

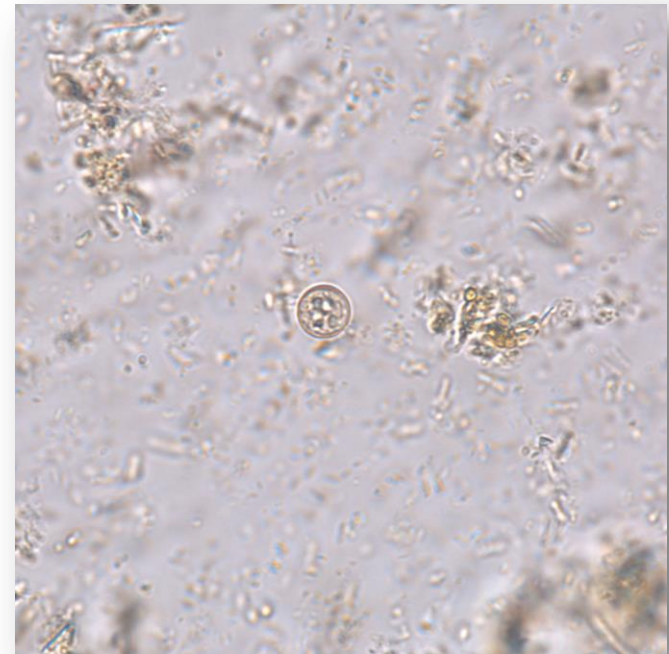
In case the diarrhoea goes on.....

What has been the diagnostic approach?

Lisette van Lieshout

Clinical microbiology/Parasitology

LUMC - LEIDEN



Pictures: Eric Brienens - LUMC

Introduction case

21 Sep 2015 – LUMC/KML receives a stool sample

Info: 59-year-old Dutch male

Regional hospital, via GP

Information to the lab: *duration of diarrhoea unknown*

Agreement: only microscopy (FEC)

FEC – performed: negative

Together with report: *Because of limited clinical information, only basic microscopy has been performed.*

Introduction case

25 Nov 2015 – LUMC/KML receives 2nd stool sample

Info: *ongoing diarrhoea after visit Indonesia, Cyclospora?*

<based on previous experiences of physician>

FEC (Ridley): negative

Auto-fluorescence: negative

PCR *Cyclospora*: Ct 33.5

Retrospectively sample of 21 Sept: **Ct 26.1**

Patient treated with Cotrim (Trimethoprim-sulfamethoxazole; TMP-SMX)

