Human cyclosporiasis

Annunziata Giangaspero, Robin B Gasser

Cyclospora species are socioeconomically important protistan pathogens. _Cyclospora cayetanensis_ is usually transmitted via food or water to a human host via the faecal–oral route and can cause the gastrointestinal disease cyclosporiasis, which can be complicated by extra-intestinal disorders, particularly in immune-compromised people. Although more than 2 million children die each year from diarrhoeal diseases worldwide, it is not known to what extent cyclosporiasis is involved. Few epidemiological data are available on _Cyclospora_ as a water-borne and food-borne pathogen in both underprivileged communities and developed countries. To gain an improved understanding of human cyclosporiasis, this Review describes the background of _Cyclospora_, summarises salient aspects of the pathogenesis, epidemiology, diagnosis, treatment, and control of cyclosporiasis, and explores what is known about its prevalence and geographical distribution. The findings show that the effect on human health of cyclosporiasis is likely underestimated, and recommendations are made about areas of future research and the prevention and control of this disease within an international collaborative context.

Lancet Infect Dis 2019; 19: e226–36

Published **Online** March 15, 2019 http://dx.doi.org/10.1016/S1473-3099(18)30789-8

Department of Science of Agriculture, Food and Environment, University of Foggia, Foggia, Italy (Prof A Giangaspero PhD); **and Department of Veterinary Biosciences, Melbourne Veterinary School, The University of Melbourne, Parkville, VIC, Australia** (Prof R B Gasser PhD)

Correspondence to: Prof Robin B Gasser, Department of Veterinary Biosciences, Melbourne Veterinary School, The University of Melbourne, Parkville, VIC 3010, Australia **robinbg@unimelb.edu.au**

Background

Water-borne and food-borne protistan pathogens, such as those of the genera *Giardia*, *Cryptosporidium*, and *Cyclospora* are of major public health importance because they cause intestinal diseases. They particularly affect very young, elderly, and immunocompromised or immunosuppressed people in both developed and developing countries.[1–4] Of these pathogen groups, the apicomplexan *Cyclospora cayetanensis* (Eimeriidae) has received less attention, despite its known importance as a pathogen in some industrialised countries.[5–7] In humans, this protist is transmitted via the ingestion of water or food contaminated by infective oocysts, and is responsible for cyclosporiasis, an enteric disease usually associated with clinical signs including nausea, abdominal cramps, and profuse, watery diarrhoea.[6,8,9] Currently, 19 taxa of *Cyclospora* are recognised, and these infect reptiles, insectivores,[2] and primates.[10,11] Humans are reported to be infected only by a single species, *C cayetanensis*,[12,13] and it is still unclear whether animals other than humans can harbour this species[2] (table).

Outbreaks of cyclosporiasis in humans have been reported mostly from North America,[6,14–17] from the infection sources of contaminated fresh food products, such as soft fruits (raspberries), leafy vegetables (coriander, basil, and mixed salad), and herbs[2,14,17] (figure 1). Soil is another possible infection source, particularly in areas with poor environmental sanitation.[18–20]

The dissemination of infective *Cyclospora* oocysts via water, soil, and unprocessed foods (eg, fruits and vegetables, including ready-to-eat salads)[21,22] is enabled by their small size (8–10 µm), low specific gravity, and high infectivity.[1,6,23] Such oocysts can survive for weeks to months in water and food, depending on the environmental temperature,[24,25] and are resistant to the routine sanitisation or chemical disinfection procedures used in irrigation systems, recreational waters, or drinking water treatment plants.[2,25] Although *C cayetanensis* is recognised as an important contaminant of fresh produce in the USA,[26] this is not the case for many developing and underprivileged countries, where usually there is a dearth of epidemiological information on this pathogen; increasing our understanding of *Cyclospora* and cyclosporiasis and their public health significance is therefore crucial. Other than the important contributions covered by three key review articles,[1,2,6] there have been some substantial research and technological advances in this field since 2010. Here, we undertook a comprehensive, global review of cyclosporiasis, covering the clinical, pathological, diagnostic, epidemiological, and interventional aspects, to identify key deficiencies and knowledge gaps, draw conclusions, and make key recommendations for future research. To achieve this, we screened 1025 articles on *Cyclospora* and cyclosporiasis published

	Hosts
C glomericola	Diplopods
C caryolitica	Insectivores
C viperae	Snakes
C babaulti	Snakes
C tropidonoti	Snakes
C scinci	Snakes
C zamenis	Snakes
C niniae	Snakes
C talpae	Insectivores
C ashtabulensis	Insectivores
C megacephali	Insectivores
C parascalopi	Insectivores
C angimurinensis	Rodents
C cercopitheci	Green monkeys
C colobi	Colobus monkey
C papionis	Baboons
C macacae	Rhesus monkeys
C shneideri	Snakes
C cayetanensis	Humans*

*There is uncertainty as to whether non-human primates, dogs, cattle, and chickens represent hosts for *C cayetanensis*.

***Table*: Species of *Cyclospora* and their hosts[2,10,11]**

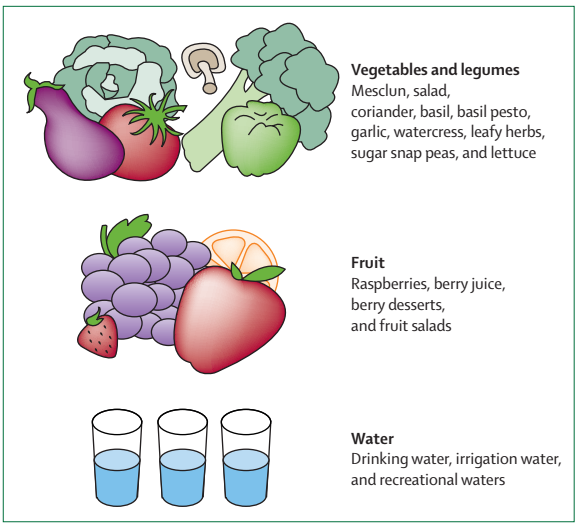

Figure 1: **Confirmed sources of cyclosporiasis worldwide**
The clinical cases in returning travellers and outbreaks of cyclosporiasis in the USA spurred investigation into the sources of infection(s). Consumption of fresh produce (fruits, herbs, and vegetables) is recognised as the main source, followed by water, usually in under-privileged countries or communities. The possibility that vertebrate animals can represent hosts and are thus amplifiers of the infection is intriguing, but zoonotic transmission remains a contentious issue.

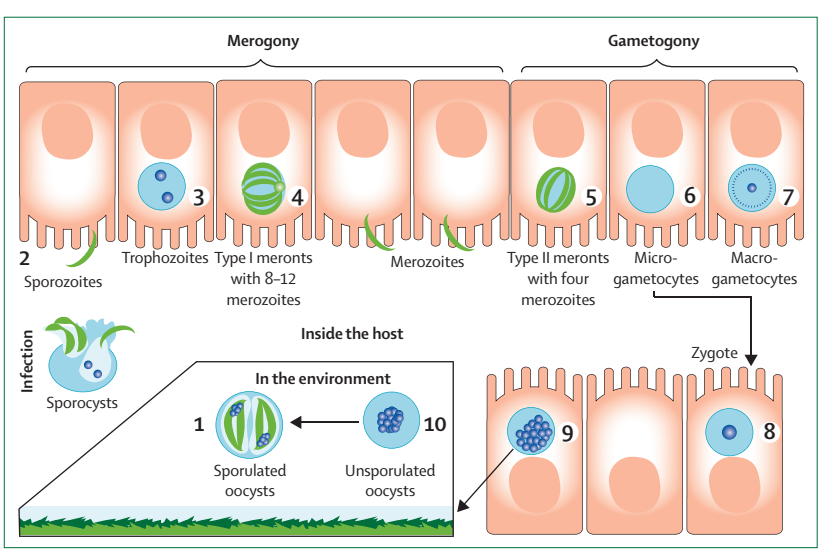

Figure 2: **Life cycle of *Cyclospora cayetanensis* in humans**
Infection commences when a susceptible individual ingests food or water contaminated with sporulated oocysts (1). In the upper gastrointestinal tract, the oocysts excyst and release sporozoites (2), which then infect the epithelium cells lining the small intestine, particularly in the jejunum. The sporozoites and subsequent stages invade the enterocytes, situated in the cytoplasm in a supranuclear position surrounded by parasitophorous vacuoles. Once inside the enterocytes, the sporozoites transform into trophozoites (3), which proliferate asexually in the merogony (or schizogony) phase to form meronts, which contain merozoites. Two types of meronts develop: type I that contain eight to 12 merozoites (4), which penetrate host cells and form type II meronts, which contain four merozoites (5). Once released, the type II merozoites enter the intestinal cells and initiate the sexual (gametogony) phase in which some meronts form (male) microgametocytes (6) or (female) macrogametocytes (7). The microgametocytes fertilise the macrogametocytes to produce zygotes (8), which differentiate into unsporulated oocysts (9) containing the sporont and are eventually released into the lumen of the intestine. From here they pass, as a non-infectious stage, into the faeces (10) and then into the environment, where they sporulate within days (in the sporogony phase) and become infective. Adapted and modified from the scientific literature.[2,11,25,33]

between May, 1961, and April, 2018, with no language restrictions, including 344 papers published since 2010.

