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Cyclospora cayetanensis: diagnosis and situation in Belgium

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Abstract

Cyclospora is an emerging or a re-emerging parasite, gaining some interest in the travel related pathology and in opportunistic infections in AIDS patients. The name Cyclospora cayetanensis was introduced in 1992. The complete morphological description was published only in 1994. Cyclospora presents in faeces as spherical oocysts with a diameter of 8-10 µm. The cell wall is clearly delineated with a double membrane enveloping several round refractile granules. Cyclospora is distributed worldwide. Some outbreaks were described in the US, linked to consumption of raspberries from Guatemala, consumption of lettuce, and drinking of contaminated water. The parasite appears to be endemic in Nepal, Haïti, and Peru. In the period between January and June 1998, 15 Cyclospora infections were reported in Belgium, all of which were probably imported. The geographical origin of the infections was obtained for 10 patients: 5 patients had travelled to Indonesia, 3 to Turkey, 1 to Mexico, and 1 to Tibet. The Institute for Tropical Medicine in

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Antwerp registered in 1997 13 cases of Cyclospora infection. Most of these infections were contracted in Indonesia, Central America, Africa, and Turkey. Many laboratories were not aware of the new parasite. They needed to be informed and instructed about the clinical and diagnostic features of Cyclospora. Therefore a stool specimen with Cyclospora was included twice in the National External Quality Control scheme (January 1997 and 1998). The detection rate improved significantly, approximately by 30%, for the second scheme. In June 1998 the participants were invited to fill in a questionnaire about the detection of Cyclospora. The questionnaire revealed the following: half of the participants were unaware of the existence of Cyclospora before their participation to the External Quality Control scheme in January 1997. Approximately two thirds of the participants detected a Cyclospora oocyste for the first time in the sample during the first EQC round. Only half of the laboratories will routinely search for Cyclospora. In conclusion, to improve the detection capability of parasites by the laboratories and to ensure that Cyclospora will be detected even in routine samples further training is still needed.

Key words

Cyclospora cayetanensis, coccidia, EQA, stool examination.

Introduction

Cyclospora organisms were first noted in the intestines of moles in 1870 by Eimer. It was Schneider who first named the genus *Cyclospora* in 1881. Schaudinn reported in 1902 the life cycle which showed that *C. caryolytica* developed in the intestinal epithelia of moles and produced severe enteritis (1, 2).

The first published report of *Cyclospora cayetanensis* infection in humans appears to be by Ashford (1979), who found unidentified *Isospora*-like coccidia in the faeces of 3 individuals in New Guinea. Narango in 1989 reported what may be the same organism from several Peruvians with chronic diarrhoea and termed the organism *Cryptosporidium muris*-like. For a while they were thought to be blue-green algae or cyanobacterium-like bodies (CLB), due to the photosynthesizing organelles within the organism (2).

The name *Cyclospora cayetanensis* was created in 1992. It was in 1993 that Ortega published the success in inducing the sporulation of *Cyclospora*, so that the parasite was placed in the genus of *Cyclospora* (3). But it wasn't until 1994 that a complete morphological description was published to validate the name (2).

Epidemiology

Cyclospora is widely distributed throughout the world. With the exemption of some outbreaks, the overall prevalence in most populations appears to be far less than 1% (Steve Upton, Kansas State University).

It has been identified in both residents and travellers from various regions including North America, Central America, South America, the Caribbean islands, Eastern Europe, India, South Africa, and Southeast Asia. In Nepal, Haiti, and Peru the parasite appears to be endemic. Persons of all ages have been infected (1). Outbreaks in the US seem to occur most frequently in spring and summer. Warmer temperatures are clearly needed to get oocysts to sporulate with any rapidity. The outbreaks have been linked to the ingestion of unwashed berries, of contaminated water, of lettuce, and of fresh basil (4, 5, 6). The mode of transmission is unknown and transmission via contaminated food, lettuce, undercooked meat, and raw beef has been suggested but not proven (1).