Immunocompetent adults: 960 mg, per os, 2dd, 7-10 days

Cyclospora cayetanensis

Coccidia, protozoa

Sporulation occurs after days or weeks at temperatures between 22°C to 32°C

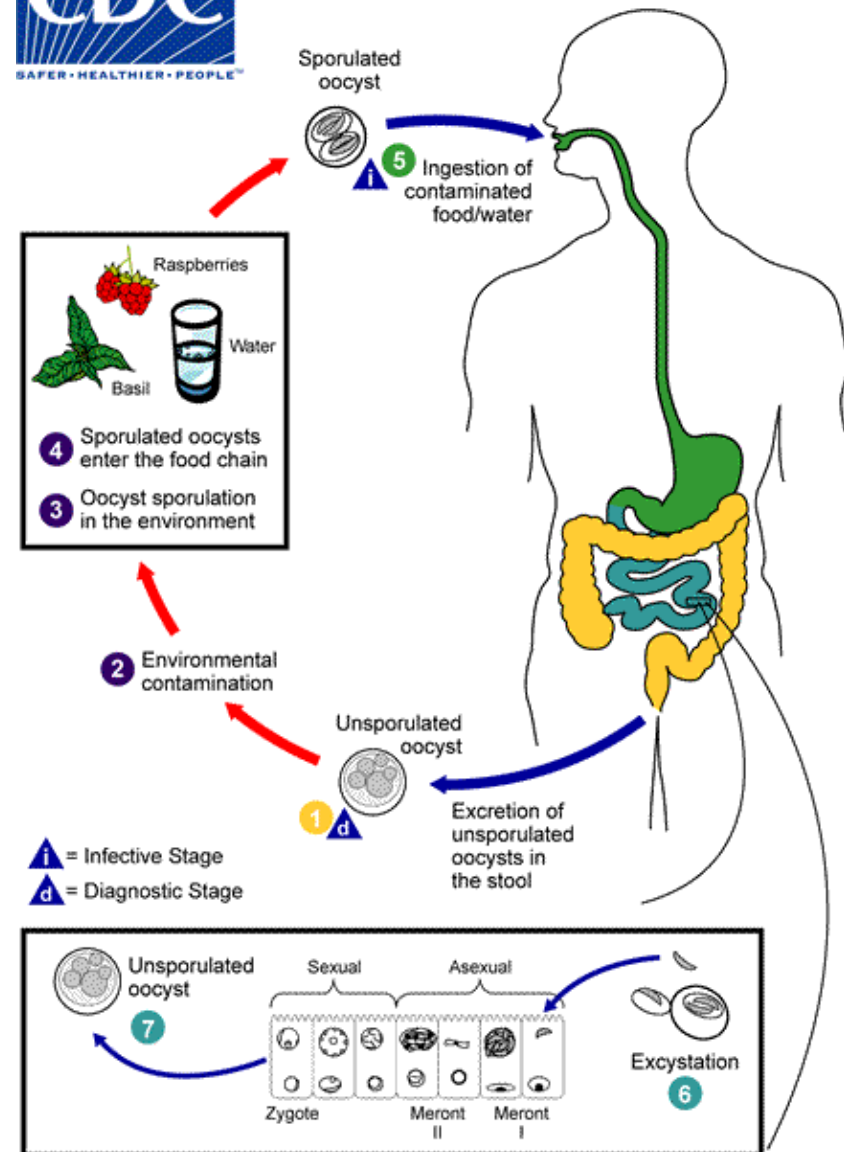
Two sporocysts, each containing two elongate sporozoites

Fresh produce and water can serve as vehicles for transmission

<http://abcnews.go.com/topics/lifestyle/health/cyclospora.htm>

The oöcysts excyst in the gastrointestinal tract, freeing the sporozoites which invade the epithelial cells of the small intestine

Inside the cells they undergo asexual multiplication and sexual development to mature into oöcysts, which will be shed in stools



