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Outbreak of cyclosporiasis in British Columbia associated with imported Thai basil

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SUMMARY

Sporadic outbreaks of cyclosporiasis, a common cause of protracted diarrhoea in underdeveloped countries, are often undetected and undiagnosed in industrial countries. In May 2001, an outbreak of *Cyclospora cayetanensis* gastroenteritis was identified in British Columbia, Canada, with 17 reported cases. We conducted a case-control study involving 12 out of the 17 reported and confirmed case patients. Eleven (92%) of the patients had consumed Thai basil, an essential ingredient in Vietnamese cuisine, compared to 3 out of 16 (19%) of the control patients ($P=0{\cdot}003$). Trace-back investigations implicated Thai basil imported via the United States as the vehicle for this outbreak. This is the first documented sporadic outbreak of cyclosporiasis linked to Thai basil in Canada, and the first outbreak of cyclosporiasis identified in an ethnic immigrant population. This outbreak provides the opportunity to increase our understanding of this emerging pathogen and improve on our prevention and control for future outbreaks.

INTRODUCTION

Cyclospora cayetanensis is a coccidian parasite that causes a protracted and often, recurrent gastroenteritis resulting from foodborne and waterborne transmission. Previously thought to be limited to travellers or natives from countries of endemic areas such as Central and South America, Southeast Asia, the Caribbean islands and parts of Eastern Europe, this intestinal protozoan has been implicated in a number of documented foodborne and waterborne outbreaks in North America and Europe [1]. The largest documented outbreak occurred in May–June of 1996, affecting over 1400 people in both Canada and the United States, and was associated with consumption of fresh raspberries imported from Guatemala [1]. Other potential vehicles identified in outbreaks of cyclosporiasis in North America and Europe included fresh Mesclun lettuce imported from Peru (December 1997, Florida) [2], fresh mixed salad and herbs from Italy and France (December 2000, Germany) [3] and fresh basil from Mexico or the United States (July 1999, Missouri) [4]. Cyclosporiasis has only been a reportable disease in British Columbia (BC) since 1999, with a background incidence averaging approximately 20 new cases, mainly travel related, per year in a total population of 4 million.

In early May 2001, four initial cases of cyclosporiasis were reported to the BC Centre for Disease Control (BCCDC) Epidemiology Services. Public

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health follow-up investigations identified that three of the individuals were of Vietnamese origin and one was Caucasian. All individuals resided in Vancouver and Greater Vancouver, and all had a negative travel history. These patients presented to their family doctors with protracted watery diarrhoea. Initial exposure assessment interviews revealed that all four patients had consumed Vietnamese foods either at home or at a restaurant within the 14 days prior to the onset of symptoms. The objectives of this investigation were to (1) determine the extent of this outbreak, (2) perform a case-control study to implicate the source, (3) perform trace-back investigations to identify the supplier of the contaminated food source, and (4) perform laboratory studies to identify the organism in the food source.

METHODS

All cases were identified through passive reporting from accredited laboratories in the province of BC to public health. A case-control study was conducted on non-travel-related cases of laboratory-confirmed cyclosporiasis between 1 April and 15 June 2001. Case-patients or their physicians were requested to nominate one unrelated control per patient who were of the same age group (0–5, 6–12, 13–19, 20–40, 41–60, 61–100 years), gender, and ethnicity as the case, who had not shared meals with the case, had no symptoms of gastroenteritis and had a negative travel history. All subjects were interviewed using a structured questionnaire about symptoms, event-related exposures and consumption of raw produce, including herbs popular in Vietnamese cuisine, during the 2 weeks prior to illness onset. Collected data were entered and statistically analysed using the software package Epi-Info 6.04b (Centers for Disease Control and Prevention, Atlanta, GA, USA). Matched odds ratios and χ^2 tests were calculated. Conditional multiple logistic regressions were performed using LogExact 1.1 (Cytel Corp., USA).

To determine the sources of the implicated foods, environmental health officers (EHOs) interviewed staff of all identified restaurants and grocers and reviewed purchasing receipts to trace these food items back to the importers and the suppliers. The EHOs also reviewed the implicated restaurants' menu items to determine ingredients.