Clinical presentation

The presentation of cyclosporiasis varies depending on the host's age and immune status, endemicity in a particular region, and probably other as yet unknown factors. This disease can be symptomatic (mostly acute and self-limiting) or non-symptomatic. Symptoms usually include watery, sometimes explosive, foul-smelling diarrhoea, abdominal cramping, bloating, headache, nausea, loss of appetite, and weight loss or reduced weight gain (or both); other signs can include vomiting and low-grade fever.[3,9,27,28] Clinical symptoms can persist for a few days to a month or longer, if patients are not treated,[29] and usually resolve quicker if treated with specific drugs, such as trimethoprim plus sulfamethoxazole.[30,31] In endemic countries, clinical signs can vary from severe in very young and elderly people, to non-symptomatic or mild in some individuals. Travellers from non-endemic countries visiting endemic regions are usually severely affected by cyclosporiasis.[32] Although human cyclosporiasis is usually not fatal in developed countries, protracted diarrhoea often leads to dehydration,[28] particularly in infants who are at greatest risk of severe dehydration and death, especially if cyclosporiasis is complicated by infections with other pathogens (viral, bacterial, or parasitic—eg, *Cryptosporidium* and *Giardia*), malnutrition, or malabsoption, particularly in underprivileged communities.[33] This aspect should be considered in the context that, in the developing world, diarrhoeal diseases are the second-leading cause of death in children younger than 5 years.[34]

Pathogenesis

Cyclosporiasis usually affects the small intestine,[9,35] although extraintestinal forms of disease can occur.[36]

Cyclospora infection of the intestine is initiated when sporozoites attach to and penetrate enterocytes, replicate asexually and spread to surrounding enterocytes in villi and crypts[9] (figure 2). Although the mechanisms are not fully understood, the replication of endogenous stages of *Cyclospora* during merogony (schizogony) and, to a lesser extent during gametogony, destroys the brush border and leads to altered mucosal architecture with a shortening and widening of the intestinal villi, atrophy, oedema of the villi, hyperplasia of the crypts, and reactive hyperaemia, with vascular dilatation and congestion of villous capillaries.[9,27,37] These pathological changes likely result in the loss of membrane-bound digestive enzymes and diminish the surface area of the intestine, leading to diarrhoea and a decreased uptake of electrolytes, water, and nutrients.[27] Inflammation occurs to a substantial degree, and relates to mixed cellular infiltration (of lymphocytes, plasma cells, and, to a lesser extent, eosinophils) in infected tissues as a result of the host immune attack against the parasite.[27] Besides children and elderly people, disease can be particularly severe in

immunosuppressed or immunocompromised people, such as individuals with HIV/AIDS[38–40] who might also develop extraintestinal forms of cyclosporiasis, including biliary disease, such as acalculous cholecystitis; Guillain-Barré syndrome; reactive arthritis syndrome; inflammatory oligoarthritis; ocular inflammation (conjunctivitis, iritis, or episcleritis); and sterile urethritis. These extraintestinal forms of the disease are thought to occur several weeks following intestinal *Cyclospora* infection and associated diarrhoeal illness.[41–45] Cyclosporiasis can be particularly challenging in patients who have received an organ transplantation and are undergoing immunosuppressive treatment.[28,45]

Conventional methods of diagnosis

Clinical diagnosis of cyclosporiasis is based on cardinal symptoms, including watery diarrhoea, abdominal cramping, and bloating. In untreated, immunocompetent people the diarrhoea can last from days to weeks to a month or more, and can wax and wane, with variable oocyst shedding.[46–49] Oocysts can continue to be shed (intermittently or continuously) by non-symptomatic people, and symptoms can also persist in the absence of oocysts in faeces.[49] In a clinical context, conventional diagnosis usually involves microscopic examination of stained (eg, with haematoxylin and eosin) intestinal tissue biopsy sections,[50,51] stool samples[52] for the presence of developmental stages of *Cyclospora*, or advanced molecular testing for DNA.[53]

Because of the intermittent nature of oocyst shedding and the low numbers of this stage in faeces,[54] it is recommended that multiple faecal specimens are collected from patients at 2–3 day intervals over a period of more than a week.[55] Wherever possible, oocysts should be concentrated from clinical or biological samples to maximise the sensitivity of detection.[2] Such concentrations can be achieved by using ethyl acetate-formalin sedimentation,[56,57] followed by discontinuous sugar gradients, Renocal-sucrose gradient sedimentation,[58] or perhaps more efficiently by discontinuous Percoll gradient centrifugation.[59]

Cyclospora oocysts are spherical, smooth, thin-walled, and refractile. Given their size and similarity to some other coccidian microorganisms, such as *Cryptosporidium* (4–6 µm), they should be stained on wet or dry mounts to achieve an increased specificity of detection.[3] Staining techniques include modified Ziehl-Neelsen (acid fast), safranin, auramine, rhodamine, Kinyoun, Giemsa, and trichrome.[3] The sensitivity of detection varies markedly among these techniques, depending on the protocol used and whether the oocysts are stained positively or not.[60]

Although acid-fast trichrome staining is appropriate for detection of some protozoans,[61] it is not reliable for detection of *Cyclospora* oocysts, which can remain unstained (so-called ghosts) on slides.[60] The modified Ziehl-Neelsen method, which stains oocysts pink to red, appears to achieve the best sensitivity, estimated at 95–100% compared with safranin stains (98% sensitivity),[62] and is typically used in clinical settings. Fluorescence-based microscopy provides an alternative means of detection;[13,63] with this technique, oocysts autofluoresce and can be better detected using a 330–380 nm wavelength (bluish-white);[64] however, a 450–490 nm wavelength (green) dichronic mirror excitation filter can be advantageous for detection.[12,64] Each of these techniques has its advantages or disadvantages and needs to be critically assessed before being used as a diagnostic tool. Although the advantage of such methods is that they are reasonably simple technically, time-efficient, and inexpensive, their disadvantages predominantly relate to limited sensitivity and specificity, and their inability to identify the species of stained *Cyclospora* oocysts and to differentiate among the species based on size and morphology of oocysts.[2]

The US Environmental Protection Agency and the US Centers for Disease Control and Prevention (CDC) have recommended the development and use of methods that are based on fluorescence and differential interference for microscopic detection of *Cryptosporidium* and *Giardia* in water samples (ie, methods 1622 and 1623).[65,66] These techniques were designed specifically for detecting *Cryptosporidium* oocysts in water samples, and rely on filtration or purification by immunomagnetic separation from small volumes of water (20 L) or large (around 1000 L), followed by the labelling of oocysts with the fluorogen 4'6-diamidino-2-phenyl indole and epifluorescence and differential interference microscopic detection. Similar techniques for detection of *Cyclospora* oocysts are not yet available. Although these approaches are used for detecting oocysts to the genus level, they have low diagnostic sensitivity and specificity, and do not allow specific identification or delineation of *Cryptosporidium*. Other diagnostic tools, such as agglutination assays, enzyme-linked immunoassays in a cartridge or dip-stick format, and flow cytometry or fluorescent in-situ hybridisation techniques are established for *Cryptosporidium* detection,[67] some of which have been adapted to detect other protozoans[68] including *C cayetanensis*,[69,70] but are not widely used.

Molecular detection, diagnosis, and analysis

Although there have been attempts to develop serological and immunological techniques to detect *Cyclospora*,[71] none of these achieve a specific diagnosis of infection on an individual patient level. By contrast, modern molecular methods can achieve a specific and sensitive diagnosis in clinical settings (with faecal or tissue samples) or detection in epidemiological settings (from food and environmental samples).[53] The specific and sensitive detection of *Cyclospora*, the accurate identification of *Cyclospora* species, and the specific diagnosis of cyclosporiasis are central to clinical management and understanding the epidemiology, prevention, and control of this disease. Nucleic acid-based methods provide an enhanced diagnostic and analytical performance,

enabling the specific and genotypic detection and identification of *Cyclospora*. Improved specificity and sensitivity have been possible largely through the use of PCR,[72] which enables the specific amplification of genetic loci from tiny amounts of genomic DNA of *Cyclospora* in complex biological samples using a thermostable DNA polymerase. Genetic loci commonly used in PCR-based methods include regions within the small subunit of nuclear ribosomal RNA gene, the second internal transcribed spacer, or the 70 kDa heat shock protein gene.[72–76] PCR-based techniques such as microsatellite analysis, restriction fragment length polymorphism analysis, mutation scanning, and direct DNA sequencing have been developed for the detection, identification, and characterisation of *Cyclospora*.[77–81] The combined use of mutation scanning and direct DNA sequencing has proven to be particularly useful for genetic analysis and the accurate detection of mutations, enabling the genetic categorisation of *Cyclospora*.[80]

Multiplexed PCR and high-resolution melting approaches have also been established[82–84] and show considerable promise for specific identification using suitable genetic loci. Nested PCR methods provide the analytical sensitivity required for subsequent DNA sequencing for specific and genotypic identification,[85] and real-time (rt) PCR-based methods usually allow DNA quantitation and are amenable to automation with high throughput capacity.[86] Four rtPCR assays were assessed for *C cayetanensis*; two use a species-specific TaqMan probe to detect unique regions in the small subunit ribosomal RNA (18S rRNA) gene,[87,88] and the other two rely on DNA-binding dyes and melting curve analysis of amplicons.[75,80] Each of these assays can achieve specific identification in a single PCR. The comparison of these four assays for detecting *C cayetanensis* in human stool samples that were test-positive by microscopy indicated that all methods have a high diagnostic specificity (98–100%) and sensitivity (93–100%), and that the TaqMan probe assay[88] had the best specificity (100%) and sensitivity (100%)[89] and seems well suited for the molecular detection of *C cayetanensis* in stool samples. An interlaboratory validation of an improved rtPCR assay shows promise for the specific detection and quantitation of *C cayetanensis* oocysts in food during cyclosporiasis outbreaks.[90] However, some caution is required when assigning species status to *Cyclospora*, because amplicons produced using these methods can be distinct in sequence from *C cayetanensis*.[80] For this reason, we would recommend that mutation scanning or sequencing-based methods are consistently used in molecular epidemiological surveys to define species and genotypes of *Cyclospora*.