Update on *Cyclospora cayetanensis*, a Food-Borne and Waterborne Parasite

CLINICAL MICROBIOLOGY REVIEWS, Jan. 2010, p. 218–234

ORTEGA AND SANCHEZ

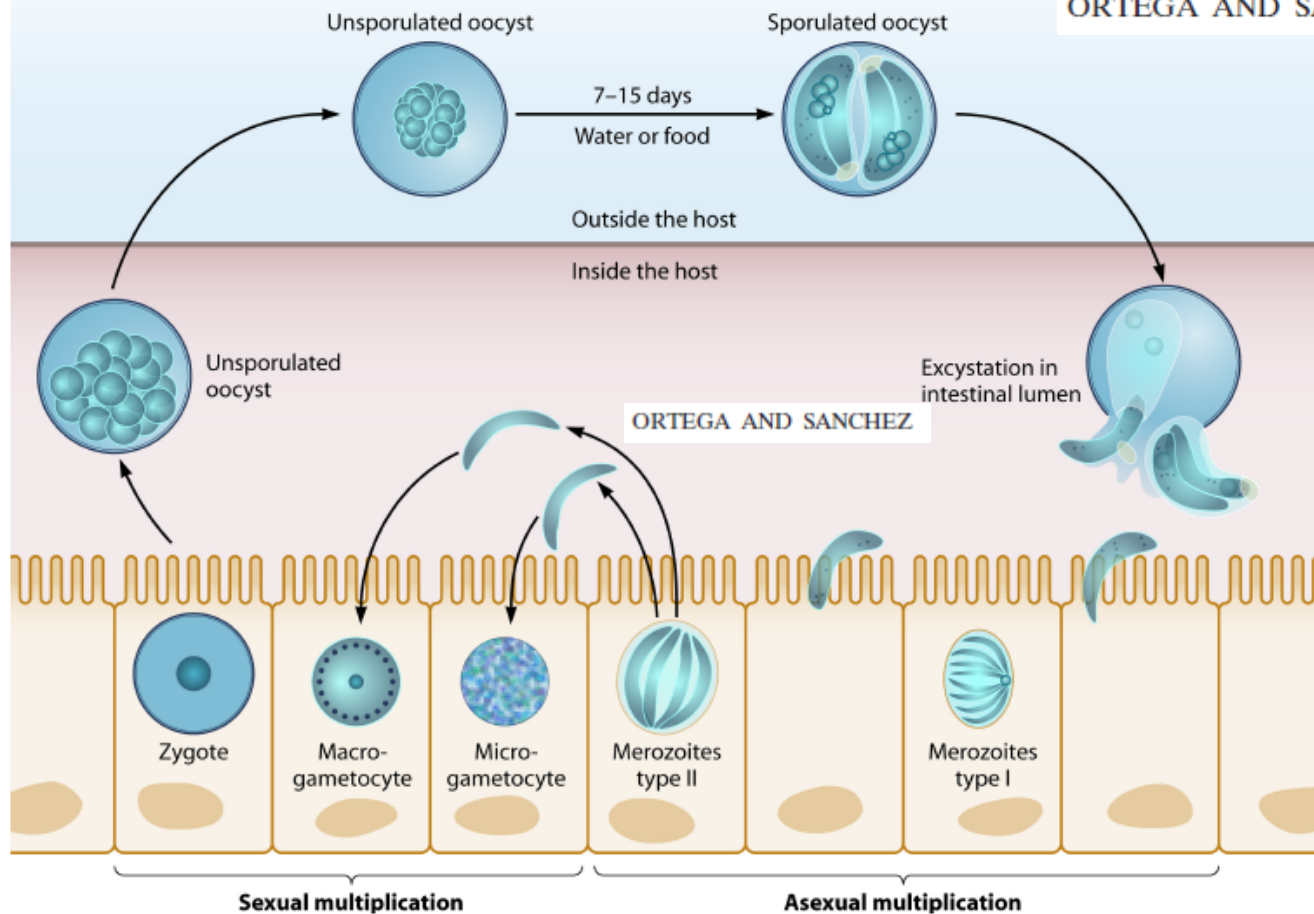
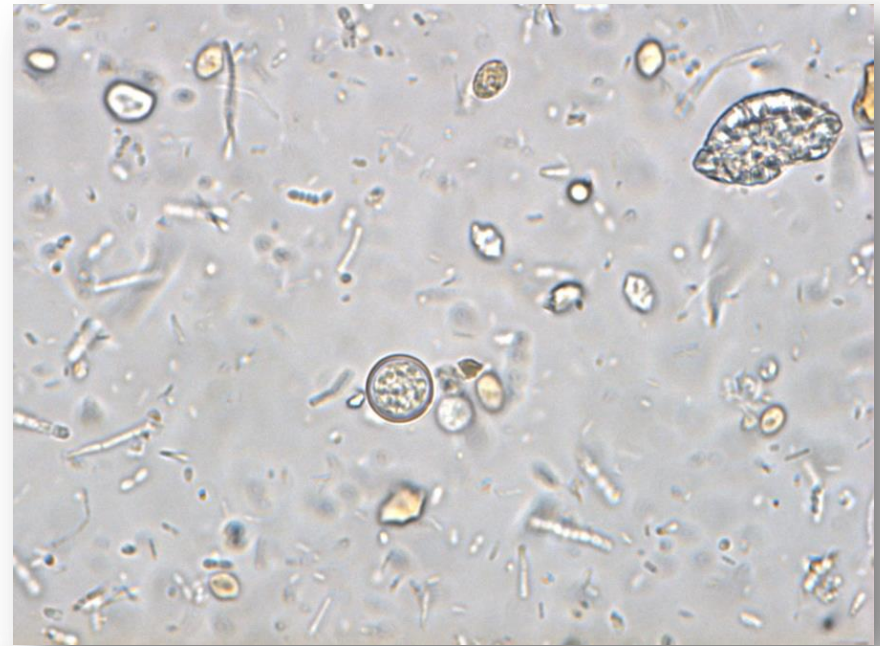


FIG. 1. Life cycle of *Cyclospora cayetanensis*. Unsporulated oocysts differentiate into sporulated oocysts, which undergo the excystation process. Sporozoites infect cells to form type I merozoites, and these form type II merozoites. The sexual-stage microgametocyte fertilizes the macrogametocyte to become a zygote and thus to differentiate as an unsporulated oocyst.