Prevalence studies from Nepal, Haiti, and Peru suggest that, to date, symptomatic infection is more common in children older than 18 months but that younger children may have asymptomatic infection, and the rates of infection are lower among natives than among non-native residents. Whether the differences in the distribution of symptomatic infections in different age groups and in various geographic areas are due to the acquisition of protective immunity, different exposures, different species or strains, the sensitivity of diagnostic techniques, or other as yet undescribed factors is not known (1).

Life cycle

Coccidia may complete their life cycle within a single host (*Cryptosporidium*) or require a second host (*Toxoplasma*) or a period of time outside the host for maturation (*Isospora*). *Cyclospora* resembles

Isospora in that oocysts are excreted unsporulated and require a time outside the host for maturation to occur (1).

The life cycle starts with the ingestion of a sporulated oocyst. The sporulated oocyst contains 2 sporocysts each enclosing 2 sporozoites. Once inside the gut the sporozoites exit from the sporocysts and oocysts, penetrating epithelial cells along the small intestine. Sporozoites undergo multiple fission inside the cells to form meronts. Each meront produces merozoites that are released to infect new cells. The final generation of merozoites penetrates new cells to form gametes. Most gametes enlarge to form macrogametes (females) and some become microgametocytes (males) which produce microgametes. The fertile microgametes exit the microgametocyte to fertilise the macrogametes and a resistant oocyst wall is laid down around the zygote. The unsporulated oocyst passes into the external environment with the faeces. It is outside the host that maturation of the oocysts occurs (Steve Upton, Kansas State University).

Clinical manifestation

The incubation period for cyclospora infection ranges from 2 to 11 days (1). The infection may persist for a month or more. Mild infections may produce few or no clinical signs. Clinical manifestations of immunocompetent patients include watery diarrhoea with a cyclical pattern, sometimes alternating with constipation. Leucocytes and erythrocytes are absent in this diarrhoea. Associated symptoms include abdominal cramp, weight loss, anorexia, myalgia, and occasionally vomiting and/or fever. The illness is self- limited, but may be prolonged for weeks (7).

Among immunocompromised hosts *Cyclospora* infection has occurred primarily in HIV-infected individuals, who are predisposed to more frequent and prolonged infections with spore-forming intestinal protozoa (7).

Two studies conducted by Pape (8) in Haiti with 51 HIV patients and by Hoge (9) in Nepal with 40 immunocompetent patients (expatriates), showed the efficacy of cotrimoxazole (trimethoprim 160 mg, sulfamethoxazole 800 mg) for the treatment of cyclosporiasis. The treatment was taken twice daily for 7 days by the immunocompetent patients and four times daily for 10 days, followed by three times weekly by the immunodeficient patients.

Diagnosis

In stool examination *Cyclospora* oocysts are spherical and measure 8-10 µm in diameter. They are passed unsporulated in low to moderate numbers.

They are easily recognised using conventional microscopy. The *Cyclospora* present in faeces as spherical oocysts with a clearly delineated cell wall. A double membrane envelops several refractile granules, giving the aspect of a morula. These granules have a greenish shade in fresh stool specimens (3).

To concentrate oocysts from faeces, flotation and sedimentation techniques are used. The usual simple ether-sedimentation methods such as the Ritchie or Bailenger method are too rough and poorly efficient (10).

Deluol recommends the following techniques. For lipid poor faeces, a flotation with an iodine-potassium mercurate solution (d = 1.20) of a faeces suspension in Sodium acetate-Acetic acid-Formalin (SAF) (11).

The best technique is the combined ether/flotation method in potassium mercurate (12). Their is no need to perform a coloration. Like the oocysts of *Cryptosporidium*, the oocysts of *Cyclospora* are acid-fast and can been seen using the acid-fast staining techniques (e.g. Ziehl-Neelsen) on which the oocysts have a variable appearance.