The Biofire FilmArray gastrointestinal panel (BioFire Diagnostics, Salt Lake City, UT, USA) also has application for detecting *Cyclospora* and 21 other enteric pathogens (including bacteria, viruses, and parasites).[91] This closed-system method can efficiently test individual faecal samples in around 1 h with 2 min of hands-on time, and includes DNA extraction and nested, multiplex PCR followed by a melting-curve analysis using FilmArray software.[4,91] The specificity and sensitivity of this assay for *Cyclospora* have both been reported as 100%.[91] Limitations of this system are that it requires stool fixed in Cary Blair transport medium and is expensive to set up and use for routine testing, but it would be helpful in outbreak situations where a rapid intervention response is required.[4]

Clearly, molecular diagnostic or analytical tools are crucial for routine monitoring in reference and diagnostic centres, as well as in the water industries; such tools should be used in combination with microscopic methods, as part of cyclosporiasis surveillance, prevention, control, and risk management efforts. It is expected that high throughput sequencing combined with informatics[53] will become the tool of choice for the specific and genotypic identification and quantification of pathogenic protists within biological matrices.

The need to test for viability and infectivity

Although no accurate assays are available that assess the viability or infectivity of *C cayetanensis*,[54] oocysts sporulate in 2·5% potassium dichromate[12] within 7–13 days at 25°C or 32°C.[25] Although unsporulated oocysts contain developing sporocysts (resembling a raspberry), sporulated oocysts contain two ovoid sporocysts, each with two sporozoites (figure 2). Oocysts excyst in vitro when exposed to trypsin (0·5%) and sodium taurocholate (1·5%) in phosphate-buffered saline, following mechanical disruption.[12] Electrorotation is another method that relies on detecting changes in the morphology and physicochemical properties of oocysts upon rotation,[92,93] but this technique is complex technically and is not entirely specific. An ideal method for assessing viability and infectivity would combine sporulation with infectivity testing in animals. However, in the absence of effective in-vitro cultivation and in-vivo experimental infection systems for *C cayetanensis*,[94] sporulation in potassium dichromate is presently the only indicator used for oocyst viability.

Treatment

Since no vaccine is available for the prevention of cyclosporiasis, chemotherapeutic treatment is crucial, particularly in immunodeficient individuals, such as those with HIV/AIDS, patients receiving organ transplantation, and individuals undergoing immunosuppressive or anticancer treatments (or both).[28,95,96] Early detection and treatment of cyclosporiasis can provide a favourable clinical outcome.

Sulfamethoxazole plus trimethoprim (co-trimoxazole) is recognised as first-line treatment against cyclosporiasis[30] and is recommended by the US Centers for Disease Control and Prevention (CDC). This combination is highly effective after 7 days of treatment,[97,98] and can also be used by patients with HIV/AIDS[50] although some cannot tolerate sulphonamides. Combined treatment with 800 mg sulfamethoxazole and 160 mg trimethoprim, taken twice

For more on **CDC advice** see http://www.cdc.gov/parasites/cyclosporiasis/treatment.html

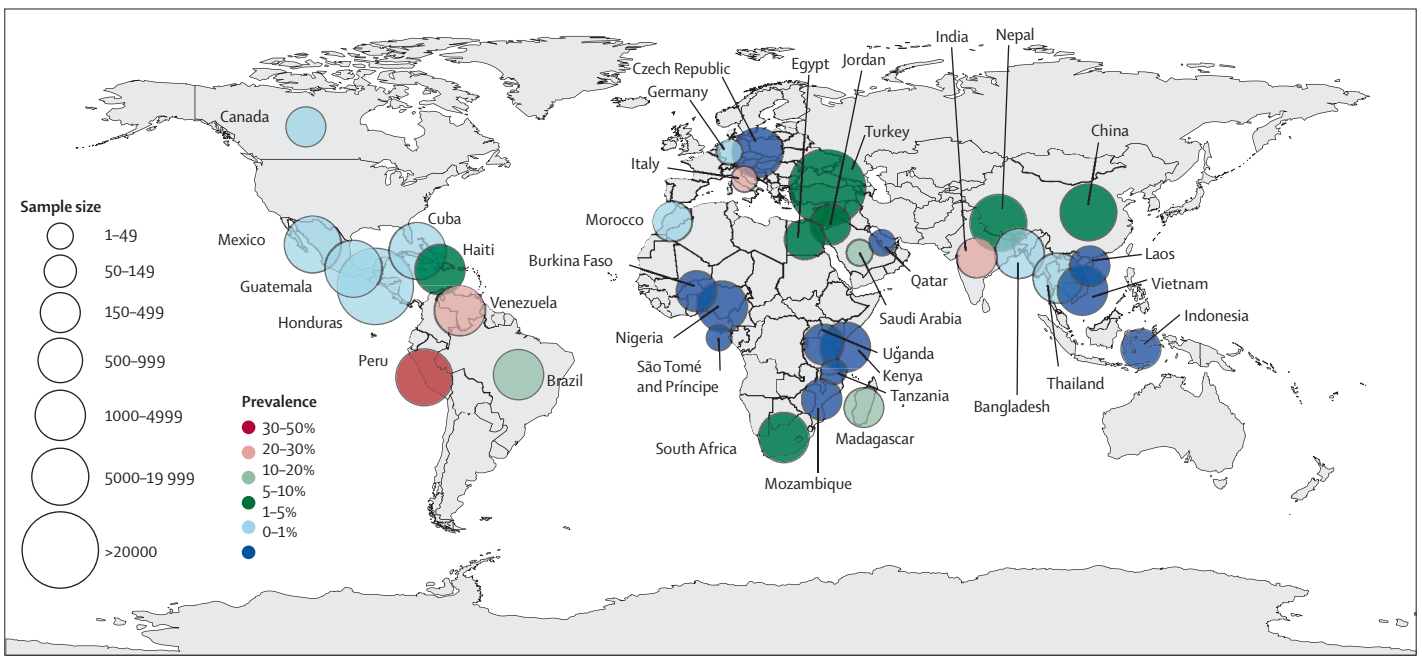

Figure 3: **Estimated prevalence and distribution of *Cyclospora* infection in immunocompetent people**
Maximum prevalences recorded in different countries and numbers of individuals tested are shown (compare with appendix for detailed data).

per day for 1 week,[98] or four times per day for 10 days and then twice per day for 3 weeks[3] usually resolves clinical symptoms and oocyst shedding within 1–2 days. In Peru, for example, excellent results were achieved when children with *Cyclospora* infection received a 3-day course of trimethoprim plus sulfamethoxazole (5–25 mg/kg bodyweight).[99,100]

Ciprofloxacin (500 mg administered orally twice per day for 1 week and then three times per week for 2 weeks) is an alternative treatment in patients who have received a transplantation. Nitazoxanide might be used in patients who are sulphonamide-intolerant[101] or who do not respond to ciprofloxacin treatment,[96] although it is not always effective.[28,101] Because of the close genetic relationship of *C cayetanensis* with *Eimeria* spp, cyclosporiasis might be treated with drugs such as sulphonamides, quinolones, and ionophores, which have been used against avian coccidioses[102] but have yet to be critically assessed against *Cyclospora*. Moreover, trials with drugs targeting mitochondrial or apicoplast metabolism might be explored for their activity against *Cyclospora*.[103–105]

Epidemiology and public health importance

Although *Cyclospora* appears to have a worldwide distribution,[32] detailed epidemiological information on this pathogen group is still scarce for most countries around the world. To provide an assessment of the prevalence of *C cayetanensis* infection in humans, determined using coprological or molecular tests, we critically appraised the relevant scientific literature (figure 3; appendix). Despite limitations in the design and execution of some published studies, large-scale epidemiological investigations indicate that, in immunocompetent people, cyclosporiasis mostly affects those in endemic countries and unprivileged communities, with prevalences of 5·6% in China,[106] 9·2% in Nepal,[107] 17·4% in Turkey,[108] and up to 22% in India.[109] Latin America is considered another important endemic area, with prevalences varying from 7·9% in Haiti,[110] 10·8% in Brazil,[111] 24·2% in Venezuela,[112] to 41·6% in Peru.[113] In African countries, highest prevalences recorded to date are in Egypt (10%)[114] and South Africa (7·2%),[115] and a systematic review and meta-analysis revealed an overall prevalence of 18% in 14 sub-Saharan countries.[116] In endemic countries, immunocompromised people, particularly children, are at high risk of cyclosporiasis. The highest prevalences have been recorded in Haiti (36%)[117] and Saudi Arabia (≤52%),[118] and in children in rural settings in Madagascar (16·5%),[119] although prevalences in some endemic countries, including India, China, Honduras, Mexico, Nepal, Peru, Thailand, and Venezuela, seem to have decreased in recent years (appendix).[120–124]

In the USA, from 1997 to 2008, the CDC recorded 1110 laboratory confirmed, sporadic cases of cyclosporiasis, about a third of which were linked to overseas travel.[125] Some evidence indicates that the incidence of infection increased substantially between 2014 and 2017.[126] In other non-endemic countries, prevalences varied from 1·9% in Canada[127] to 0·1% in Czech Republic,[128] to 2·6% in Germany[129] (mostly detected in travellers returning to

See Online for appendix

Europe), although a much higher prevalence (27·5%) was recorded in Italy in 2015.[130] Although no official outbreaks of cyclosporiasis have been recorded in Italy, autochthonous cases of the disease have been reported,[131–134] and *Cyclospora* has been detected at high prevalence (6–21%) in environmental samples such as water and soil as well as vegetable samples.[130] These examples clearly signal the need for investigations into the public health significance of *Cyclospora* in Italy and other developed countries, where the reporting of cyclosporiasis is currently not required.