Cyclospora cayetanensis - microscopy

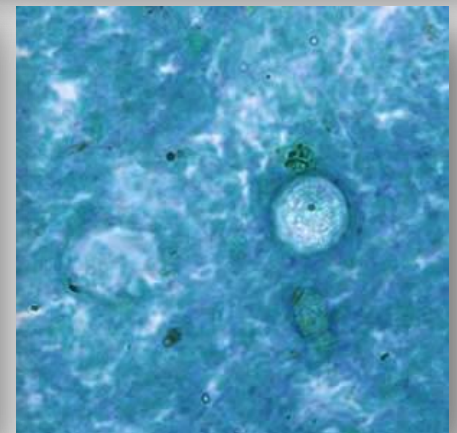
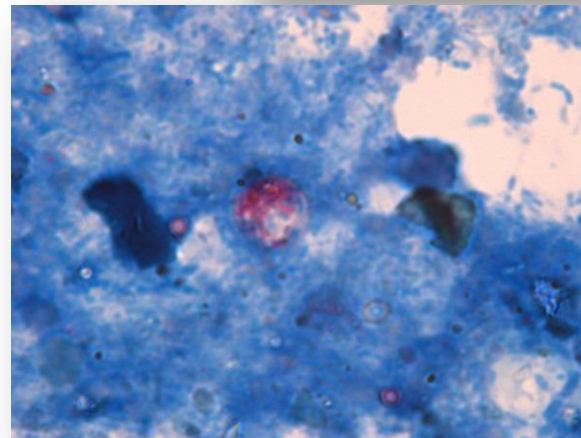
Direct smear/Ridley sediment

- oöcysts often not recognized
- 8-10 µm, round



Modified acid-fast staining

- variability of staining
- wrinkled appearance

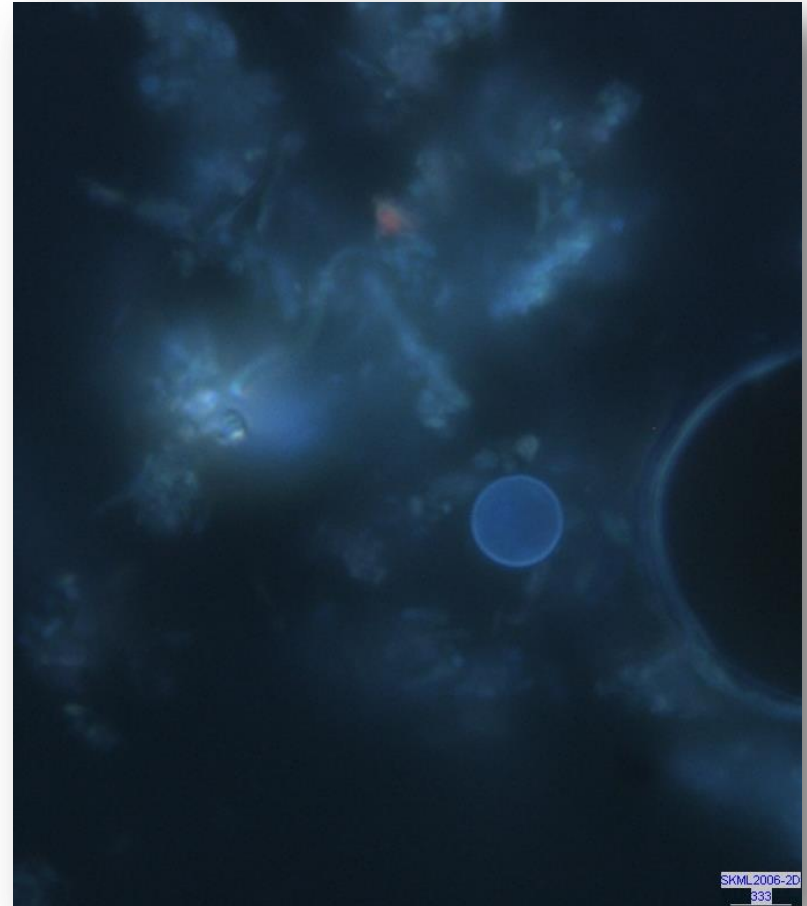


Cyclospora cayetanensis - microscopy

Autofluorescence (UV microscopy)

