Cyclospora cayetanensis infection: vegetables and water as possible vehicles for its transmission in Lagos, Nigeria

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Cases of *Cyclospora cayetanensis* infection increasingly are being reported worldwide. Although the route of transmission is not yet fully understood, the oral-faecal route remains the only known and agreed route of transmission.[1] The methods in which water and food become contaminated remain speculative, apart from their contamination with human faeces.

Most of the recently reported outbreaks of cyclosporiasis, especially from the United States and Canada, have been attributed to the consumption of contaminated fruits such as berries.[2,3] The possible involvement of vegetables, such as lettuce and basil, as vehicles for transmission have also been documented.[4]

It has been suggested that vegetables be thoroughly washed to prevent the spread of infection, although this has been reported to remove less than 15% of the oocysts from vegetables.[5] However, washing may prove effective with fruits, especially when their surface is not open or cut.

Cyclosporiasis has recently been described in Nigeria[6] but to our knowledge no investigation has been undertaken to determine whether or not vegetables are a source of *C. cayetanensis* infection in that country. Therefore, the present study aims to determine the possible presence of *C. cayetanensis* in common edible vegetables and water in Lagos, Nigeria.

Common vegetables were obtained from markets in Yaba, Ojo, Gbagada, Cele-Oshodi and Idi-Araba (all cities in Lagos) and directly from vegetable farms. A total of 84 vegetable samples comprised a mixture green leaf, water leaf, ugwu leaf, bitter leaf, sokoyokoto, lettuce and igbagba.

Three types of water sample were obtained: commercial pure water sold in sachets and used widely by the Lagos public; public water collected from taps situated around the Yaba, Ojo and Idi-Araba areas; and, well-water used by farmers for the irrigation of vegetable beds. A total of 51 water samples were collected.

Vegetables were washed thoroughly with prefiltered distilled water in clean plastic bowls and the washed suspensions from each were transferred to sterile plastic universal tubes and concentrated by centrifugation at 1800 x**g** for 15 min. The water samples were passed through conventional filter papers (3µm pore size) into clean plastic bowls. The filter apparatus consisted of a 1 L conical flask and U-shaped glass funnel fixed with a cork through which the vacuum was applied by negative pressure. Suspension particles on the filter paper were washed off using prefiltered distilled water into a plastic beaker and transferred to centrifuge tubes. These were centrifuged at 1800 x**g** for 15 min.

Deposits from both the vegetable and water samples were examined for the presence of *C. cayetanensis* oocysts by direct light microscopy and after staining with a modified Ziehl-

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Table 1. Sample used in the study by type

Samples (*n*=135)	Number (%)	Number positive (%)
Vegetables (*n*=84 [62.2%])		
Amarantus cuadanis (greens)	21 (15.6)	0 (0)
Talinum triangulare (water leaf)	14 (10.4)	1 (0.7)
Cucurbita pep (Ugwu leaf)	11 (8.1)	0 (0)
Corchorus olitorius (Bitter leaf)	9 (6.7)	0 (0)
Vermonia amygdalina (Ewedu leaf)	9 (6.7)	0 (0)
Celosia spp. (Sokoyokoto)	8 (5.9)	0 (0)
Launace toraxacofolia (lettuce)	7 (5.1)	1 (0.7)
Solanum spp. (Igbagba)	5 (3.7)	0 (0)
Water (*n*=51 [37.8%])		
Pure water	29 (21.5)	0 (0)
Irrigation water	15 (11.1)	1 (0.7)
Tap water	7 (5.2)	0 (0)

Table 2. Samples used in the study by location

Location	Number (%) of vegetable samples	Number (%) of water samples	Total number (%)	Number positive (%)
Cele-Oshodi	16 (19.0)	0 (0.0)	16 (11.9)	0 (0)
Gbagada	14 (16.7)	0 (0.0)	14 (10.4)	0 (0)
Idi-Araba	12 (14.3)	8 (15.7)	20 (14.8)	2 (1.5)
Lagos	0 (0.0)	29 (56.8)	29 (21.5)	0 (0)
Ojo	25 (29.8)	6 (11.8)	31 (22.9)	0 (0)
Yaba	17 (20.2)	8 (15.7)	25 (18.5)	1 (0.7)
	84	51	135	3 (2.2)

