmic protozoan parasite that belongs to the phylum Apicomplexa (Ryan et al. 2016). It is the causative agent of cryptosporidiosis, which affects both humans and animals. Two species of *Cryptosporidium* most commonly cause the disease in humans and other vertebrate hosts: *C. parvum* and *C. hominis* (Widmer et al. 2020; Pinto et al. 2022). Symptoms in humans range from self-limiting nausea and abdominal cramps to life-threatening disease in children under 5 years

of age and immunocompromised hosts, such as HIV-positive individuals (Wang et al. 2018). It is the second most common cause of diarrhoea in children under 12 months of age and the second most common cause of diarrhoea-associated deaths in children between 12 and 23 months (Wang et al. 2018; Khalil et al. 2018; Kotloff et al. 2013). The parasite is transmitted through the faecal-oral route typically acquired via ingestion of food or water contaminated with oocysts, the transmission form of the parasite. Studies also suggest that *Cryptosporidium* can also be transmitted through inhalation of oocysts (Pinto et al. 2022; Nyangulu et al. 2019).

In industrialized countries such as the USA and the UK, *Cryptosporidium* has been a cause of waterborne and foodborne outbreaks. In the UK, between 1992 and 2003, *Cryptosporidium* caused 67 outbreaks in drinking water and swimming pools with the main causes being *C. parvum* and *C. hominis* (Smith et al. 2006). To date, the largest foodborne outbreak of the parasite reported in England and Scotland was from pre-cut vegetables sold in supermarkets with 74 confirmed lab cases of *C. parvum* (McKerr et al. 2015).

One of the reasons *Cryptosporidium* is prone to causing waterborne and foodborne outbreaks is due to its ability to form oocysts that resist environmental pressures. The formation of oocysts enables *Cryptosporidium* to survive outside a host for several months (Weir 2001). Moreover, the oocysts

Handling Editor: Julia Walochnik

Aisha Jamo Suleiman, Daphne E. Mavrides and Sadiya Maxamhud have equal contribution.

✉ Anastasios D. Tsaousis
A.Tsaousis@kent.ac.uk

¹ Laboratory of Molecular and Evolutionary Parasitology, RAPID Group, School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, UK

² Department of Veterinary Medicine, University of Nicosia School of Veterinary Medicine, 2414 Nicosia, Cyprus

³ Department of Basic and Clinical Sciences, University of Nicosia Medical School, Nicosia, Cyprus

are resistant to disinfection with chlorine and can only be reliably removed by boiling water or filtration (Weir 2001).

More recently, a spike in *Cryptosporidium* cases was noted during routine surveillance between August and October 2023 across various regions of the UK with about 2411 laboratory-confirmed cases (Peake et al. 2023). Current data shows *C. hominis* accounted for most of the cases and was associated with exposure to foreign travel, swimming, farm animals, and food consumption (Peake et al. 2023). However, no single exposure accounts for the widespread exceedance in cases, and investigations are ongoing. In recent years, ready-to-eat (RTE) vegetables have become increasingly attractive to consumers by readily providing healthy, no-preparation-needed nutrition. However, concerns have been raised regarding the detection of microorganisms such as bacteria, viruses, fungi, and parasites in RTE vegetables (Zhang et al. 2020; Gizzie and Adukwu 2023). In light of this, we wanted to investigate pre-washed and RTE vegetables as a means for *Cryptosporidium* spreading. Hence, this pilot study aims to assess

the presence of *Cryptosporidium* contamination in RTE from 36 prepacked bags containing a variety of vegetables (Table 1) from different supermarkets in the UK.

Materials and methods

In the period between May 2023 and July 2023, a total of 36 pre-washed vegetables were purchased from four major supermarkets (A, B, C, D) in Canterbury, Kent County, UK. Samples were randomly chosen from packaged RTE vegetables and duplicates from each variety were obtained. Two hundred and fifty grams of vegetables per package were placed in a beaker with 0.9% NaCl and thereafter placed in a shaking incubator at 37 °C for 60 min. The resultant fluid was collected into 50-ml falcon tubes and centrifuged at 3000 rcf for 10 min at 4 °C. The supernatant was then discarded, and pellets were frozen at – 20 °C or used immediately for DNA extraction as previously described (Jinatham et al. 2023). Genomic DNA was extracted from all samples

Table 1 Component ingredients in RTE samples. Nested PCR samples positive for *Cryptosporidium* spp. with *SSU* rRNA and *gp60* sequencing results. N/A (Not available)