microscopes must be fitted with a 340-380nm excitation filter

- clear ring
- round



Cyclospora cayetanensis – molecular diagnosis

SSU rRNA gene => real-time diagnostic PCR

Int. J. Med. Microbiol. 293, 1–4 (2003)
© Urban & Fischer Verlag
<http://www.urbanfischer.de/journals/ijmm>

IJMM

Detection of *Cyclospora cayetanensis* in travellers returning from the tropics and subtropics using microscopy and real-time PCR

Jaco J. Verweij, Daphne Laeijendecker, Eric A. T. Brienen, Lisette van Lieshout, Anton M. Polderman

(Cysto)isopora positive stool samples => *Cyclospora* signal

Infectious Diseases, 2015; Early Online: 1–8

informa
healthcare

ORIGINAL ARTICLE

PCR for enteric pathogens in high-prevalence settings. What does a positive signal tell us?

HAGEN FRICKMANN^{1,2}, NORBERT G. SCHWARZ³, RAPHAEL RAKOTOZANDRINDRAIN⁴,
JÜRGEN MAY³, RALF M. HAGEN¹

Cyclospora cayetanensis

Travel and food related:
(sub) tropics – sporadic in Africa

Periods (intermittent) of:

- explosive watery diarrhoea
- vomiting
- severe weight loss

(abdominal pain, myalgias, low-grade fever, and fatigue)

Incubation \approx 1 week

Self limiting in healthy cases (10-12 weeks)

Treatable

Severe in immuno-compromised

Canada investigating source of 83 cyclospora illnesses



By Joe Whitworth+
10-Aug-2015



Source of the illnesses is still being investigated

Related tags: Cyclospora, Cyclosporiasis, PHAC, CFIA, PHE, FDA, CDC, Health Canada, Salad, Cilantro, Puebla, Produce

The Public Health Agency of Canada (PHAC) is investigating 83 cases of cyclospora infections and said the source is unknown.

Cases have been reported in British Columbia (3), Alberta (1), Ontario (74), and Quebec (5) and two people have been hospitalized.

Geographical distribution

Geographic Distribution

Cyclosporiasis has been reported in many countries, but is most common in tropical and subtropical areas.



What is detected by the *Cyclospora* PCR?

Infectious Diseases, 2015; Early Online: 1–8

informa
healthcare

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PCR for enteric pathogens in high-prevalence settings. What does a positive signal tell us?