Although human cyclosporiasis is expected to be more common than currently acknowledged, particularly in communities where sanitary conditions are compromised, it is challenging to assess the global impact of this parasitic disease, mainly because of the limited application of accurate and standardised molecular-diagnostic and molecular-epidemiology tools.[53] Routine coproscopic methods do not allow a specific or genotypic identification of *Cyclospora*, which hinders an improved understanding of the epidemiology of human cyclosporiasis. Well coordinated surveillance programmes for pathogenic protists, including *Cyclospora*, should be implemented to monitor sanitation and to work towards desirable improvements.[135] Clearly, there is a need to estimate the actual burden of cyclosporiasis and to gain a detailed understanding of the geographical and host distributions of *Cyclospora*, and the dynamics of disease dissemination during cyclosporiasis outbreaks using advanced molecular analytical and diagnostic tools, to justify investment in large-scale prevention and control programmes.

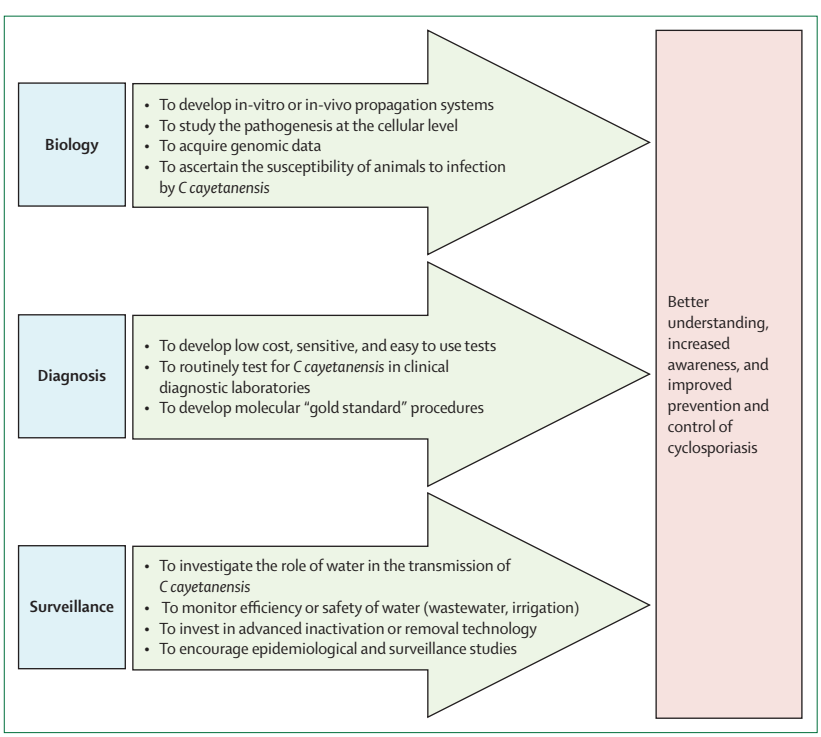

Figure 4: **Future collaborative research needed on *Cyclospora***
A OneHealth strategy, including an efficient multidisciplinary research approach dealing with key areas of biology, epidemiology, diagnosis, and treatment, is needed to enhance the knowledge base and improve the prevention and control of human cyclosporiasis.

Prevention and control

Measures to prevent or control cyclosporiasis include health education, good personal hygiene (including adequate handwashing), changes in habits (including prevention of geophagia by children in developing countries), drinking boiled or bottled water, and not eating raw produce (for immunocompromised people or those travelling to endemic areas), proper sanitary infrastructure (including toilet facilities, particularly in poor countries), and the treatment of human sewage. However, implementation of these measures can be challenging in socioeconomically disadvantaged communities[32] and some developed countries, including those in Europe. Prevention and control can be assisted by killing or removing oocysts from water or fresh foods. Since oocysts of *Cyclospora* are resistant to many disinfectants[136] used in traditional water-treatment plants and are difficult to remove from water,[130,137,138] physical treatments (eg, membrane filtration), ultraviolet disinfection, or managed aquifer recharge are possible approaches for their removal.[138]

Although sodium dichloroisocyanurate solution reduces the viability and infectivity of parasites including *Cyclospora*,[139] chlorine as sodium hypochlorite is the most widely used disinfectant by industrial producers of fresh produce (raw vegetables and fruits), because it is inexpensive and easy to use. However, it has little effectiveness against protists, because the concentration commonly used (80–100 mg/L) is too low to kill them, and an increased concentration is not permissible.[140,141] Chlorine dioxide is more effective at inactivating protists than sodium hypochlorite, but it is five to ten times more expensive and is also hazardous by comparison with sodium hypochlorite. Ozone treatment is more effective than chlorination, but is not widely used in the food industry because it is unstable and hazardous to operators.[142] In the food chain, disinfection by ultraviolet light might be an alternative approach to kill *Cyclospora* oocysts, because it is inexpensive, requires short illumination times, has a low environmental impact (ie, no toxic byproducts), and there are no chemical additives or changes to the taste or smell of the product.[142] However, this proposed approach requires rigorous assessment before implementation.

Conclusions and future prospects

The discovery of the coccidian-like parasite in humans in Papua New Guinea in the late 1970s[143] went largely unnoticed for 10 years until a documented outbreak of diarrhoea in a physician's dormitory in the USA in 1990 was linked to this microorganism.[17] It was not until 1992 that this microorganism found in some faecal samples from Peruvians was classified as *C cayetanensis*,[12] later

recognised as being closely related to members of the genus *Eimeria*.[74] Since then, food-borne transmission of *C cayetanensis* has become a substantial public health concern in the USA.[6,26] The understanding of *Cyclospora* has improved through research of its biology, pathogenesis, transmission, and epidemiology, but, despite this research there are key deficiencies and knowledge gaps for most other countries around the world. This Review shows that *Cyclospora* remains enigmatic in many respects, particularly in relation to its biology, epidemiology, and proposed zoonotic role. Despite the increasing numbers of reports on the detection of *C cayetanensis* in animals, particularly non-human primates, other mammals, and even birds, the roles of animals as true hosts—in which the protist completes its life cycle—are still unclear.

Molecular tools for the accurate identification of species and genotypes and the measurement of genetic variation within and among populations (and potentially individuals) of *Cyclospora* are pivotal to exploring many aspects of the biology and epidemiology of representatives of this genus and are crucial for tracking cyclosporiasis dissemination and outbreaks in human populations. Although substantial progress has been made in the development of techniques for detecting oocysts in biological samples and PCR-based methods for the diagnosis of infection and cyclosporiasis, there are no assays to assess the viability, infectivity, or virulence of *C cayetanensis*. The virulence aspect could be of particular importance if the initial hypothesis that distinct variants of *Cyclospora* have variable pathogenicity in the human host[69,144,145] is supported. Moreover, the number of genetic markers in current use is insufficient to reliably establish the extent of genetic diversity within and among *Cyclospora* populations from the same and distinct host animals. In our opinion, there needs to be an increased focus on genomic studies of *Cyclospora* spp as a foundation for molecular, genetic, and epidemiological investigations, and the establishment of improved diagnostic and analytical tools using extensive panels of genetic markers.

Although the draft genome resources available for *C cayetanensis*[146–148] are useful, additional genome sequencing projects of other taxa (including indeterminate species and genotypes) of *Cyclospora* are required. The availability of such nuclear and mitochondrial data would enable comparative genomic analyses, leading to a better understanding of the relationships of *Cyclospora* species and genotypes. Such analyses would also assist in the identification of genes that are conserved among species, and genes that are under positive selection, being specific to *Cyclospora* species or genotypes. Genomic comparisons of different operational taxonomic units (or species and genotypes) would lead to the identification of genes that are associated with their adaptation to different host species and enable the reliable differentiation of zoonotic from non-zoonotic taxa of *Cyclospora*. Such studies might also assist in identifying genes that are specific to zoonotic members of this genus and are essential for their survival, thus representing candidates for treatments, vaccines, or diagnostic targets.