Implicated foods were collected from identified grocers and restaurants for laboratory investigations. Leftover implicated food from patients' refrigerators

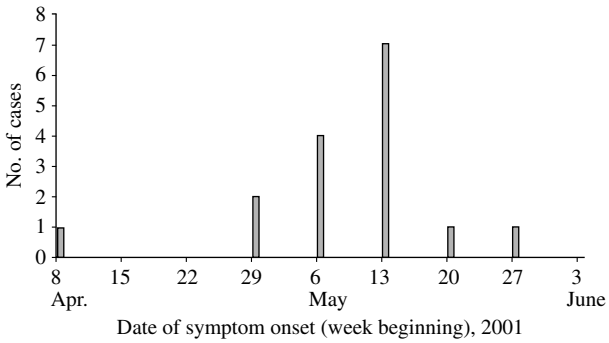

Fig. 1. Epidemic curve of an outbreak of cyclosporiasis in British Columbia in 2001.

was sought for all cases. Patients and laboratories were encouraged to submit preserved and unpreserved stool samples to the BCCDC Laboratory Services for confirmation of diagnosis.

The method used for recovery of cyclospora oocyst from food sources was based on the FDA protocol [5]. Implicated produce items were collected from restaurants A–F and markets 1, 2 and 4. Fifty grams of the submitted specimens were weighed into resealable plastic bags containing 50 ml deionized water. The samples were gently agitated for 60 min on a platform shaker set at 60–150 cycles per minute. The bags were inverted at 30 min to ensure thorough washing. The suspension was carefully removed into 50 ml tubes which were centrifuged at 1500 ***g*** for 10 min. Wet mounts of the supernatant from specimens obtained from markets 1 and 2 and restaurant A were prepared and viewed under UV light at 400× magnification. Differential interference contrast microscopy at 400× magnification was used on the same field to confirm internal structures of presumptive cyclospora oocysts. Cyclospora oocysts from known positive, patient stool samples were used as controls. These environmental specimens were also tested in duplicate using microscopy in a separate laboratory.

RESULTS

Thirty cases of cyclosporiasis were reported between 1 January and 15 June 2001. Sixteen of these individuals reported a negative travel history. The age of the case-patients ranged from 25 to 81 years, with a mean of 40 ± 3 years. Five (31%) persons out of 16 were Vietnamese, three (19%) Chinese and eight (50%) Caucasian. Their illness onset dates ranged from 13 April to 2 June 2001. The epidemic curve as shown in Figure 1 identifies the peak of this outbreak was the

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Table. *Culinary exposures in patients with cyclosporiasis and their matched controls during an outbreak of cyclosporiasis in British Columbia, Canada in May 2001*

Culinary exposure	Cases exposed $N=12$; n (%)	Controls exposed $N=16$; n (%)	Matched OR	P value
Berries				
Strawberries	7 (58·3)	13 (81·3)	0·4	0·43
Blackberries	0 (0)	0 (0)	—	—
Blueberries	0 (0)	0 (0)	—	—
Raspberries	2 (16·7)	0 (0)	—	0·48
Other fruit				
Mango	5 (41·7)	7 (43·8)	0·9	0·84
Dessert with fruits	0 (0)	7 (43·8)	—	0·04
Fruit salad	1 (8·3)	8 (50·0)	0·2	0·12
Lettuce and raw vegetables				
Mesclun lettuce	7 (58·3)	6 (37·5)	4·0	0·61
Butter leaf lettuce	4 (33·3)	6 (37·5)	0·2	0·50
Red leaf lettuce	2 (16·7)	7 (43·8)	0·1	0·23
Spinach	2 (16·7)	4 (25·0)	0·7	0·94
Watercress	1 (8·3)	1 (6·3)	1·0	0·48
Green onions	9 (75·0)	9 (56·3)	3·5	0·69
Herbs				
Thai basil	11 (91·7)	2 (12·5)	—	0·003
Sweet basil	5 (41·7)	5 (31·3)	1·7	0·82
Cilantro	10 (83·3)	9 (56·3)	2·5	0·44
Dill	1 (8·3)	2 (12·5)	0·5	1·0
Cumin	1 (8·3)	3 (18·8)	0·3	0·62
Chives	1 (8·3)	2 (12·5)	0·5	1·0
Vietnamese spinach	0 (0)	0 (0)	—	—
Mint	5 (41·7)	3 (18·8)	2·7	0·44
Peppermint	2 (16·7)	2 (12·5)	1·0	0·48
'Fish mint'	2 (16·7)	0 (0)	—	0·48
'Vietnamese mint'	4 (33·3)	1 (6·3)	—	0·25
'Vietnamese rosemary'	3 (33·3)	0 (0)	—	0·25
'Purple mint'	1 (8·3)	0 (0)	—	1·0
Sprouts				
Alfalfa sprouts	1 (8·3)	3 (18·8)	0·5	0·93
Bean sprouts	10 (83·3)	4 (25·0)	8·5	0·03