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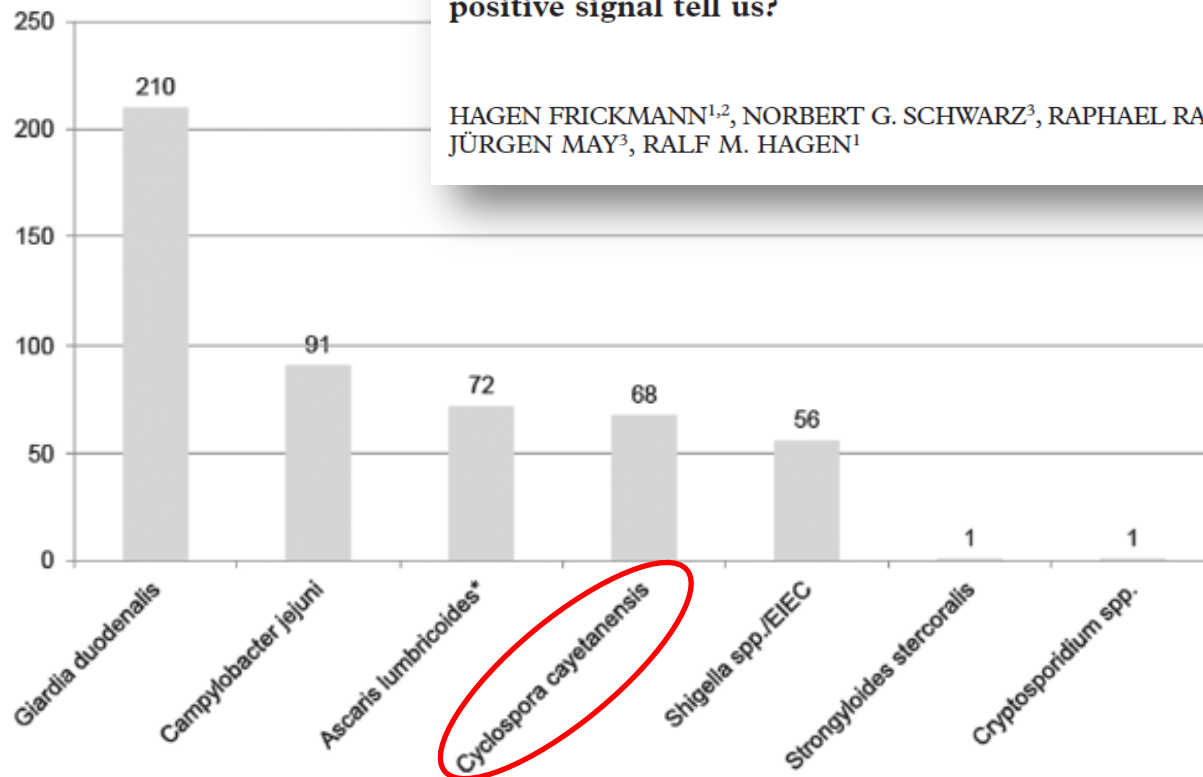


Figure 2. Distribution of detected pathogens in the study population of 410 schoolchildren from a Madagascan highland village. *Due to the expected low sensitivity of the helminth PCR, a 'positive' result was considered if PCR alone, microscopy alone, or both methods yielded a positive result.

Geographical distribution

Geographic Distribution

Cyclosporiasis has been reported in many countries, but is most common in tropical and subtropical areas.



Since 1990, at least 11 foodborne outbreaks of cyclosporiasis, affecting approximately 3600 persons, have been documented in the **United States** and **Canada**.

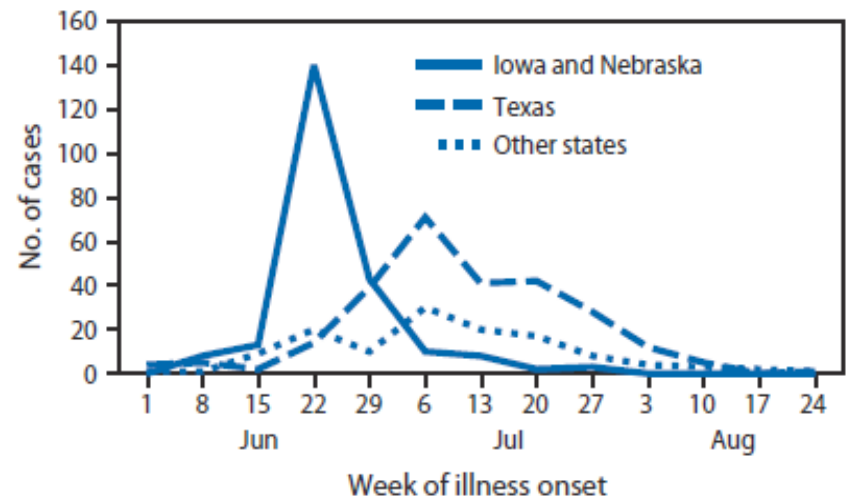
Notes from the Field

Outbreaks of Cyclosporiasis — United States, June–August 2013

During June–August 2013, CDC, state and local public health officials, and the Food and Drug Administration (FDA) investigated an unusually large number of reports of cyclosporiasis (compared with annual reports to the National Notifiable Disease Surveillance System [e.g., 123 cases in 2012]), an intestinal infection caused by the parasite *Cyclospora cayentanensis* (1). By September 20, CDC had been notified of 643 cases from 25 states, primarily Texas (278 cases), Iowa (153), and Nebraska (86). Investigations in Iowa and Nebraska showed that restaurant-associated cases in these two states were linked to a salad mix that contained iceberg lettuce, romaine lettuce, red cabbage, and carrots (2). Most patients in Iowa and Nebraska became ill during June 15–29; cases reported during July and August were primarily from Texas (Figure).