Compared with *Cryptosporidium* and *Giardia*, progress in the genomics of *C cayetanensis* has been slow, mainly because of the challenges in obtaining a sufficient amount of oocyst material, and the absence of in-vitro cultivation and in-vivo experimental infection systems. However, major advances in informatics, genomics, and genetics,[149,150] and being able to work on tiny amounts of material provide exciting prospects for future investigations of representatives of this genus. This focus should also enable the development of reliable methods for the detection of *C cayetanensis* and other *Cyclospora* taxa in fresh produce (eg, fruit and vegetables), edible shellfish (eg, filter feeders), the environment (water and soil), and in humans and animals. In this context, we believe that a collaborative, OneHealth approach should be applied to *Cyclospora* (figure 4), as has been the case for other key protists such as *Giardia* and *Cryptosporidium*. Future collaborative research efforts could focus on several key areas relating particularly to biology, diagnosis, and surveillance (figure 4), which we believe would contribute toward establishing effective preventive and control measures against cyclosporiasis.

Clearly, protistan diseases linked to the consumption of contaminated fresh produce and water are an emerging issue,[26] but cyclosporiasis is, based on this Review, a neglected disease, both in developing and many developed countries. Considering current global challenges, such as political instability, wars, migration, an increasing demand for fresh foods, and climate change, there is a need for better prevention, surveillance, and control strategies, which is why a multidisciplinary

Search strategy and selection criteria

The publications cited in this Review were identified through comprehensive searches of the PubMed and Scopus databases (up to May 1, 2018). For references cited in the whole text/contribution, searches were conducted using the terms "cyclospor*" AND ("taxonomy" OR "nomenclature"); "cyclospor*" AND (life-cycle" OR "transmission" OR "host"); "cyclospor*" AND ("histo*" OR "clinical manifestation" OR "syndrome"); "cyclospor*" AND ("epidemiology" OR "prevalence"); "cyclospor*" AND "travel" OR "outbreak" OR "public health"); "cyclospor*" AND ("immunocomp*" OR "non-immunocom" OR "immunological status"); "cyclospor*" AND ("age"); "cyclospor*" AND "gender"); "cyclospor*" AND ("season"); "cyclospor*" AND "source"); "cyclospor*" AND ("detection" OR "diagnosis" OR "microscopy"); "cyclospor*" AND ("sporulation" OR "viability"); "cyclospor*" AND "resistance"); "cyclospor*" AND ("treatment" OR "chemotherapy"); "cyclospor*" AND ("immunol*" OR "vaccine"); "cyclospor*" AND ("surveillance" OR "risk assessment"); "cyclospor*" AND "sanitation" OR "hygiene"); "cyclospor*" AND ("prevention" OR "control"; "cyclospor*" AND ("molecular" OR "genom*"). A total of 1025 publications were identified; we screened the titles and abstracts and identified articles with relevant content and context. The full texts of these articles were read to verify their relevance to the present topics in this Review.

approach is emphasised here to fill the knowledge gaps surrounding cyclosporiasis and *Cyclospora* (figure 4). Addressing these areas should provide crucial data and information for risk assessments and for the implementation of preventive and control measures to tackle what is still a mysterious pathogen–disease complex of considerable public health importance worldwide.

Contributors

AG undertook the literature search and prepared the tables and figures. AG and RBG wrote the manuscript.

Declaration of interests

We declare no competing interests.

Acknowledgments

AG is mainly supported by grants from the Laboratories for Innovation of Functional Food: Rete di laboratori per l'innovazione nel campo degli alimenti funzionali (codice n 47), PO Puglia FESR 2007–13, Asse I, Linea 12, Accordo di Programma Quadro in materia di Ricerca Scientifica; Intervento Reti di Laboratori Pubblici di Ricerca. RBG is supported by grants from the Australian Research Council, the National Health and Medical Research Council of Australia, Melbourne Water Corporation, and Yourgene Bioscience. Although we acknowledge all contributions in the field of *Cyclospora* and cyclosporiasis, we were not able to cite all peer-reviewed publications in this Review due to a restriction in the number of references that can be cited and listed. We thank Vito Santacesarea for his contributions to figure 3.

References

1 Chacín-Bonilla L. Epidemiology of *Cyclospora cayetanensis*: a review focusing in endemic areas. *Acta Trop* 2010; **115:** 181–93.

2 Ortega YR, Sanchez R. Update on *Cyclospora cayetanensis*, a food-borne and waterborne parasite. *Clin Microbiol Rev* 2010; **23:** 218–34.

3 Fletcher SM, Stark D, Harkness J, Ellis J. Enteric protozoa in the developed world: a public health perspective. *Clin Microbiol Rev* 2012; **25:** 420–49.

4 Ryan U, Paparini A, Oskam C. New technologies for detection of enteric parasites. *Trends Parasitol* 2017; **33:** 532–46.

5 Insulander M, Svenungsson B, Lebbad M, Karlsson L, de Jong B. A foodborne outbreak of *Cyclospora* infection in Stockholm, Sweden. *Foodborne Pathog Dis* 2010; **7:** 1585–87.

6 Herwaldt B. *Cyclospora cayetanensis*: a review, focusing on the outbreaks of cyclosporiasis in the 1990s. *Clin Infect Dis* 2000; **31:** 1040–57.

7 Plutzer J, Karanis P. Neglected waterborne parasitic protozoa and their detection in water. *Water Res* 2016; **101:** 318–32.

8 Ortega YR, Roxas CR, Gilman RH, et al. Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemic region in Peru. *Am J Trop Med Hyg* 1997; **57:** 683–86.

9 Connor BA, Reidy J, Soave R. Cyclosporiasis: clinical and histopathologic correlates. *Clin Infect Dis* 1999; **28:** 1216–22.

10 Lainson R. The genus *Cyclospora* (Apicomplexa: Eimeriidae), with a description of *Cyclospora schneideri* n sp in the snake *Anilius scytale scytale* (Aniliidae) from Amazonian Brazil: a review. *Mem do Inst Oswaldo Cruz* 2005; **100:** 103–10.

11 Li N, Ye J, Arrowood MJ, et al. Identification and morphologic and molecular characterization of *Cyclospora macacae* n. sp. from rhesus monkeys in China. *Parasitol Res* 2015; **114:** 1811–16.

12 Ortega YR, Sterling CR, Gilman RH, Cama VA, Diaz F. *Cyclospora* species—a new protozoan pathogen of humans. *N Engl J Med* 1993; **328:** 1308–12.

13 Ortega YR, Gilman RH, Sterling CR. A new coccidian parasite (Apicomplexa: Eimeriidae) from humans. *J Parasitol* 1994; **80:** 625–29.

14 Hall RL, Jones JL, Hurd S, Smith G, Mahonì BE, Herwaldt BL. Population-based active surveillance for *Cyclospora* infection-United States, Foodborne Diseases Active Surveillance Network (FoodNet), 1997–2009. *Clin Infect Dis* 2012; **54:** S411–17.

15 Kozak GK, MacDonald D, Landry L, Farber JM. Foodborne outbreaks in Canada linked to produce: 2001 through 2009. *J Food Prot* 2013; **76:** 173–83.

16 Centers for Disease Control and Prevention. Cyclosporiasis update. 2016. https://www.cdc.gov/parasites/cyclosporiasis/outbreaks/2016/index.html (accessed April 27, 2018).

17 Huang P, Weber JT, Sosin DM, et al. The first reported outbreak of diarrheal illness associated with *Cyclospora* in the United States. *Ann Intern Med* 1995; **123:** 409–14.

18 Mansfield LS, Gajadhar AA. *Cyclospora cayetanensis*, a food- and waterborne coccidian parasite. *Vet Parasitol* 2004; **126:** 73–90.

19 Chacin-Bonilla L. Transmission of *Cyclospora cayetanensis* infection: a review focusing on soil-borne infection. *Trans R Soc Trop Med Hyg* 2008; **102:** 215–16.

20 Gomez Martínez E, Figuera L, Guilarte DV, et al. First report of *Cyclospora cayetanensis* in an indigenous Kariña A community in Sucre state, Venezuela. *B Malariol Salud Amb* 2016; **56:** 19–29.

21 Caradonna T, Marangi M, Del Chierico F, et al. Detection and prevalence of protozoan parasites in ready-to-eat packaged salads on sale in Italy. *Food Microbiol* 2017; **67:** 67–75.

22 Dixon B, Parrington L, Cook A, Pollari F, Farber J. Detection of *Cyclospora*, *Cryptosporidium*, and *Giardia* in ready-to-eat packaged leafy greens in Ontario, Canada. *J Food Prot* 2013; **76:** 307–13.

23 Sterling CR, Ortega YR. *Cyclospora*: an enigma worth unravelling. *Emerg Infect Dis* 1999; **5:** 48–53.

24 Smith HV, Paton CA, Mitambo MM, Girdwood RW. Sporulation of *Cyclospora* sp oocysts. *Appl Environ Microbiol* 1997; **63:** 1631–32.

25 Ortega YR, Sterling CR, Gilman RH. *Cyclospora cayetanensis*. *Adv Parasitol* 1998; **40:** 399–418.

26 Dixon BR. Parasitic illness associated with the consumption of fresh produce—an emerging issue in developed countries. *Current Opinion Food Sci* 2016; **8:** 104–09

27 Ortega YR, Nagle R, Gilman RH, et al. Pathologic and clinical findings in patients with cyclosporiasis and a description of intracellular parasite life-cycle stages. *J Infect Dis* 1997; **176:** 1584–89.