The *Cyclospora* oocysts are autofluorescent and appear as neon-blue circles when examined by microscopy in ultraviolet light. This characteristic will wane over time for the nonpreserved oocysts (9).

It is rather easy to obtain sporulation of the oocysts, used to confirm the diagnosis. A small amount of faeces is suspended in a solution of 2.5% of potassium dichromate. The suspension is left at ambient temperature, after a maturation period of 5 till 13 days, 2 sporocysts with each 2 sporozoites are observed in the oocysts (3). Sporulated oocysts of *C. cayetanensis* collapse and distort fairly readily in many sucrose or salt solutions (due to osmolarity problems), used in the flotation techniques. It is recommended to first use the flotation technique to concentrate oocysts, then to wash them off the coverslip into a conical centrifuge tube with a stream of water. The pellet obtained by centrifugation is suspended again in a little water and observed by wet mount (Steve Upton, Kansas State University).

Situation in Belgium

Epidemiology

In 1995 the first 2 cases of intestinal *Cyclospora* infections were reported in Belgium. Both patients had travelled to Indonesia. The publication included a characteristic photograph of the parasite (13).

The Institute for Tropical Medicine in Antwerp registered in 1997 13 cases of *Cyclospora* infection. The infections were contracted in Haïti (1), Nigeria (1), Mexico (2), Indonesia (3), Turkey (1), Venezuela (1), El Salvador (1), and Congo (1). One of the infection was notified as a mixed infection (Nigeria). For the period of January till June 1998 a total of 15 *Cyclospora* infections were registered by the Department of Epidemiology of the Institute of Public Health – Louis Pasteur (IPH) in Brussels. Following geographic distribution was noted for 10 patients, 1 had travelled to Mexico, 5 to Indonesia, 1 to Tibet, and 3 to Turkey. Most of the patients were male and the ages ranged from 23 to 67 years old. HIV status is not known.

Diagnosis

As *Cyclospora* is a newly (re)discovered parasite, detection capability by the clinical laboratories is not evident. In the aim to introduce this parasite to the clinical laboratories in Belgium, a faeces suspension containing *Cyclospora* oocysts was included in the National External Quality Assessment scheme of Parasitology. The participants of the schemes received the samples in January 1997 and 1998, illustrated with respectively the following clinical information: female, 26 years old, went for a journey to Indonesia; 8 days afters her return in Belgium she complains of abdominal cramp and diarrhoea with periods of constipation; male patient 27 years old, with diarrhoea after a holiday in Nepal. The diarrhoea lasts for 3 weeks with complaints of nausea, abdominal cramp, and severe weight loss. Before inclusion in the schemes, both samples were carefully examined by at least 3 experts in parasitology. The samples were approved based on the distinctive identification characteristics of the parasite and his concentration. Mixed infections were not observed by the experts. After each evaluation a short description of the parasite and a summary of the results was sent to the participants.

A total of 255 laboratories participated in the first scheme in January 1997, 135 laboratories of them were able to detect *Cyclospora* (135/255,

52.9%). The correct stage of the parasite was reported by 73.3% (99/135). The absence of parasites was mentioned by 52 (20.4%) participants. Microsporidia 5.9% (15/255), *Entamoeba hartmanni* 4.7% (12/255), *Entamoeba histolytica* 4.3% (11/255), and other parasites 16.9% (43/255) were reported. For the scheme in January 1998 a total of 263 laboratories participated and 214 detected *Cyclospora* (214/263, 81.4%), 182 (85%) of them mentioned correctly the stage of oocyst. Ten (3.8%) participants did not find any parasite. Analogous to the previous scheme, other misidentified parasites were reported: Microsporidia 1.5% (4/263), *Cryptosporidium* 3.4% (9/263), *Entamoeba hartmanni* 3.4% (9/263), *Entamoeba histolytica* 3.0% (8/263), *Entamoeba coli* 2.7% (7/263), and others 2.7% (7/263).