CDC collaborated with state and local public health officials in Texas and the FDA to investigate a cluster of illnesses among patrons of a Mexican-style restaurant in Fort Bend County, Texas (restaurant A). A case of restaurant A–associated gastroenteritis was defined as gastrointestinal illness in a person who

FIGURE. Laboratory-confirmed cyclosporiasis cases by week of onset — United States, June 1–September 10, 2013



Lettuce served at restaurant A was neither sourced from the same producer implicated in the outbreak investigation in Iowa and Nebraska nor was it associated with illness. Additionally, restaurant A did not use red cabbage or carrots. Taken together, data from tracebacks and epidemiologic investigations in Texas, Iowa, and Nebraska indicate that more than one outbreak of

UK outbreak in summer 2015

RAPID COMMUNICATIONS

Cyclospora infection linked to travel to Mexico, June to September 2015

GL Nichols ¹, J Freedman ¹, KG Pollock ², C Rumble ¹, RM Chalmers ³, P Chiodini ⁴, G Hawkins ², CL Alexander ⁵, G Godbole ^{1,4}, C Williams ⁶, HA Kirkbride ¹, M Hamel ⁷, JI Hawker ¹

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5. Scottish Parasite Diagnostic and Reference Laboratory, Glasgow, United Kingdom

6. Public Health Wales, Cardiff, United Kingdom

7. Public Health Agency of Canada, Ottawa, Canada

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Article s

Outbreak description

Between 1 June and 22 September 2015, 79 probable or confirmed case-patients (hereafter called cases) were reported in England (n = 55), Scotland (n = 21) and Wales (n = 3). No further cases linked to Mexico have been identified in the period since that date (as at 28 October 2015). Symptom onset dates were available for 62 confirmed or probable cases and ranged from 8 June to 19 August 2015 (Figure 1). Travel information was available for 60 cases; the earliest departure date from the UK to Mexico was 22 May and the latest date of return was 30 August 2015. The median age of cases

Epidemiol. Infect., Page 1 of 4. © Cambridge University Press 2015
doi:10.1017/S0950268815002356

COMMENTARY

Foodborne outbreaks caused by *Cyclospora*: the message is more important than the messenger

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Received 29 August 2015; Accepted 9 September 2015

Take-home messages:

- *Cyclospora* is easily missed
- PCR > microscopy
- PCR often not routinely performed
- Outbreaks
 - Seasonal
 - Regional
 - Food related
- Commercial platforms, based on outbreaks: trend to include *Cyclospora*

FilmArray™ Gastrointestinal Panel

1 Test. 22 Targets. All in about an hour.



Bacteria

Campylobacter (jejuni, coli and upsaliensis)

Clostridium difficile (toxin A/B)

Plesiomonas shigelloides

Salmonella

Yersinia enterocolitica

Vibrio (parahaemolyticus, vulnificus and cholerae)

Vibrio cholerae

Diarrheagenic *E. coli*/Shigella

Enteraggregative *E. coli* (EAEC)

Enteropathogenic *E. coli* (EPEC)

Enterotoxigenic *E. coli* (ETEC) *lt/st*

Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*

E. coli O157

Shigella/Enteroinvasive *E. coli* (EIEC)