28 Bednarska M, Bajer A, Welc-Faleciak R, Pawelas A. *Cyclospora cayetanensis* infection in transplant traveller: a case report of outbreak. *Parasit Vectors* 2015; **8:** 411.

29 Thapa SS, Basnyat B. Chronic diarrhea in a traveler: cyclosporiasis. *Am J Med* 2017; **130:** e535–36

30 Goldberg E, Bishara J. Contemporary unconventional clinical use of co-trimoxazole. *Clin Microbiol Infect* 2012; **18:** 8–17.

31 Centers for Disease Control and Prevention. 2015. Cyclosporiasis outbreak investigations—United States, 2015. https://www.cdc.gov/parasites/cyclosporiasis/outbreaks/2015/index.html (accessed April 27, 2018).

32 Chacin-Bonilla L. *Cyclospora cayetanensis*. In Rose JB, Jiménez-Cisneros B, eds. Global water pathogens project. Michigan State University: UNESCO, 2017: 1–43

33 Behera B, Mirdha BR, Makharia GK, Bhatnagar S, Dattagupta S, Samantaray JC. Parasites in patients with malabsorption syndrome: a clinical study in children and adults. *Dig Dis Sci* 2008; **53:** 672–79.

34 WHO. Diarrhoeal disease. Key facts. Geneva: World Health Organization, May 2, 2017. http://www.who.int/en/news-room/fact-sheets/detail/diarrhoeal-disease (accessed April 28, 2018).

35 Sun T, Ilardi CF, Asnis D, et al. Light and electron microscopic identification of *Cyclospora* species in the small intestine. Evidence of the presence of asexual life cycle in human host. *Am J Clin Pathol* 1996; **105:** 216–20.

36 de Górgolas M, Fortés J, Fernández Guerrero ML. *Cyclospora cayetanensis* cholecystitis in a patient with AIDS. *Ann Intern Med* 2001; **134:** 166.

37 Connor BA, Shlim DR, Scholes JV. Pathologic changes in the small bowel in nine patients with diarrhoea associated with a coccidian-like body. *Ann Intern Med* 1993; **119:** 377–82.

38 Pape JW, Verdier RI, Boncy M, Boncy J, Johnson WD. *Cyclospora* infection in adults infected with HIV. Clinical manifestations, treatment, and prophylaxis. *Ann Intern Med* 1994; **121:** 654–57.

39 Sifuentes-Osornio J, Porras-Cortes G, Bendall RP, Morales-Villarreal F, Reyes-Teran G, Ruiz-Palacios GM. *Cyclospora cayetanensis* infection in patients with and without AIDS: biliary disease as another clinical manifestation. *Clin Infect Dis* 1995; **21:** 1092–97.

40 Chacín-Bonilla L, Estevez J, Monsalve F, Quijada L. *Cyclospora cayetanensis* infections among diarrheal patients from Venezuela. *Am J Trop Med Hyg* 2001; **65:** 351–54.

41 Richardson RF Jr, Remler BF, Katirji B, Murad MH. Guillain-Barré syndrome after *Cyclospora* infection. *Muscle Nerve* 1998; **21:** 669–71.

42 Connor BA, Johnson EJ, Soave R. Reiter syndrome following protracted symptoms of *Cyclospora* infection. *Emerg Infect Dis* 2001; **7:** 453–54.

43 Zar FA, El-Bayoumi E, Yungbluth MM. Histologic proof of acalculous cholecystitis due to *Cyclospora cayetanensis*. *Clin Infect Dis* 2001; **33:** 140–41.

44 Marcos LA, Gotuzzo E. Intestinal protozoan infections in the immunocompromised host. *Curr Opin Infect Dis* 2013; **26:** 295–301.

45 Visvesvara GM, Arrowood MJ, Qvarnstrom Y, et al. Concurrent parasitic infections in a renal transplant patient. *Emerg Infect Dis* 2013; **19:** 2044–45.

46 Soave R, Dubey JP, Ramos LJ, Tummings M. A new intestinal pathogen? *Clin Res* 1986; **34:** 533A.

47 Sancak B, Akyon Y, Erguven S. *Cyclospora* infection in five immunocompetent patients in a Turkish university hospital. *J Med Microbiol* 2006; **55:** 459–62.

48 Shlim DR, Cohen MT, Eaton M, Rajah R, Long EG, Unger BLP. An alga like organism associated with an outbreak of prolonged diarrhea among foreigners in Nepal. *Am J Trop Med Hyg* 1991; **45:** 383–89.

49 Hoge CW, Shlim DR, Rajah R, et al. Epidemiology of diarrhoeal illness associated with coccidian-like organism among travellers and foreign residents in Nepal. *Lancet* 1993; **341:** 1175–79.

50 Tsang OT, Wong RW, Lam BH, Chan JM, Tsang KY, Leung WS. *Cyclospora* infection in a young woman with human immunodeficiency virus in Hong Kong: a case report. *BMC Res Notes* 2013; **6:** 521.

51 Nath J, Hussain G, Singha B, Paul J, Ghosh SK. Burden of major diarrheagenic protozoan parasitic co-infection among amoebic dysentery cases from North East India: a case report. *Parasitology* 2015; **142:** 1318–25.

52 Clarke CS, McInyre M. Modified detergent Ziehl-Nelseen technique for the staining of *Cyclospora cayetanensis*. *J Clin Pathol* 1996; **49:** 511–12.

53 Ortega YR, Robertson LJ. *Cyclospora cayetanensis* as a foodborne pathogen. Cham: Springer International Publishing AV, 2017.

54 Eberhard ML, Pieniazek NJ, Arrowood MJ. Laboratory diagnosis of *Cyclospora* infections. *Arch Pathol Lab Med* 1997; **121:** 792–97.

55 Cann KJ, Chalmers RM, Nichols G, O'Brien SJ. *Cyclospora* infections in England and Wales: 1993 to 1998. *Commun Dis Public Health* 2000; **3:** 46–49.

56 Negm AY. Identification of *Cyclospora cayetanensis* in stool using different stains. *J Egypt Soc Parasitol* 1998; **28:** 429–36.

57 Kimura K, Kumar SR, Takemasa K, et al. Comparison of three microscopic techniques for diagnosis of *Cyclospora cayetanensis*. *FEMS Microbiol Lett* 2004; **238:** 263–66.

58 Riner DK, Mullin AS, Lucas SY, Cross JH, Lindquist HD. Enhanced concentration and isolation of *Cyclospora cayetanensis* oocysts from human fecal samples. *J Microbiol Methods* 2007; **71:** 75–77.

59 Medina-De la Garza CE, García-López HL, Salinas-Carmona MC, González-Spencer DJ. Use of discontinuous Percoll gradients to isolate *Cyclospora* oocysts. *Ann Trop Med Parasitol* 1997; **91:** 319–21.

60 Harrington BJ. Microscopy of 4 pathogenic enteric protozoan parasites: a review. *Lab Med* 2008; **39:** 231–38.

61 Rigo CR, Franco RM. Comparison between the modified Ziehl-Neelsen and Acid-Fast-Trichrome methods for fecal screening of *Cryptosporidium parvum* and *Isospora belli*. *Rev Soc Bras Med Trop* 2002; **35:** 209–14 (in Portuguese).

62 Galvan-Díaz AL, Herrera-Jaramillo V, Santos-Rodriguez ZM, Delgado-Naranjo M. Coloraciones Ziehl-Neelsen y Safranina modificadas para el diagnóstico de *Cyclospora cayetanensis*. *Rev Salud Publica (Bogota)* 2008; **10:** 488–93.

63 Long EG, White EH, Carmichael WW, et al. Morphologic and staining characteristics of a cyanobacterium-like organism associated with diarrhea. *J Infect Dis* 1991; **164:** 199–202.

64 Berlin OG, Peter JB, Gagne C. Autofluorescence and the detection of *Cyclospora* oocysts. *Emerg Infect Dis* 1998; **4:** 127–28.

65 US Environmental Protection Agency. Method 1622: *Cryptosporidium* in water by filtration/IMS/FA. December 2005, EPA 815–R–05–001.

66 US Environmental Protection Agency. Method 1623: *Cryptosporidium* and *Giardia* in water by filtration/IMS/FA. 2005, EPA 815–R–05–002.

67 Jex AR, Smith HV, Nolan MJ, et al. Cryptic parasite revealed improved prospects for treatment and control of human cryptosporidiosis through advanced technologies. *Adv Parasitol* 2011; **77:** 141–73.

68 Karanis P, Aldeyarbi HM, Mirhashemi ME, Khalil KM. The importance of the waterborne transmission of *Toxoplasma gondii* and analysis efforts for water detection: an overview and update. *Environ Sci Pollut Res* 2013; **20:** 86–99.

69 Hussein EM, El-Moamly AA, Dawoud HA, Fahmy H, El-Shal HE, Sabek NA. Real-time PCR and flow cytometry in detection of *Cyclospora* oocysts in fecal samples of symptomatic and asymptomatic pediatrics patients. *J Egypt Soc Parasitol* 2007; **37:** 151–70.

70 Plutzer J, Karanis P. Neglected waterborne parasitic protozoa and their detection in water. *Water Res* 2016; **101:** 318–32.

71 Clarke CS, McInyre M. An attempt to demonstrate a serological immune response in patients infected with *Cyclospora cayetanensis*. *Br J Biomed Sci* 1997; **54:** 73–74.

