Blastocystis in raw vegetables from street markets in northern Thailand

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Short Report

Keywords: Blastocystis, Subtype, Small subunit ribosomal RNA, Raw vegetables, Thailand

Posted Date: November 15th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2259198/v1

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Abstract

*Blastocystis* is a stramenopile protist of controversial pathogenicity. The organism colonizes a broad range of vertebrate and invertebrate hosts. *Blastocystis* has also been found in the environment both in water and soil. Several routes of transmission have been proposed including human-to-human, animal-to-human and via contaminated food and water. In recent years, the presence of *Blastocystis* in vegetables has started to be explored. However, most studies have focused on microscopic detection. Moreover, works of this type from Asia are barely available. Hence, the aim of this preliminary study was to examine the occurrence of *Blastocystis* in raw vegetables sold in markets in northern Thailand. Fresh produce (n=20) commonly used in Thai cuisine (Lanna) was purchased from two street markets and screened for *Blastocystis* using qPCR. *Blastocystis* was detected in 45% of the samples with the dominant subtype being ST3. Produce growing underground, such as galangal, carrot and beetroot were positive for the organism suggesting soil or inadequately composted manure as the source of contamination. To our knowledge, our study is the first to perform subtyping of *Blastocystis* in vegetables. Our results hint towards fresh produce being an, as yet, not widely explored, transmission route of *Blastocystis* in the studied community. Looking forward, large-scale investigations should on the prevalence of this and other organisms under the One Health umbrella should be undertaken.

Introduction

Fresh vegetables have been identified as contributors to foodborne transmission of micro-organisms (Abougrain et al. 2010; Al-Megrin 2010; El Said Said 2012; Rodrigues et al. 2020). Presence of parasites such as *Cryptosporidium* spp., *Giardia duodenalis*, *Cyclospora cayetanensis*, *Entamoeba* spp., *Toxoplasma gondii*, *Balantioides coli*, *Cystoisospora belli* and *Enterocytozoon bieneusi* has been reported on produce (Alemu et al. 2019; Amorós, Alonso, and Cuesta 2010; El Bakri et al. 2020; Duedu et al. 2014; Nyarango et al. 2008; Rodrigues et al. 2020; Utaaker et al. 2017). One of the less examined organisms with regard to foodborne transmission is *Blastocystis*, a common intestinal protozoan of controversial pathogenicity. *Blastocystis* has been found in a broad range of hosts, but also in the environment including water and soil (Elseadawy et al. 2022; Ithoi et al. 2011; Jinatham et al. 2021). Nonetheless, the food transmission route is only now beginning to be explored (Li et al. 2020; Siwila et al. 2020). *Blastocystis* has been detected in lettuce leaves and to a lesser extent in other salad vegetables, radish, spearmint and coriander (Caradonna et al. 2017; Heidar Nejadi 2021; Isazadeh et al. 2020; Rodrigues et al. 2020). Most studies exploring presence of *Blastocystis* in vegetables come from countries in the Middle East and South America. Information on its occurrence in fresh vegetables sold in markets in Asian countries is barely available (Punsawad et al. 2019). Previous works from our laboratory showed high prevalence of *Blastocystis* in the environment suggesting potential contamination of drinking and/or edible sources (Jinatham et al. 2021; 2022). Hence, the objective of this pilot study was to determine the presence of *Blastocystis* and its subtypes in raw, commonly consumed vegetables sold in street markets in Chiang Rai province, northern Thailand.
Methods

The study took place in Chiang Rai province, which borders Myanmar in the north. Two street markets located in Muang district were selected, 10 kilometers apart. Twenty raw produce samples were randomly purchased. These consisted of vegetables, tubers and herbs commonly consumed in northern Thai (Lanna) cuisine (Fig. 1). Vegetables were placed separately in clean plastic bags and transported to the laboratory for immediate processing. A total of 200 grams of each vegetable sample was soaked in physiological saline solution (0.95% NaCl) and shaken for 10 minutes as previously described with some modifications (Fallah, Makhtumi, and Pirali-Kheirabadi 2016). In 16 cases, the part of the produce above the ground was sampled, in two cases the root was sampled, while in two (i.e. coriander and long coriander) both parts were sampled. One mL of eluent was placed in LYSGM media and incubated at 37°C. Cultures were examined using a light microscope. A total of 500 µL of culture sediment was used to extract genomic DNA using the high molecular weight extraction protocol (Matsuki et al. 2002).