In June 1998 the participants were invited to answer a questionnaire regarding their experiences with the *C. cayetanensis* detection. Almost half (92/189) of those who answered were informed about the existence of the parasite through the National External Quality Assessment. Most of them (116 versus 46) saw the parasite for the first time through their participation in the scheme. Nearly 50% (90/187) will search for the parasite only on request of the physician.

Before 1997 13 laboratories detected a total of 19 *Cyclospora*, the detections were performed on routine stool specimens. This amount increased in 1997, when 27 laboratories found a total of 61 cases of *Cyclospora*. Of these 27 laboratories, 20 look for the parasite in routine stool samples and 8 learned about the parasite through the scheme. For the period January-June 1998, 39 *Cyclospora* infections were detected by 23 laboratories, 7 of them learned about the parasite through the scheme. The educational objective of the External Quality Assessment in Parasitology has been considered as achieved by 170 (96,6%) versus 6 participants.

Discussion

The parasite *C. cayetanensis* is rather unknown in Belgium, resulting in misdiagnosis and underreporting. The misidentifications observed in the Belgian External Quality Assessment schemes could easily have been avoided by using a micrometer. Comparing the results obtained in 1997 and in 1998, we observe a significant ($\chi^2 = 47.6$, $p<0,001$) improvement for the identification of the parasite by approximately 30%. Since 1996 the detection of *Cyclospora* is increasing, in 1997 and 1998 respectively 40% and 30% of the laboratories which identified the para-

site in stool specimens, where informed about the parasite through the schemes. Most of the laboratories detecting *Cyclospora* look for the parasite on a routine basis.

Conclusion

In western Europe, *Cyclospora* remains a rare parasite. In travellers with diarrhoea however, *Cyclospora* is more frequently detected. Further training is still needed to improve the detection capability, to ensure that laboratories will become familiar with this pathogen and will be able to recognise it in stool specimens submitted for routine parasitologic examination. Training and updating can be organised through External Quality Assessment schemes as illustrated above, and stimulate the laboratories to participate constructively. This study has been limited to the experiences of the laboratories, it does not reflect if clinicians are aware of the existence of this parasite, maybe a more general information and updating should be needed.

Samenvatting

Cyclospora is een nieuwe of een oude herontdekte parasiet, belangrijk voor importpathologie en opportunistische infecties bij aids-patiënten.

De naam *Cyclospora cayetanensis* ontstond in 1992, maar pas in 1994 werd een volledige morfologische beschrijving van de parasiet gepubliceerd. In faeces zijn de *Cyclospora* oöcysten sferisch en bedraagt hun diameter 8-10 µm. De celwand is duidelijk afgetekend en de dubbele membraan omsluit meerdere ronde en licht weerkaatsende granulen. *Cyclospora* is wereldwijd verspreid. Sommige outbreaks werden beschreven in de V.S. en in verband gebracht met het eten van frambozen uit Guatemala, het eten van sla en het drinken van besmet water. In Nepal, Haïti en Peru zou de parasiet endemisch zijn.

Tijdens de periode januari 1998 - juni 1998, werden in België 15 infecties ten gevolge van *Cyclospora* geregistreerd. Deze infecties werden waarschijnlijk allen geïmporteerd. Informatie omtrent de geografische oorsprong van de infectie werd meegedeeld door 10 patiënten: 5 hadden een reis ondernomen naar Indonesië, 3 naar Turkije, 1 naar Mexico en 1 naar Tibet.