72 Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988; **239:** 487–91.

73 Lalonde LF, Gajadhar AA. Highly sensitive and specific PCR assay for reliable detection of *Cyclospora cayetanensis* oocysts. *Appl Environ Microbiol* 2008; **74:** 4354–58.

74 Relman DA, Schmidt TM, Gajadhar A, et al. Molecular phylogenetic analysis of *Cyclospora*, the human intestinal pathogen, suggests that it is closely related to *Eimeria* species. *J Infect Dis* 1996; **173:** 440–45.

75 Lalonde LF, Gajadhar AA. Detection and differentiation of coccidian oocysts by real-time PCR and melting curve analysis. *J Parasitol* 2011; **97:** 725–30.

76 Sulaiman IM, Torres P, Simpson S, Kerdahi K, Ortega Y. Sequence characterization of heat shock protein gene of *Cyclospora cayetanensis* isolates from Nepal, Mexico, and Peru. *J Parasitol* 2013; **99:** 379–82.

77 Jinneman KC, Wetherington JH, Hill WE, et al. Template preparation for PCR and RFLP of amplification products for the detection and identification of *Cyclospora* sp and *Eimeria* spp oocysts directly from raspberries. *J Food Prot* 1998; **61:** 1497–503.

78 Quintero-Betancourt W, Peele ER, Rose JB. *Cryptosporidium parvum* and *Cyclospora cayetanensis*: a review of laboratory methods for detection of these waterborne parasites. *J Microbiol Methods* 2002; **49:** 209–24.

79 Shields JM, Olson BH. PCR-restriction fragment length polymorphism method for detection of *Cyclospora cayetanensis* in environmental waters without microscopic confirmation. *Appl Environ Microbiol* 2003; **69:** 4662–69.

80 Marangi M, Koehler AV, Zanzani SA, et al. Detection of *Cyclospora* in captive chimpanzees and macaques by a quantitative PCR-based mutation scanning approach. *Parasit Vectors* 2015; **15:** 274.

81 Li J, Dong H, Wang R, et al. An investigation of parasitic infections and review of molecular characterization of the intestinal protozoa in nonhuman primates in China from 2009 to 2015. *Int J Parasitol Parasites Wildl* 2017; **6:** 8–15

82 Taniuchi M, Verweij JJ, Sethabutr O, et al. Multiplex polymerase chain reaction method to detect *Cyclospora*, *Cystoisospora*, and microsporidia in stool samples. *Diagn Microbiol Infect Dis* 2011; **71:** 386–90.

83 Shin JH, Lee SE, Kim TS, Ma DW, Chai JY, Shin EH. Multiplex-touchdown PCR to simultaneously detect *Cryptosporidium parvum*, *Giardia lamblia*, and *Cyclospora cayetanensis*, the major causes of traveler's diarrhea. *Korean J Parasitol* 2016; **54:** 631–36

84 Sim S, Won J, Kim JW, Kim W, Park WY, Yu YR. Simultaneous molecular detection of *Cryptosporidium* and *Cyclospora* from raw vegetables in Korea. *Korean J Parasitol* 2017; **55:** 137–42.

85 Liu H, Shen Y, Yin J, et al. Prevalence and genetic characterization of *Cryptosporidium*, *Enterocytozoon*, *Giardia* and *Cyclospora* in diarrheal outpatients in China. *BMC Infect Dis* 2014; **14:** 25.

86 Chalmers RM. *Cyclospora cayetanensis*. In: Percival SL, Yates MV, Williams DW, Chalmers RM, Gray NF, eds. Microbiology of waterborne diseases: microbiological aspects and risks, 2nd edn. London: Elsevier, 2013: 327–53.

87 Varma M, Hester JD, Schaefer FW, Ware MW, Lindquist HD. Detection of *Cyclospora cayetanensis* using a quantitative real-time PCR assay. *J Microbiol Methods* 2003; **53:** 27–36.

88 Verweij JJ, Laeijendecker D, Brienen EA, van Lieshout L, Polderman AM. Detection of *Cyclospora cayetanensis* in travellers returning from the tropics and subtropics using microscopy and real-time PCR. *Int J Med Microbiol* 2003; **293:** 199–202.

89 Qvarnstrom Y, Benedict T, Marcet PL, Wiegand RE, Herwaldt BL, da Silva AJ. Molecular detection of *Cyclospora cayetanensis* in human stool specimens using UNEX-based DNA extraction and real-time PCR. *Parasitology* 2017; **8:** 1–6.

90 Murphy HR, Cinar HN, Gopinath G, et al. Interlaboratory validation of an improved method for detection of *Cyclospora cayetanensis* in produce using a real-time PCR assay. *Food Microbiol* 2018; **69:** 170–78.

91 Buss SN, Leber A, Chapin K, et al. Multicentre evaluation of the BioFire Film Array gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. *J Clin Microbiol* 2015; **53:** 915–25.

92 Dalton C, Goater AD, Pethig R, Smith HV. Viability of *Giardia intestinalis* cysts and viability and sporulation state of *Cyclospora cayetanensis* oocysts determined by electrorotation. *Appl Environ Microbiol* 2001; **67:** 586–90.

93 Dalton C, Goater AD, Burt JP, Smith HV. Analysis of parasites by electrorotation. *J Appl Microbiol* 2004; **96:** 24–32.

94 Eberhard ML, Ortega YR, Hanes DE, et al. Attempts to establish experimental *Cyclospora cayetanensis* infection in laboratory animals. *J Parasitol* 2000; **86:** 577–82.

95 Zerpa R, Uchima N, Huicho L. *Cyclospora cayetanensis* associated with watery diarrhoea in Peruvian patients. *J Trop Med Hyg* 1995; **98:** 325–29.

96 Verdier RI, Fitzgerald DW, Johnson WD, Pape JW. Trimethoprim-sulfamethoxazole compared with ciprofloxacin for treatment and prophylaxis of *Isospora belli* and *Cyclospora cayetanensis* infection in HIV-infected patients. A randomized, controlled trial. *Ann Intern Med* 2000; **132:** 885–88.

97 Soave R, Johnson WD Jr. Cyclospora: conquest of an emerging pathogen. *Lancet* 1995; **345:** 667–68.

98 Hoge CW, Shlim DR, Ghimire M, et al. Placebo-controlled trial of co-trimoxazole for Cyclospora infections among travellers and foreign residents in Nepal. *Lancet* 1995; **345:** 1060.

99 Madico G, Gilman RH, Miranda E, Cabrera L, Sterling CR. Treatment of Cyclospora infections with co-trimoxazole. *Lancet* 1993; **342:** 122–23.

100 Madico G, McDonald J, Gilman RH, Cabrera L, Sterling CR. Epidemiology and treatment of *Cyclospora cayetanensis* infection in Peruvian children. *Clin Infect Dis* 1997; **24:** 977–81.

101 Zimmer SM, Schuetz AN, Franco-Paredes C. Efficacy of nitazoxanide for cyclosporiasis in patients with sulfa allergy. *Clin Infect Dis* 2007; **44:** 466–67.

102 Tang K, Guo Y, Zhang L, et al. Genetic similarities between *Cyclospora cayetanensis* and cecum-infecting avian *Eimeria* spp in apicoplast and mitochondrial genomes. *Parasit Vectors* 2015; **8:** 358.

103 Saremy S, Boroujeni ME, Bhattacharjee B, Mittal V, Chatterjee J. Identification of potential apicoplast associated therapeutic targets in human and animal pathogen *Toxoplasma gondii* ME49. *Bioinformation* 2011; **7:** 379–83.

104 Goodman CD, McFadden GI. Targeting apicoplasts in malaria parasites. *Expert Opin Ther Targets* 2013; **17:** 167–77.

105 Stocks PA, Barton V, Antoine T, Biagini GA, Ward SA, O'Neill PM. Novel inhibitors of the *Plasmodium falciparum* electron transport chain. *Parasitology* 2014; **14:** 50–65.

106 Wang KX, Li CP, Wang J, Tian Y. *Cyclospora cayetanensis* in Anhui China. *World J Gastroenterol* 2002; **15:** 1144–48.

107 Kimura K, Rai SK, Rai G, et al. Study on *Cyclospora cayetanensis*, associated with diarrheal disease in Nepal and Lao PDR. *Southeast Asian J Trop Med Public Health* 2005; **36:** 1371–76.

108 Turgay N, Unver-Yolasığmaz A, Oyur S, Bardak-Özcem T, Töz S. Monthly distribution of intestinal parasites detected in a part of western Turkey between May 2009–April 2010–results of acid fast and modified trichrome staining methods. *Turkiye Parazitol Derg* 2012; **36:** 71–74.

109 Dhanabal J, Selvadoss PP, Muthuswamy K. Comparative study of the prevalence of intestinal parasites in low socioeconomic areas from South Chennai, India. *J Parasitol Res* 2014; **2014:** 630968.

110 Eberhard ML, Nace EK, Freeman AR, Streit TG, da Silva AJ, Lammie PJ. *Cyclospora cayetanensis* infections in Haiti: a common occurrence in the absence of watery diarrhea. *Am J Trop Med Hyg* 1999; **60:** 584–86.

111 Días Borges J, Rodriguez Alarcón RS, Amato Neto V, Gakiya E. Parasitoses intestinalis de indígenas da comunida de Mapuera (Oriximiná, Estado do Pará, Brasil): elevada prevalência de *Blastocystis hominis* and finding of *Cryptosporidium* sp and *Cyclospora cayetanensis*. *Rev Soc Brás Med Trop* 2009; **42:** 348–50.