The primer pair PPF1; 5’-AGTAGTCATACGCTCGTCTCAAA-3 and R2PP; 5’-TCTTCGTTACCCGTTACTGC-3’ was used to detect the presence of *Blastocystis* using qPCR as previously described (Jinatham et al. 2021, 2022; Poirier et al. 2011). The qPCR conditions were as follows: initial denaturation at 95°C for 5 min followed by 49 cycles of denaturation at 95°C for 5 sec, annealing at 68°C for 10 sec, and 72°C for 15 sec. Reactions were run on a Bio-Rad/CFX96 Touch Real-Time PCR Detection System. The qPCR products were purified using the GeneJET Gel Extraction Kit (Thermo Scientific; Wardmedic, Thailand) according to the manufacturer’s protocol. The sequences of the purified products were obtained using Sanger sequencing [Bionics Company, Korea].

Raw reads were checked individually using the 4Peaks software and ambiguous bases from both ends were removed. The subtype of the sequences was determined using a combination of blast, the *Blastocystis* typing database (https://pubmlst.org/bigsdb?db=pubmlst_blastocystis_seqdef) and phylogeny (data not shown).

Results And Discussion

In this study, *Blastocystis* was detected in 45% (9/20) of the produce using qPCR, including the three that were positive by microscopy. Of the 18 above ground samples eight (44%) were positive for *Blastocystis*. Of these, four were from produce with edible peel. Only one out of four (25%) root samples was positive. Sequences have been submitted to GenBank under accession numbers xxx-xxxx. Foodborne transmission of *Blastocystis* has been previously speculated. A study in Riyadh, Saudi Arabia reported the common occurrence of *Blastocystis* in leafy vegetables, most especially lettuce and parsley (Al-Megrin 2010). Similarly, the organism was detected in lettuce sold in markets of northern Brazil and in ready-to-eat (prewashed) packaged salads in Italy (Caradonna et al. 2017). In Iran, more than 20% of raw herbs (Heidar Nejadi 2021) and 16% of fresh vegetables (Isazadeh et al. 2020) were contaminated with *Blastocystis*. Our results revealed a higher percentage of *Blastocystis* in fresh produce than previous works elsewhere, likely due to the more sensitive nature of qPCR.
Of the *Blastocystis* positive samples, subtype (ST) 3 was the most common, comprising 78% (n = 7) of the total (Table 1). We have designated two of the sequences (22%) as unknown. In the blast search and in the phylogeny, both of these sequences group with subtype 30, however this placement is not entirely robust. Since there are only very few ST30 sequences in the database and the fragments generated in this study are relatively short, a longer fragment is necessary to conclusively determine the subtype. Subtype three is the most frequent and widely distributed globally having been found in humans, animals, and also the environment (Adamska 2020; Banaticla and Rivera 2011; I Lee et al. 2012; Ithoi et al. 2011; Javanmard et al. 2019; Jinatham et al. 2021, 2022; Koloren, Gulabi, and Karanis 2018; Leelayoova et al. 2008.) Previous studies with a similar focus used microscopic detection, hence it is not possible at this time to speculate whether ST3 is a common occurrence on vegetables.
Table 1
Occurrence of *Blastocystis* in vegetables bought at two street markets in Chiang Rai, Thailand. Asterisks denote samples that were sampled in the root and in the above ground part.