Supermarket	Sample ID	Component vegetables	Country of origin/place of packaging	<i>Cryptosporidium gp60</i>
A	Mild and tender mixed baby leaf (MT1)	Baby spinach, green butterhead lettuce, red chard, ruby red chard	N/A / UK	
A	Mild and tender mixed baby leaf (MT2)	Baby spinach, green butterhead lettuce, red chard, ruby red chard	N/A / UK	
B	Sweet leaf salad (SLS1)	Iceberg lettuce, carrot, romaine lettuce, white cabbage	UK / UK	
B	Sweet leaf salad (SLS2)	Iceberg lettuce, carrot, romaine lettuce, white cabbage	UK / UK	
C	Sweet and crunchy salad (SCS1)	Iceberg lettuce, red cabbage, carrot	N/A	
C	Sweet and crunchy salad (SCS2)	Iceberg lettuce, red cabbage, carrot	N/A	<i>C. parvum</i>
C	Beetroot salad (BRS1)	Spinach, chard, lamb's lettuce	N/A	<i>C. parvum</i>
C	Beetroot salad (BRS2)	Spinach, chard, lamb's lettuce	N/A	<i>C. parvum</i>
D	British butterhead salad (BBS1)	Red butterhead lettuce, green butterhead lettuce, baby spinach	UK / UK	
D	British butterhead salad (BBS2)	Red butterhead lettuce, green butterhead lettuce, baby spinach	UK / UK	<i>C. parvum</i> IIaA17G2R1
D	Rocket and baby leaf salad (RBS1)	Rocket, baby red leaves, baby spinach, mizuna	A mix of UK and non-UK produce	
D	Rocket and baby leaf salad (RBS2)	Rocket, baby red leaves, baby spinach, mizuna	A mix of UK and non-UK produce	
D	British pea shoot salad (BPS1)	Baby spinach, pea shoots, baby red leaves	UK / UK	
D	Bistro salad (BS2)	Shredded beetroot, baby spinach, lambs' lettuce, ruby red chard	A mix of UK and non-UK produce	

using Purelink™ Microbiome DNA Purification Kit (ThermoFisher Scientific, UK) (soil samples) according to the manufacturer's instructions. A nested PCR was done on the extracted DNA to amplify the small subunit (*SSU*) rRNA gene sequence (Supplementary information). The 60-kDa glycoprotein (*gp60*) gene was also amplified by nested PCR on samples that were PCR-positive for *SSU* rRNA (Supplementary information) (Pinto et al. 2021). Gel extraction of the amplified products was carried out using a QIAquick Gel Extraction Kit (cat. Nos. 28704 and 28,706) according to the manufacturer's protocol. Purified products were sent off for Sanger sequencing at Eurofins Genomics (Cologne, Germany). The obtained chromatograms were viewed and edited using the Snap Gene Viewer software. Ambiguous nucleotides at the ends of each read were removed. We used nucleotide sequences as queries to Basic Local Alignment Search Tool (BLASTn) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) against the nr database in GenBank. Cloning was performed on sequences of unclear chromatographs as previously described (Betts et al. 2020).

Results

Twelve out of 36 samples did not yield sufficient DNA (less than 0.01 ng/μl) and were excluded from further analyses. Of the remaining 24, 14 were PCR-positive using *SSU* rRNA gene sequence amplification by nested PCR. All 14 were genotyped using *gp60*. Finally, a total of 4/24 were sequence positive for *Cryptosporidium*: BBS2, BRS1, BRS2 and SCS2. The percent identity was 99.28% (CP082114), 99.16% (CP141123), 97.05% (CP141123) and 94.60% (MK391454), respectively.

The sequences have been submitted to GenBank under accession numbers PP502149, PP828699-PP828701.

Discussion

Infection with *Cryptosporidium* causes long-term sequelae such as diarrhea, abdominal pain, nausea, headaches, and fatigue. Infection is associated with failure to thrive, malnutrition, cognitive deficits, and stunting in infants and children (Khalil et al 2018; Vanathy et al. 2017). Therefore, efforts to prevent infection and outbreaks should be strictly enforced. In Western countries, one of the sources of infection that is frequently overlooked is the consumption of RTE vegetables. The Food Standards Agency (FSA) in the UK has recommended rewashing RTE (ACM/891 n.d.). The findings of our study could have implications for public health in the UK. Notably, the species identified herein was the zoonotic *C. parvum* rather than the anthroponotic *C. hominis* as is the

case with the current exceedance. As such, further investigation is warranted.

