

Prevalence of infection with *Cryptosporidium parvum* and *Cyclospora cayetanensis* among international travellers

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Abstract

Background—*Cryptosporidium parvum* and *Cyclospora cayetanensis* are recognised as possible pathogens of traveller's diarrhoea.

Aims—To identify the prevalence of *C parvum* and *Cyc cayetanensis* in travellers returning from developing countries. **Patients**—Nine hundred and seventy eight stool samples were taken from 795 patients returning from developing countries.

Methods—Microscopy (iron-haematoxylin stain, SAF concentration, modified acid fast stain) and a commercially available enzyme linked immunosorbent assay (ELISA) kit for the detection of *Cryptosporidium* antigen in stool.

Results—Of the 795 patients in the study, 469 suffered from diarrhoea. Infection with *Cyc cayetanensis* could be detected in five subjects (1.1%) by acid fast stain, and 13 patients (2.8%) were infected with *C parvum*. On evaluation, the antigen capture ELISA turned out to be clearly less sensitive for detection of *C parvum* than microscopy. All patients with either *C parvum* or *Cyc cayetanensis* infection suffered from watery diarrhoea.

Conclusions—*C parvum* and *Cyc cayetanensis* are not major causes of diarrhoea in international travellers. In cases of persistent watery diarrhoea, however, these pathogens should be taken into account in the differential diagnosis.

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Keywords: diarrhoea; *Cryptosporidium parvum*; *Cyclospora cayetanensis*

Diarrhoea seems to be a more or less accepted part of the travelling experience for many tourists. This has some justification, as most episodes of traveller's diarrhoea are self limiting, and the pathogens responsible do not cause major harm to the intestines. However, some patients develop diarrhoea as the result of more obstinate pathogens, and continue to suffer from symptoms for a considerable time after their return home. On evaluation of the symptoms, two protozoa that only recently have been recognised as possible causes of traveller's diarrhoea should be considered: *Cryptosporidium parvum* and *Cyclospora cayetanensis*.

Cyc cayetanensis was first described in 1986 as a pathogen and is now recognised as a world wide cause of diarrhoea in humans.¹ The most common clinical features caused by this parasite include watery diarrhoea, fatigue, anorexia, weight loss, and upper intestinal pain.² Infection with *Cyclospora* appears to be common in some areas of the world: 6–18% of children in Lima, Peru, were found to be infected with the parasite but only 22% of these were symptomatic.³ It has been assumed that populations in endemic areas may be less susceptible to the organism than populations in non-endemic areas, who may suffer a 100% attack rate.³ A common source of infection appears to be contaminated water,² and transmission rates seem to peak between April and September.^{2,3} Various reports on *Cyclospora* infections in travellers have been published.^{1,2,4–13} However, to our knowledge, the actual prevalence of this pathogen among travellers returning from developing countries has not been investigated in a prospective study.

In addition to being a frequent cause of diarrhoea in HIV-infected patients and being the causative agent of several water-borne outbreaks of diarrhoea in recent years, *C parvum* has been recognised as an important agent of traveller's diarrhoea.¹ Clinically immunocompetent patients usually suffer from watery diarrhoea, occasionally accompanied by nausea, vomiting, weight loss, anorexia, and dehydration. The duration of symptoms and excretion of oocysts might last from 1 day to 8 weeks.^{14,15} In a review of pooled data from 78 studies, 6.1% of diarrhoeal illnesses in immunocompetent patients in developing countries were found to be due to *C parvum*, compared with 2.1% in developed countries.¹⁶ In HIV-infected patients, the same study showed that 24% of diarrhoeal illnesses in developing countries and 13.8% in developed countries are caused by *C parvum*. Reservoirs for infection of humans have been identified in calves, cats, and dogs, but faecal-oral person to person transmission remains very important.^{1,17,18} *C parvum* has been identified repeatedly as the causative agent of watery diarrhoea in travellers.^{1,11,15,19–28} However, there is still a lack of prospective studies investigating the epidemiology of *C parvum* infection in international travellers.