Het Instituut voor Tropische Geneeskunde in Antwerpen heeft in de loop van 1997 13 gevallen van cyclosporiasis gedetecteerd. De meeste infecties werden opgelopen tijdens een reis in Indonesië, Midden-Amerika, Afrika of Turkije. Aangezien de meeste laboratoria niet vertrouwd waren met deze nieuwe parasiet, was het onontbeerlijk hen de nodige informatie omtrent kliniek en diagnostiek te verschaffen. Daarom werden faecessuspensies met *Cyclospora* tweemaal achtereenvolgens uitgestuurd in de nationale Externe

KwaliteitsEvaluaties (EKE) (januari 1997 en 1998). Tijdens de tweede ronde werd er een significante stijging met ongeveer 30% juiste antwoorden bekomen. In juni 1998 werden de laboratoria uitgenodigd een vragenlijst omtrent de detectie van de parasiet te beantwoorden. Het volgende werd waargenomen: ongeveer de helft van de deelnemers was niet op de hoogte van het bestaan van *Cyclospora* vóór hun deelname aan de EKE en bij benadering tweederde van de deelnemers observeerde voor het eerst tijdens de enquête in 1997 een *Cyclospora* oöcyst. Slechts de helft van de laboratoria zoekt routinematig naar *Cyclospora*.

Tot besluit, het lijkt ons noodzakelijk de opleiding van het laboratoriumpersoneel verder te zetten om het vermogen tot detectie en identificatie van de parasieten nog te verbeteren, zodat detectie ook bij routineonderzoek mogelijk wordt.

Sleutelwoorden

Cyclospora cayetanensis, coccidia, EKE, faecesanalyse.

Résumé

Cyclospora est un nouveau parasite ou un ancien redécouvert, qui apparemment se présente comme un agent important de la pathologie d'importation ainsi que des infections opportunistes chez les patients atteints du SIDA.

Le nom *Cyclospora cayetanensis* apparaît en 1992, mais ce n'est qu'en 1994 qu'une description complète de la morphologie du parasite fut publiée. Dans les selles les oocystes du *Cyclospora* sont sphériques avec un diamètre de 8-10 µm. La paroi est nette et une double membrane bien visible entoure plusieurs granulations arrondies très réfringentes.

Cyclospora est cosmopolite. Quelques épidémies ont été décrites aux Etats-Unis, elles étaient liées à l'ingestion de framboises provenant du Guatemala, de salade et d'eau contaminées. Le parasite paraît endémique au Népal, à Haïti et au Pérou.

Pendant la période de janvier à juin 1998, un total de 15 infections dues à un *Cyclospora* ont été signalées en Belgique. Ces quelques infections étaient probablement toutes importées. Des informations quant à l'origine géographique de l'infection ont été obtenues pour 10 patients: 5 revenaient d'un voyage en Indonésie, 3 revenaient de Turquie, 1 du Mexique et 1 du Tibet. L'Institut de Médecine Tropicale à Antwerpen a détecté 13 cas de Cyclosporiases en 1997. La plupart de ces infections ont été contractées au cours de voyages en Indonésie, Amérique centrale, Afrique ou Turquie. La plupart des laboratoires n'étant pas encore familiarisés avec ce nouveau parasite, il apparaissait indispensable de leur fournir des informations tant au niveau de l'intérêt clinique que du diagnostic. Dans ce but, une suspension de selles avec des *Cyclospora* a été envoyée à deux reprises à l'occasion des enquêtes nationales des évaluations externes de la qualité (janvier 1997 et 1998). A l'occasion de la deuxième enquête, une amélioration très significati-

ve des résultats a pu être observée (environ 30%). En juin 1998, les participants ont été invités à remplir un questionnaire concernant la détection du parasite. Cette enquête a permis de mettre en évidence les observations suivantes: la moitié des laboratoires n'étaient pas au courant de l'existence du parasite avant leur participation à l'enquête; deux tiers des participants ont observé pour la première fois un oocyste de *Cyclospora* lors de la première enquête; seule la moitié des laboratoires effectue l'analyse en routine.

En conclusion, il semble nécessaire de poursuivre la formation du personnel de laboratoire pour améliorer la détection et l'identification du parasite afin d'en garantir la détection en routine.

Mots-clés

Cyclospora cayetanensis, coccidia, évaluation externe de la qualité, analyse des selles.

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