Pre-washed and ready-to-eat vegetables undergo more thorough washing with water containing chlorine disinfectants as compared to unpackaged vegetables and are expected to be free from parasites and therefore, can be eaten straight from the packaging. However, due to the resistance of oocysts to chlorine disinfection and their persistence on the vegetable surface, some remain, and are likely to cause illness (Barlaam et al. 2022). This is in keeping with previous evidence of *Cryptosporidium* contamination in pre-washed and ready-to-eat vegetables. Dixon et al. identified *Cryptosporidium parvum* in 5.9% of RTE vegetables in Canada (Dixon et al. 2013), in line with the results herein. Another study identified *Cryptosporidium* as the second most prevalent protozoan parasite present in 0.9% of RTE vegetables in Italy (Caradonna et al. 2017). As Italy is the second largest supplier of vegetables in Europe (Caradonna et al. 2017), it would be assumed that any contamination arising from production could lead to widespread distribution throughout Europe.

Herein, the parasite could originate from external contamination of vegetables happening along the chain of production including oocyst present in irrigation water, fertilizers, or transfer by handlers at the point of harvesting, processing, packaging, and/or transportation (Barlaam et al. 2022). Deciphering the point in the chain where contamination occurred is further complicated by the presence of multiple vegetable varieties in a package. Moreover, mixing contaminated and non-contaminated vegetables and recycling wash water could all lead to cross-contamination (Koutsoumanis et al. 2018). The presence of *Cryptosporidium* in pre-washed vegetables could mean revisiting the sanitation methods employed by suppliers along the chain of production such as improved hygiene measures during harvesting, processing, packaging, transportation, and storage (Jung et al. 2014). Other methods of sanitizing irrigation and washing water such as filtration, boiling, and the use of ozone could be explored (Gérard et al. 2019). There is also a need for increased awareness among consumers on adequate storage of vegetables and handwashing before eating. Better monitoring and regulation of pre-washed vegetables by appropriate authorities should also be encouraged.

This pilot study could unlock a potential common source of *Cryptosporidium* infection across the various regions in the UK. The vegetables from individual chain supermarkets are packaged in their own central facilities and subsequently distributed nationwide. Our study could contribute to developing better strategies to prevent infection with *Cryptosporidium*. Further studies could focus on tracking the exact point of contamination and addressing it.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00436-024-08250-w>.

Acknowledgements We would like to thank the technical services of the School of Biosciences at the University of Kent for supporting Aisha Jamo and Daphne E. Mavrides during their tenure at Tsaousis Lab.

Author contribution Conceptualisation, A.D.T.; methodology, A.J.S., D.E.M. and S.M.; validation, S.M., E.G. and A.D.T.; formal analysis, A.J.S., D.E.M. and S.M.; investigation, A.J.S., D.E.M. and S.M.; resources, A.D.T.; data curation, E.G.; writing—original draft preparation, A.J.S., D.E.M. and S.M.; writing—review and editing, E.G. and A.D.T.; visualisation, A.D.T.; supervision, E.G. and A.D.T.; project administration, A.D.T.. All authors have read and agreed to the published version of the manuscript.

Data Availability The sequences have been submitted to GenBank under accession numbers PP502149, PP828699-PP828701.