Both *Cyc cayetanensis* and *C parvum* can be diagnosed microscopically by using a modified acid fast stain.^{3,29,30} Having a size of 8–10 µm in diameter, the variably staining oocysts of *Cyc cayetanensis* (fig 1) are larger than the usually

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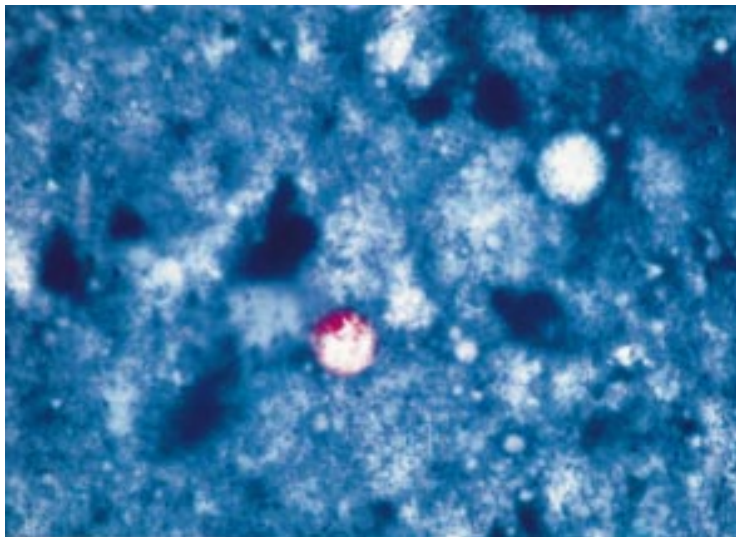


Figure 1: *Cyclospora cayetanensis* in stool samples from symptomatic travellers (modified acid fast stain, bright field microscopy, original magnification $\times 630$).

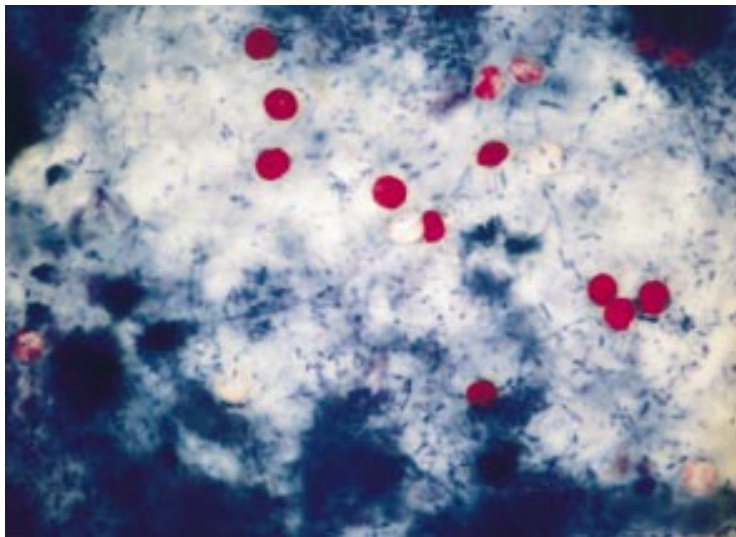


Figure 2: *Cryptosporidium parvum* in stool samples from symptomatic travellers (modified acid fast stain, bright field microscopy, original magnification $\times 630$).

nicely staining oocysts of *C parvum* (3–6 μm) (fig 2). In addition, oocysts of *Cyc cayetanensis* show blue autofluorescence under ultraviolet light.³ Enzyme linked immunosorbent assay (ELISA) kits for the detection of *C parvum* oocysts in stools have been reported to have good sensitivity and specificity,^{31 32} but microscopy remains currently the most reliable method for diagnosing *Cyc cayetanensis* from a stool sample.

To identify the prevalence of *C parvum* and *Cyc cayetanensis* infection in travellers returning from developing countries, a prospective study was carried out at the travel clinic and outpatient department of the Department of Infectious Diseases and Tropical Medicine, University of Munich, Germany.

Materials and Methods

PATIENTS

From May to September 1995, 795 patients who presented with various medical complaints were recruited from our outpatient

clinic. The main symptoms were diarrhoea in 469 (59%), fever in 167 (21%), and various skin problems in 83 (10.4%), while 76 (9.6%) wanted a post-travel medical check up but did not complain of any symptoms. All patients were German nationals returning from vacation trips abroad and gave their informed consent to the investigation.