112 Cazorla D, Acosta ME, Morales P. Estudio clínico-epidemiológico de coccidiosis intestinales en una población rural de región semiárida del estado Falcón, Venezuela. *Invest Clin* 2012; **53:** 273–88.

113 Burnstein Alva S. Ciclosporosis: una parasitosis emergente (I). Aspectos clínicos y epidemiologicos. *Rev Gastroenterol Peru* 2005; **25:** 328–35.

114 Nassef NE, el-Ahl SA, el-Shafee OK, Nawar M. *Cyclospora*: a newly identified protozoan pathogen of man. *J Egypt Soc Parasitol* 1998; **28:** 213–19.

115 Samie A, Guerrant RL, Barrett L, Bessong PO, Igumbor EO, Obi CL. Prevalence of intestinal parasitic and bacterial pathogens in diarrhoeal and non-diarrhoeal human stools from Vhembe district, South Africa. *J Health Popul Nutr* 2009; **27:** 739–45.

116 Fletcher SM, Stark D, Ellis J. Prevalence of gastrointestinal pathogens in sub-Saharan Africa: systematic review and meta-analysis. *J Public Health Africa* 2011; **30:** 127–37.

117 Raccurt CP, Fouché B, Agnamey P, et al. Presence of *Enterocytozoon bieneusi* associated with intestinal coccidia in patients with chronic diarrhea visiting an HIV center in Haiti. *Am J Trop Med Hyg* 2008; **79:** 579–80.

118 Sanad MM, Thagfan FA, Al Olayan EM, et al. Opportunistic coccidian parasites among Saudi cancer patients presenting with diarrhea: prevalence and immune status. *Res J Parasitol* 2014; **9:** 55–63

119 Frickmann H, Schwarz NG, Rakotozandrindrainy R, May J, Hagen RM. PCR for enteric pathogens in high-prevalence settings. What does a positive signal tell us? *Infect Dis (Lond)* 2015; **47:** 491–94

120 Pandey S, Lo AL, Shrestha RB. Intestinal parasitic infections among school children of northern Kathmandu, Nepal. *Asian Pacific J Trop Dis* 2015; **5:** S89–92

121 Silva-Díaz H, Campos-Flores H, Llagas-Linares JP, Cancino D. Intestinal coccidiosis in children admitted to a hospital in Peru and comparison of two methods for detecting *Cryptosporidium* spp. *Rev Peruana de Med Expud Publica* 2016; **33:** 739–44

122 Ghoshal U, Dey A, Ranjan P, Khanduja S, Agarwal V, Ghoshal UC. Identification of opportunistic enteric parasites among immunocompetent patients with diarrhoea from northern India and genetic characterization of *Cryptosporidium* and microsporidia. *Indian J Med Microbiol* 2016; **34:** 60–66.

123 Dahlal M, Dahal RH, Chaudhary DK. Prevalence of *Cyclospora cayetanensis* and other enteropathogen among children under the age of 15 years in Biratnagar, *Nepal Asian Pac J Trop Dis* 2017; **7:** 75–79.

124 Kumar P, Vats O, Kumar D, Singh S. Coccidian intestinal parasites among immunocompetent children presenting with diarrhea: are we missing them? *Trop Parasitol* 2017; **7:** 37–40.

125 Hall RL, Jones JL, Herwaldt BL. Surveillance for laboratory-confirmed sporadic cases of cyclosporiasis–United States, 1997–2008. *MMWR Surveill Summ* 2011; **60:** 1–11.

126 Marder EP, Griffin PM, Cieslak PR, et al. Preliminary incidence and trends of infections with pathogens transmitted commonly through food–Foodborne Diseases Active Surveillance Network, 10 US sites, 2006–2017. *Morb Mortal Wkly Rep* 2018; **67:** 324–28.

127 Salehi L, Lofters AK, Hoffmann SM, Polsky JY, Rouleau KD. Health and growth status of immigrant and refugee children in Toronto, Ontario: a retrospective chart review. *Pediatr Child Health* 2015; **20:** e38–42.

128 Jelínková A, Kašičková D, Valkoun A. *Cyclospora cayetanensis*—the rare causal agent of diarrhoeal diseases. *Klin Mikrobiol Infekc Lek* 2011; **17:** 86–88.

129 Paschke C, Apelt N, Fleischmann E, et al. Controlled study on enteropathogens in travellers returning from the tropics with and without diarrhoea. *Clin Microbiol Infect* 2011; **17:** 1194–200.

130 Giangaspero A, Marangi M, Koehler AV, et al. Molecular detection of *Cyclospora* in water, soil, vegetables and humans in southern Italy signals a need for improved monitoring by health authorities. *Int J Food Microbiol* 2015; **211:** 95–100.

131 Maggi P, Brandonisio O, Larocca AM, et al. *Cyclospora* in AIDS patients: not always an agent of diarrhoeic syndrome. *New Microbiol* 1995; **18:** 73–76.

132 Scaglia M, Gatti S, Bassi P, Viale PL, Novati S, Ranieri S. Intestinal co-infection by *Cyclospora* sp and *Cryptosporidium parvum*: first report in an AIDS patient. *Parasite* 1994; **1:** 387–90.

133 Masucci L, Graffeo R, Siciliano R, Franceschelli A, Bugli F, Fadda G. First Italian case of cyclosporiasis in an immunocompetent woman: local acquired infection. *New Microbiol* 2008; **31:** 281–84.

134 Masucci L, Graffeo R, Bani S, et al. Intestinal parasites isolated in a large teaching hospital, Italy, 1 May 2006 to 31 December 2008. *Euro Surveill* 2011; **16:** 19891.

135 Rosado-García FM, Guerrero-Flórez M, Karanis G, Hinojosa MDC, Karanis P. Water-borne protozoa parasites: the Latin American perspective. *Int J Hyg Environ Health* 2017; **220:** 783–98.

136 Soave R, Herwaldt BL, Relman DA. *Cyclospora*. *Infect Dis Clin North Am* 1998; **12:** 1–12.

137 Galván AL, Magnet A, Izquierdo F, et al. Molecular characterization of human pathogenic microsporidia and *Cyclospora cayetanensis* isolated from various water sources in Spain: a year-long longitudinal study. *Appl Environ Microbiol* 2013; **79:** 449–59.

138 Kitajima M, Haramoto E, Iker BC, Gerba CP. Occurrence of *Cryptosporidium*, *Giardia*, and *Cyclospora* in influent and effluent water at wastewater treatment plants in Arizona. *Sci Total Environ* 2014; **15:** 129–36.

139 El Zawawy LA, El-Said D, Ali SM, Fathy FM. Disinfection efficacy of sodium dichloroisocyanurate (NADCC) against common food-borne intestinal protozoa. *J Egypt Soc Parasitol* 2010; **40:** 165–85.

140 Parish ME, Beuchat LR, Suslow TV, et al. Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. *Comprehensive Rev Food Sci Food Safety* 2003; **2:** 161–73.

141 Soliva-Fortuny RC, Martin-Belloso O. New advances in extending the shelf-life of fresh-cut fruits: a review. *Trends Food Sci Technol* 2003; **14:** 341–53.

142 Turatti A. Processo di lavorazione delle insalate di quarta gamma. In: Angelini RML, Gullino I, Ponti I, eds. Le insalate. Bologna: ART Servizi Editoriali SpA, 2011: 420–37.

143 Ashford RW. Occurrence of an undescribed coccidian in man in Papua New Guinea. *Ann Trop Med Parasitol* 1979; **73:** 497–500.

144 Zhou Y, Lu B, Wang Q, et al. Prevalence and molecular characterization of *Cyclospora cayetanensis*, Henan, China. *Emerg Infect Dis* 2011; **17:** 1887–90.

145 Hussein EM, El-Moamly AA, Mahmoud MA, Ateek NS. Wide genetic variations at 18S ribosomal RNA locus of *Cyclospora cayetanensis* isolated from Egyptian patients using high resolution melting curve. *Parasitol Res* 2016; **115:** 2797–806.

146 Qvarnstrom Y, Wei-Pridgeon Y, Li W, et al. Draft genome sequences from *Cyclospora cayetanensis* oocysts purified from a human stool sample. *Genome Announc* 2015; **3:** e01324-15.

147 Cinar HN, Gopinath GK, Jarvis K, Murphy HR. The complete mitochondrial genome of the foodborne parasitic pathogen *Cyclospora cayetanensis*. *PLoS One* 2015; **10:** e0128645.

148 Guo Y, Li N, Ortega YR, et al. Population genetic characterization of *Cyclospora cayetanensis* from discrete geographical regions. *Exp Parasitol* 2018; **184:** 121–27.

149 Gopinath GR, Cinar HN, Murphy HR, et al. A hybrid reference-guided de novo assembly approach for generating *Cyclospora* mitochondrion genomes. *Gut Pathog* 2018; **10:** 15.

150 Cinar HN, Qvarnstrom Y, Wei-Pridgeon Y, et al. Comparative sequence analysis of *Cyclospora cayetanensis* apicoplast genomes originating from diverse geographical regions. *Parasit Vectors* 2016; **9:** 611.

© 2019 Elsevier Ltd. All rights reserved.