Parasites

Cryptosporidium

Cyclospora cayentanensis

Entamoeba histolytica

Giardia lamblia



Viruses

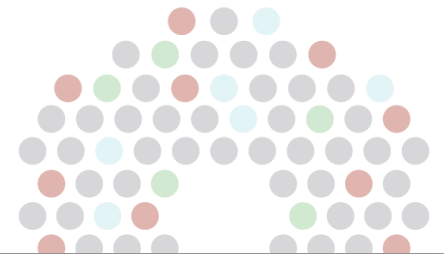
Adenovirus F 40/41

Astrovirus

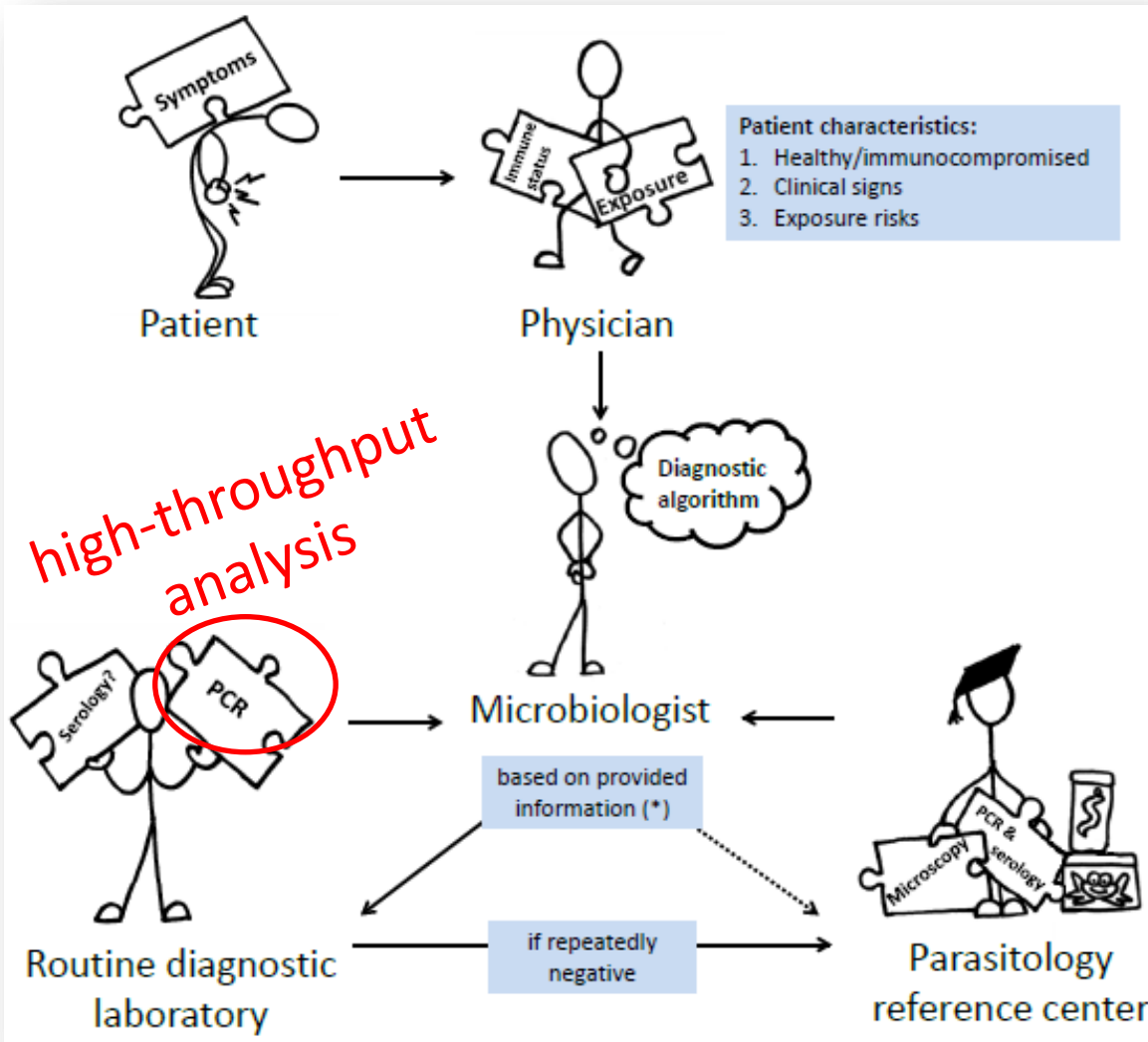
Norovirus GI/GII

Rotavirus A

Sapovirus (I, II, IV and V)



Communication with lab



Clinicians be aware:
Which targets
routinely covered?
When to ask for
more?

Van Lieshout & Roestenberg (2015)

Clinical consequences of new diagnostic tools for intestinal parasites.

Clinical Microbiology and Infection 21:520-528