week of 13 May 2001. On review of their symptoms, 100% of the patients had diarrhoea, and over 80% experienced nausea (83·3%) and fatigue (81·8%). Other symptoms included anorexia (77·8%), myalgia (42·9%), abdominal cramps (53·8%), weight loss (61·5%) and bloating (69·2%).

Twelve of the identified patients with cyclosporiasis and their appropriately matched controls participated in this case-control study to identify the food source contaminated with *Cyclospora cayetanensis*. Results of their food exposure analysis are presented in the Table. Eleven out of the 12 persons with cyclosporiasis had exposure to *Ocimum basilicum* variety 'Thai basil', also referred to as 'Siam Queen', either at home or at a restaurant, compared to 2 out of 16 in the control group ($P=0·003$ on bivariate analysis). The single case that denied consumption of Thai basil admitted to consuming food 'contaminated' with the basil. This individual disliked all herbs and recalled removing the basil from the soup served at one of the implicated restaurants. One person only ate basil and avoided bean sprouts, however, there were no cases of single exposure to bean sprouts. Ten of the 12 case-patients consumed raw bean sprouts along with the Thai basil. Four out of 16 individuals in the control group had also eaten raw bean sprouts. Bean sprouts also had a significant exposure risk on bivariate analysis, with a P value of 0·03. However, when using conditional logistic regression with Thai basil and bean sprouts as predictor variables, only

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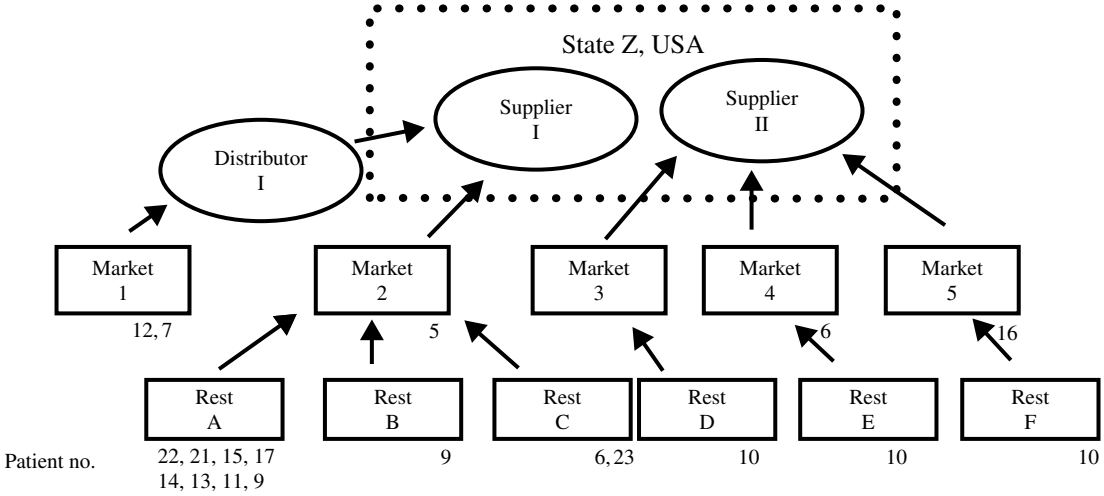

Fig. 2. Flow of Thai basil from patients to source during an outbreak of cyclosporiasis in Vancouver, British Columbia, Canada in May, 2001.