Neelsen method[7] at x1000 magnification. Identification of oocysts was based on the size, morphology and staining characteristics. Confirmation of identity was performed at the Scottish Meningococcus and Pneumococcus Reference Laboratory, Glasgow, UK using a modified Ziehl-Neelsen method and autofluorescence.[7,8]

C. cayetanensis oocysts were present in three samples (2.2%) – a lettuce sample from Idi-Araba, a water leaf sample from Yaba market, and a well-water sample taken from a farm on which the water was used for crop irrigation; co-incidentally, also in Idi-Araba.

Vegetables and water remain potential vehicles for the transmission of intestinal parasitic agents worldwide. This study revealed the presence of *C. cayetanensis* oocysts in two commonly eaten vegetables (lettuce and water leaf) in Lagos, Nigeria, and also in a well-water sample used for the irrigation of vegetables on farms. Such wells are about a metre deep and over a metre wide, and are never covered. They are dug on the farm and therefore contamination could be expected, due to surface run-off or it being located in an area of fractured bed-rock. The actual reasons for contamination in this case, however, are not known.

Of the two vegetable types implicated, lettuce is more likely to be a potential vehicle for the transmission of this pathogen because it is eaten raw in salad meals, although it may be washed before consumption. However, washing may not remove all oocysts from a contaminated sample.

The results presented here re-emphasise the need for improved hygiene, especially during the preparation of food, although more studies are necessary to determine the extent of vegetables involvement in the spread of *C. cayetanensis* oocysts.

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Sequence analysis of partial regions of the 5.8S rRNA internal transcribed region 2 and 28S rRNA of *Isospora belli*

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Isospora belli is an important gastrointestinal protozoal pathogen in several mammalian host species and is particularly important in the aetiology of diarrhoeal disease in immunocompromised humans in tropical and subtropical regions, including tropical Africa, Brazil and South East Asia.[1-3] The prevalence of this parasite is particularly high in HIV infection and HIV-AIDS, and recent studies have shown infection rates of 2.5%, 11% and 17% in such patients in northern India, eastern India and Guinea-Bissau, respectively.[4-7]

Historically, laboratory diagnosis of this disease has been difficult, especially during the early years of human isosporiasis (c. 1915), due to morphological confusion with related genera including *Toxoplasma, Eimeria* and *Sarcocystis*.[8] Presently, several morphological criteria are used to identify *I. belli*, including the presence of ellipsoidal oocysts that range in length (20-30 µm) and width (10-19 µm), where sporocysts are rarely seen broken out of oocysts.[9]

The usual diagnostic stage in faeces is the presence of immature oocysts (containing two sporocysts, each with four sporozoites), as visualised by a spherical mass of protoplasm.[9] However, a recent review has highlighted the importance of molecular techniques in overcoming current diagnostic limitations.[10] Previously, Muller *et al*.[11] designed a specific polymerase chain reaction (PCR) assay to target the small-subunit (18S) ribosomal RNA (rRNA) sequence of *I. belli*, and Franzen *et al*.[8] have examined the taxonomical position of this protozoan, based on 18S rRNA sequence analysis.

To date, there have been no published sequence data available

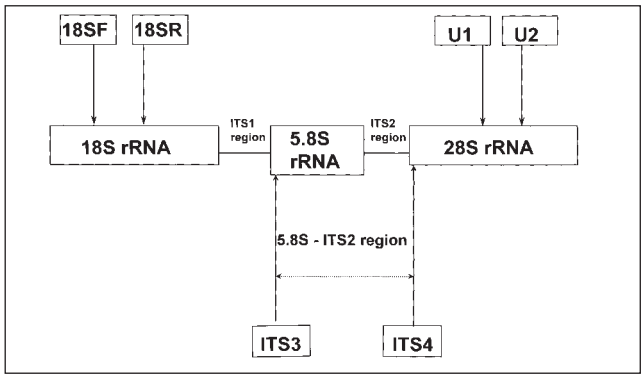

Fig. 1. Arrangement of ribosomal RNA gene loci of *Isospora belli* and location of oligonucleotide primer pairs used in the study.

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