<table>
<thead>
<tr>
<th>Vegetable samples</th>
<th><em>Blastocystis</em> positive (ST)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Above ground</strong></td>
<td></td>
</tr>
<tr>
<td>Beetroot</td>
<td><em>Beta vulgaris</em> ST3 44%</td>
</tr>
<tr>
<td>Cabbage</td>
<td><em>Brassica oleracea var. capitata</em> UNK</td>
</tr>
<tr>
<td>Carrot</td>
<td><em>Daucus carota</em> ST3</td>
</tr>
<tr>
<td>Cauliflower</td>
<td><em>Brassica oleracea var. botrytis</em> -</td>
</tr>
<tr>
<td>Chinese Cabbage</td>
<td><em>Brassica pekinensis</em> ST3</td>
</tr>
<tr>
<td>Coriander*</td>
<td><em>Coriandrum sativum</em> -</td>
</tr>
<tr>
<td>Cucumber</td>
<td><em>Cucumis sativus</em> -</td>
</tr>
<tr>
<td>False Pak Choi</td>
<td><em>Brassica chinensis</em> -</td>
</tr>
<tr>
<td>Galangal</td>
<td><em>Alpinia galanga</em> ST3</td>
</tr>
<tr>
<td>Ginger</td>
<td><em>Zingiber officinale</em> -</td>
</tr>
<tr>
<td>Kale</td>
<td><em>Brassica alboglabra</em> ST3</td>
</tr>
<tr>
<td>Lemongrass</td>
<td><em>Cymbopogon citratus</em> ST3</td>
</tr>
<tr>
<td>Lettuce</td>
<td><em>Lactuca sativa</em> -</td>
</tr>
<tr>
<td>Long Coriander*</td>
<td><em>Eryngium foetidum</em> -</td>
</tr>
<tr>
<td>Onion</td>
<td><em>Allium cepa</em> -</td>
</tr>
<tr>
<td>Pumpkin</td>
<td><em>Cucurbita moschata</em> -</td>
</tr>
<tr>
<td>Tomato</td>
<td><em>Lycopersicon esculentum</em> UNK</td>
</tr>
<tr>
<td>Turmeric</td>
<td><em>Curcuma longa</em> -</td>
</tr>
<tr>
<td><strong>Root</strong></td>
<td></td>
</tr>
<tr>
<td>Asiatic Pennywort</td>
<td><em>Centella asiatica</em> - 25%</td>
</tr>
<tr>
<td>Chinese Morning Glory</td>
<td><em>Ipomoea aquatica</em> ST3</td>
</tr>
<tr>
<td>Coriander*</td>
<td><em>Coriandrum sativum</em> -</td>
</tr>
<tr>
<td>Long Coriander*</td>
<td><em>Eryngium foetidum</em> -</td>
</tr>
</tbody>
</table>

The presence of *Blastocystis* on produce in this and other studies is surprising given the low tolerance of the organism to oxygen (Tsaousis et al. 2018). The shape and surface of vegetables has been associated...
with the degree of contamination. Micro-organisms are usually attached to green leafy vegetables with uneven surfaces, whereas their occurrence on vegetables with smooth surfaces is lower (Abougrain et al. 2010; Damen et al. 2007; El Said Said 2012). The multi-layering and roughness of vegetable stems could create micro niches encouraging Blastocystis attachment.

Parasite contamination of vegetables can occur at any point in the supply chain, both at the pre- and post-harvest phases. Its occurrence depends on several factors such as water source, soil supplementation and presence of animals (Ismail 2016; El Said Said 2012). In Thailand, crops are regularly fertilized with inadequately composted manure from animals that are known to carry Blastocystis. Produce such as galangal, carrot and beetroot grows near and/or under soil, hence it is possible that the organism was established pre-harvest in these foodstuffs. Soil was also recently proposed as a route of Blastocystis transmission (Jinatham et al. 2021). Alternatively, the organism could have been acquired following harvest. Vegetables in this study were washed/sprinkled with tap water available in the markets before being distributed to the customers. Tap water in the same study area was previously shown to be contaminated with Blastocystis (Jinatham et al. 2022). Regardless, these sources of transmission should be investigated using a combination of molecular and epidemiological approaches.

This pioneer pilot study provides the first molecular investigation of Blastocystis and its subtypes in vegetables. Our results pinpoint towards fresh produce being a transmission route of Blastocystis. These findings have important implications for the reduction or avoidance of contamination by parasites in vegetables in the various stages of the food supply chain ensuring food safety. Identification of another potential transmission route of Blastocystis highlights the need of a One Health approach in future large-scale investigations on the prevalence of this and other organisms.

Declarations

Funding

This work was funded by the Thailand Research Fund (RSA6080048) awarded to Eleni Gentekaki. This research and innovation activity was funded by the National Research Council of Thailand (NRCT) scholarship (N41D640028) for developing Ph.D. students awarded to Vasana Jinatham.

Availability of data

Sequences generated in this study are available in GenBank

Authors’ contributions

Competing interests

The authors declare no competing interests

Conflict of interest

The authors declare no conflict of interest

Consent for publication

All authors revised the manuscript and gave final approval for publication

Ethics approval

Not applicable

Consent to participate

Not applicable

References


Figures

Image not available with this version

Figure 1

Detail of vegetables and tubers that were investigated for Blastocystispresence in this study