Thai basil was significantly associated (OR 6·59, $P = 0{\cdot}04$).

A few weeks post-study completion, an additional case of non-travel-related cyclosporiasis was identified in July 2001 from a person with a Japanese surname. As it turned out, this individual was indeed of Vietnamese origin, had purchased Thai basil from a market in late May, and had kept it in her freezer until consumption in July. She was the only person within her family who had eaten this herb and the only person with confirmed cyclosporiasis, in her family. There was no residual sample of the frozen basil for laboratory analysis.

Trace-back investigations identified bean sprouts originating from multiple sources locally, while Thai basil was imported from two distributors in the United States as depicted in Figure 2. This finding was reported to the appropriate food safety authorities for follow-up. Further trace-back confirmed that the Thai basil from the two distributors was grown within a single US state. Further information about the grower(s) was not available to the investigators. Therefore, we are unable to speculate on whether the outbreak was due to a single source or was a result of two simultaneously contaminated batches of herbs. We received no information on ill employees from any of the markets or restaurants. On analysis of food items obtained from markets 1 and 2 and restaurant A, cyclospora oocysts could not be detected on UV and differential interference contrast microscopy. No cyclospora oocysts were found on duplicate samples by a second laboratory, thus suggesting oocysts were absent or in very low numbers in these environmental specimens.

CONCLUSION

This is the first documented outbreak of cyclosporiasis linked to *Ocimum basilicum* variety 'Thai basil' or 'Siam Queen', and the third outbreak related to the consumption of fresh basil [4, 6]. Previous outbreaks of cyclosporiasis have been identified through clusters associated with events where the contaminated food items were served [1, 3–7]. This, however, is the first outbreak of cyclosporiasis associated with an ethnic food type.

In Vietnamese cuisine, Thai basil is served raw as a garnish on noodle soups and salads and bean sprouts are often served alongside. Other ethnic foods such as Thai cuisine serve cooked Thai basil and were not affected by this outbreak. It was valuable to look at the specific varieties of basil that cases were exposed to as some, such as Thai basil, are unique to certain ethnic foods. Therefore, cooking of fresh produce such as basil may be needed to prevent cyclosporiasis, especially during outbreaks and in areas of endemicity.

Although the specific source of this outbreak was unknown, the speculated, potential roots of contamination of the basil in this outbreak, as identified in other outbreaks, are contamination of the produce with sporulated oocysts through irrigation with contaminated water, or by spraying with pesticides or fungicides prepared using contaminated water. Other

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possibilities include handling of the produce by workers who were infected or exposure of the produce to night soil.

Investigation of a sporadic outbreak of cyclosporiasis can be a great challenge because of the relatively prolonged incubation period. Often, case-patients are unable to recall the details of the meals that they have had 1–2 weeks prior to their symptom onset date. In addition, implicated food items often have a limited shelf life and as in this outbreak, we speculate that the contaminated food products had probably been cleared from shelves by the time the outbreak was identified. This, in part, may explain the negative microscopic findings. Alternatively, the cyclospora oocyst extraction method available may not have worked as well for green leafy vegetables as compared to raspberries. The limited sensitivity of microscopic methods may have also explained the negative results.

The success of this outbreak investigation, however, was dependent on the coordinated efforts between the medical microbiology diagnostic laboratories and epidemiological public health surveillance in BC. Careful surveillance for cases of cyclosporiasis and close communication between community laboratories and public health is essential to allow for early detection of future outbreaks followed by rapid trace-back investigation to obtain both epidemiological and microbiological evidence of contaminated food or water sources. Finally, international coordination and cooperation is essential, particularly in trace-back investigations, because of the increasing number of outbreaks that are related to imported foods.

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