METHODS

From the 795 patients, 978 stool specimens were collected and processed. All stool samples were investigated for ova and parasites by direct microscopy (iron-haematoxylin stain), the SAF (sodium acetate/acetic acid/formalin)/ethyl acetate concentration technique, iodine staining, and modified acid fast stain.^{3 29 30 33} Each slide (sample volume approximately 10 μl) was read quantitatively by two experienced microscopists before being considered negative. A portion of every fresh stool sample was stored immediately at -20°C and tested later by a commercially available antigen capture ELISA designed for the detection of *Cryptosporidium* oocysts (Alexon Inc, Mountain View, California, USA) according to the manufacturer's instructions, by one laboratory technician who was blinded to the results of the microscopy. If discordant results between microscopy and ELISA were obtained, both tests were repeated. Microscopy was defined as the reference for the final results; only samples with microscopically positive results for *C parvum* were considered as positive. The definition "true positive", however, was extended to all patients who produced at least one microscopically positive stool specimen, even if they initially presented with a series of negative specimens. Statistical analysis was performed using the EPI-Info 6.0 (CDC, Atlanta, Georgia, USA and WHO, Geneva, Switzerland) software package.

Results

All 978 specimens from 795 patients were examined by microscopy and antigen capture ELISA. Altogether, 15 specimens from 13 patients (1.6%) were considered positive for *C parvum* and a further five specimens from five patients (0.6%) were positive for *Cyc cayetanensis*. However, infection with *C parvum* occurred in 2.8% of the 469 patients presenting with diarrhoea, while *Cyclospora* accounted for 1.1% of infections in these 469 symptomatic patients. All samples from the latter group were diagnosed by modified acid-fast staining. The same staining method was used as the "gold standard" for the diagnosis of *C parvum*: of the initial specimens, eight were positive by microscopy and ELISA (table 1), and 782 were negative by both methods. Five specimens were positive by microscopy and negative by ELISA; these were considered to be false negative in the ELISA. One specimen was positive by the ELISA and negative by microscopy. This sample was initially considered to be a false positive in the ELISA. On re-presentation of the patient, two more samples were delivered and examined; they were now positive by both methods. Defining each microscopic result per

TABLE 1 Comparison of microscopy and antigen capture enzyme linked immunosorbent assay (ELISA) for the detection of *Cryptosporidium parvum* in stool ($n = 978$ samples)

| | All specimens ($n = 978$) | Initial specimens ($n = 795$) |
|--|--------------------------------|------------------------------------|
| Results | | |
| Microscopy +; ELISA + | 9 | 7 |
| Microscopy +; ELISA - | 5 | 5 |
| Microscopy -; ELISA + | 1 | 1 |
| Microscopy -; ELISA - | 963 | 782 |
| Sensitivity of ELISA <i>v</i> microscopy* | 9/14 (64%) | 7/12 (58%) |
| Sensitivity of both methods <i>v</i> all true positives† | | |
| ELISA | 10/15 (67%) | 8/13 (61%) |
| Microscopy | 14/15 (93%) | 12/13 (92%) |

*Values are number of tests positive by ELISA or microscopy/number of microscopically positive tests.

†Values are number of tests positive by ELISA or microscopy/number of "true positive" tests. This includes specimens positive for *C. parvum* by microscopy as well as ELISA positive and microscopically negative specimens from patients with subsequent microscopically positive stool samples.

specimen as the gold standard, the ELISA reached a sensitivity of 58% compared with microscopy. The use of the definition for true positive patients (a single positive specimen in a row of negatives defines a patient as *Cryptosporidium* positive) did not change the sensitivity of the ELISA much (61%), while microscopy of single specimens yielded good results with a sensitivity of 92%. Very similar results were obtained for the evaluation of all 978 samples (table 1): the sensitivity of ELISA *v* microscopy was 64%, that of ELISA *v* all true positives 67%, and that of microscopy *v* all true positives 93%.

No other parasites carried by the patients investigated stained in the modified acid-fast stain, nor were any cross reactions of the antigen capture ELISA with other antigens observed. Of the 795 patients, 201 (25.3%) carried at least one parasite other than *Cyclospora* or *Cryptosporidium* in the stool: 74 patients were infected with *Giardia duodenalis* (14 asymptomatic), 74 with *Entamoeba dispar/histolytica* (43 asymptomatic), 73 with *Blastocystis hominis* (22 asymptomatic), 54 with *Entamoeba coli* (all asymptomatic), 53 with *Endolimax nana* (all asymptomatic), and 55 with a variety of other intestinal parasites (including *Iodamoeba bitschlii*, *Trichomonas hominis*, *Chilomastix mesnili*, *Isospora belli*, *Ancylostoma duodenale/Necator americanus*, *Strongyloides stercoralis*, *Trichuris trichiura*, *Ascaris lumbricoides*; 40 asymptomatic).