Declarations

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- ACM/891 (n.d.) Advisory Committee on the Microbiological Safety of Food Fresh produce: Agency advice on re-washing ready-to-eat leafy salads. UK Food standards Agency. Issue (cited 2024 Feb 25). Available from: <http://www.food.gov.uk/multimedia/pdfs/ecolitaskfinreport.pdf>
- Barlaam A, Sannella AR, Ferrari N, Temesgen TT, Rinaldi L, Normanno G et al (2022) Ready-to-eat salads and berry fruits purchased in Italy contaminated by *Cryptosporidium* spp., *Giardia duodenalis*, and *Entamoeba histolytica*. *Int J Food Microbiol*, vol 370. p 109634
- Betts EL, Gentekaki E, Tsaousis AD (2020) Exploring micro-eukaryotic diversity in the gut: co-occurrence of Blastocystis subtypes and other protists in zoo animals. *Front Microbiol* 25(11):502350
- Caradonna T, Marangi M, Del Chierico F, Ferrari N, Reddel S, Braccaglia G et al (2017) Detection and prevalence of protozoan parasites in ready-to-eat packaged salads on sale in Italy. *Food Microbiol* 67:67–75 (cited 2023 Aug 16)
- Dixon B, Parrington L, Cook A, Pollari F, Farber J (2013) Detection of *Cyclospora*, *Cryptosporidium*, and *Giardia* in ready-to-eat packaged leafy greens in Ontario Canada. *J Food Prot* 76(2):307–313
- EFSA Panel on Biological Hazards (BIOHAZ), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S et al (2018) Public health risks associated with food-borne parasites. *E F S A Journal*. 16(12):e05495. <https://doi.org/10.2903/j.efsa.2018.5495>
- Gérard C, Franssen F, La Carbona S, Monteiro S, Cozma-Petruț A, Utaaker KS et al (2019) Inactivation of parasite transmission stages: efficacy of treatments on foods of non-animal origin. *Trends Food Sci Technol* 1(91):12–23
- Gizzie NY, Adukwu E (2023) Assessment of microbial quality and effect of rinsing on pre-packaged salads. (cited 2023 Aug 25) <https://doi.org/10.13140/RG.2.1.1525.2561>
- Jinatham V, Wandee T, Nonebudsri C, Popluechai S, Tsaousis AD, Gentekaki E (2023) Blastocystis subtypes in raw vegetables from street markets in northern Thailand. *Parasitol Res* 122(4):1027–31 (cited 2024 Feb 25)
- Jung Y, Jang H, Matthews KR (2014) Effect of the food production chain from farm practices to vegetable processing on outbreak incidence. *Microb Biotechnol* 7(6):517–27 (cited 2024 Mar 1)
- Khalil IA, Troeger C, Rao PC, Blacker BF, Brown A, Brewer TG et al (2018) Morbidity, mortality, and long-term consequences associated with diarrhoea from *Cryptosporidium* infection in children younger than 5 years: a meta-analysis study. *Lancet Glob Health* 6(7):e758–68 (cited 2023 Aug 15)
- Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S et al (2013) Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 382(9888):209–22 (cited 2023 Aug 15)
- McKerr C, Adak GK, Nichols G, Gorton R, Chalmers RM, Kafatos G, Cosford P, Charlett A, Reacher M, Pollock KG, Alexander CL, Morton S (2015) An Outbreak of *Cryptosporidium parvum* across England & Scotland Associated with Consumption of Fresh Pre-Cut Salad Leaves. *PLoS One*. 10(5):e0125955. <https://doi.org/10.1371/journal.pone.0125955>
- Nyangulu W, Van Voorhis W, Iroh Tam PY (2019) Evaluating respiratory cryptosporidiosis in pediatric diarrheal disease: protocol for a prospective, observational study in Malawi. *BMC Infect Dis* 19:728. <https://doi.org/10.1186/s12879-019-4380-x>
- Peake L, Inns T, Jarvis C, King G, Rabie H, Henderson J et al (2023) Preliminary investigation of a significant national *Cryptosporidium* exceedance in the United Kingdom, August 2023, and ongoing. *Eurosurveillance* 28(43):2300538 (cited 2023 Nov 26)
- Pinto P, Ribeiro CA, Hoque S, Hammouma O, Leruste H, Détriché S et al (2021) Cross-border investigations on the prevalence and transmission dynamics of *Cryptosporidium* species in dairy cattle farms in western mainland Europe. *Microorganisms* 9(11):2394 (cited 2023 Dec 13)
- Pinto P, Ribeiro CA, Kváč M, Tsaousis AD (2022) *Cryptosporidium*. [cited 2023 Aug 5];331–89. Available from: https://link.springer.com/chapter/10.1007/978-3-030-80682-8_7
- Ryan U, Papparini A, Monis P, Hijjawi N (2016) It's official – *Cryptosporidium* is a gregarine: what are the implications for the water industry? *Water Res* 15(105):305–313
- Smith A, Reacher M, Smerdon W, Adak GK, Nichols G, Chalmers RM (2006) Outbreaks of waterborne infectious intestinal disease in England and Wales, 1992–2003. *Epidemiol Infect* 134(6):1141–9 (cited 2023 Aug 15)
- Vanathy K, Parija S, Mandal J, Hamide A, Krishnamurthy S (2017) Cryptosporidiosis: a mini-review. *Trop Parasitol* 7(2):72 (cited 2023 Aug 5)
- Wang ZD, Liu Q, Liu HH, Li S, Zhang L, Zhao YK et al (2018) Prevalence of *Cryptosporidium*, microsporidia and *Isoospora* infection in HIV-infected people: a global systematic review and meta-analysis. *Parasit Vectors* 11(1):1–19
- Weir E (2001) Public Health: The cryptic nature of cryptosporidiosis. *CMAJ: Can Med Assoc J* 164(12):1743 (cited 2023 Aug 24)
- Widmer G, Köster PC, Carmena D (2020) *Cryptosporidium hominis* infections in non-human animal species: revisiting the concept of host specificity. *Int J Parasitol* 50(4):253–262

Zhang H, Yamamoto E, Murphy J, Locas A (2020) Microbiological safety of ready-to-eat fresh-cut fruits and vegetables sold on the Canadian retail market. *Int J Food Microbiol* 16(335):108855

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.