All five patients considered to be infected with *Cyclospora* presented with watery diarrhoea as the predominant medical complaint during or after the journey. The mean duration of symptoms was 19.8 days (range 11–32 days). Two patients complained of occasional spells of nausea and anorexia. Of the 13 patients infected with *Cryptosporidium*, ten presented with persistent watery diarrhoea as their sole complaint. The other three presented with diarrhoea and an itching exanthema on the arms and legs (two) or trunk (one). The mean duration of symptoms before presentation was 36.2 days (range 12–120 days) in these 13 patients. Four of the 13 patients infected with *Cryptosporidium* had visited India, three South East Asia (Thailand and Malaysia), two each South America (Venezuela and Brazil) and West Africa (The Gambia and Senegal), and

one each Southern Africa (Zimbabwe) and Central America (Mexico). Of the five patients infected with *Cyclospora*, two each had been to Central America (Mexico and Guatemala) and India and one to South East Asia (Thailand) before the study. In comparison, 120 (25.6%) of the 469 patients with traveller's diarrhoea had been to the Indian subcontinent, 96 (20.5%) to East Africa (mostly Kenya), 76 (16.2%) to South east Asia (predominantly Thailand), and 65 (13.9%) to Central America. Presentation of patients positive for either *Cryptosporidium* or *Cyclospora* was evenly distributed throughout the study period (May to September 1995).

Discussion

Quick, simple and reliable tests are required for the screening of large numbers of patients for intestinal parasites. Acid fast staining, which is able to detect oocysts of *Cyclospora* and *Cryptosporidium* with satisfactory sensitivity,³³ can be included in most routine parasitological laboratory procedures without much expenditure. As indicated by the low prevalence in the group of travellers investigated in this study (1.6% for *Cryptosporidium* and 0.6% for *Cyclospora*), neither parasite appears to be a major cause of diarrhoea in international travellers. However, they should be considered as causative pathogens in patients with prolonged watery diarrhoea, since infection with *C. parvum* occurred in 2.8% of the 469 patients presenting with diarrhoea and *Cyclospora* accounted for 1.1% of these infections. Consideration of these pathogens is especially warranted because diagnosis can be made readily by acid fast stain, and treatment (at least of *Cyclospora*) is available.³⁴

ELISAs for the detection of *C. parvum* antigen in stool samples have recently been developed in several laboratories,^{31, 32} and some are now commercially available. To assess its usefulness in clinical and epidemiological settings, we evaluated one of these test kits. Compared with microscopic examination of stool samples, the ELISA turned out to be markedly less sensitive (66.7% *v* 93.3%) at an optical density of ≥ 0.05 , as recommended by the manufacturer (table 1). The technical properties of an ELISA mean that many specimens can be processed and read by a single technician in a short period of time, thereby maintaining a level standard. Therefore the ELISA is potentially more suitable than microscopy in certain settings, especially in epidemiological surveys and in follow up examinations of patients known to be *Cryptosporidium* positive. However, although the small number of positives in the group investigated does not allow a definitive answer, the ELISA used in this investigation was clearly less sensitive than microscopic analysis of stool samples.

Watery diarrhoea was the main symptom in all patients positive for either *Cyclospora* or *Cryptosporidium*. An interesting, and somewhat unexpected, finding was the occurrence of an itching exanthema in three patients with *C. parvum* infection. The mean duration of symptoms was quite long: 19.8 days for patients

positive for *Cyclospora* and 36.2 days for those positive for *Cryptosporidium*. Some patients reported the presence of diarrhoeal and other gastrointestinal symptoms for up to 120 days. All of these "long-sufferers" had stool samples tested microscopically at various times without any positive result. These findings agree with findings that patients with *Cyclospora* or *Cryptosporidium* infection frequently remain undiagnosed because of a lack of routine laboratory procedures to identify these pathogens.

The data do not connect a particular travel destination with the acquisition of either *Cyclospora* or *Cryptosporidium*. The Indian subcontinent and Indonesia have been reported as being high risk areas for both pathogens.^{1 2 6 9} However, a sizeable number of our patients acquired the respective infection in Africa or the Americas. The results of the present investigation show that infection with either *Cyclospora* or *Cryptosporidium* appears to be a fairly rare event in international travellers. However, an examination of fresh stool samples by modified acid fast staining could be useful for patients with prolonged watery diarrhoea after a journey to a developing country.

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