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Cyclospora cayetanensis infection in humans: biological characteristics, clinical features, epidemiology, detection method and treatment

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Abstract

Cyclospora cayetanensis, a coccidian parasite that causes protracted and relapsing gastroenteritis, has a short recorded history. At least 54 countries have documented *C. cayetanensis* infections and 13 of them have recorded cyclosporiasis outbreaks. *Cyclospora cayetanensis* infections are commonly reported in developing countries with low-socioeconomic levels or in endemic areas, although large outbreaks have also been documented in developed countries. The overall *C. cayetanensis* prevalence in humans worldwide is 3.55%. Among susceptible populations, the highest prevalence has been documented in immunocompetent individuals with diarrhea. Infections are markedly seasonal, occurring in the rainy season or summer. *Cyclospora cayetanensis* or *Cyclospora*-like organisms have also been detected in food, water, soil and some other animals. Detection methods based on oocyst morphology, staining and molecular testing have been developed. Treatment with trimethoprim–sulfamethoxazole (TMP–SMX) effectively cures *C. cayetanensis* infection, whereas ciprofloxacin is less effective than TMP–SMX, but is suitable for patients who cannot tolerate co-trimoxazole. Here, we review the biological characteristics, clinical features, epidemiology, detection methods and treatment of *C. cayetanensis* in humans, and assess some risk factors for infection with this pathogen.

Introduction

Nearly 1.7 billion cases of diarrheal disease are reported globally every year, and its socioeconomic burden on health services has been estimated at 72.8 million disability-adjusted life years annually (Ryan *et al.*, 2017). Enteric protozoan parasites are among the major contributors to this diarrheal disease load (Fletcher *et al.*, 2012; Di Genova and Tonelli, 2016). *Cyclospora cayetanensis* is an important global pathogen in humans, typically causing prolonged diarrhea accompanied by anorexia, malaise, nausea and cramping, among other symptoms (Shields and Olson, 2003; Giangaspero and Gasser, 2019). Many large cyclosporiasis outbreaks have been documented in industrialized nations (Ortega and Sanchez, 2010). In these, food has been identified as the main vehicle for *Cyclospora* transmission, according to source-tracing studies (Herwaldt and Ackers, 1997; Ortega and Sanchez, 2010). Cilantro from Mexico was identified as one of the possible sources of a cyclosporiasis outbreak in the United States (USA) in 2013, with more than 600 cases of infection (Abanyie *et al.*, 2015). More recently, prepackaged vegetable trays and vegetable salads sold at a fast food chain have been the suspected sources of cyclosporiasis outbreaks in June and July, 2018, according to trace-back investigations (Casillas *et al.*, 2018).

Up to 31 December 2018, more than one thousand papers have been published on *Cyclospora*. Numerous studies of *Cyclospora* infections among travelers, immunodeficient patients, diarrheal and asymptomatic patients and the residents of disease-endemic areas have been reported. In this study, we review the biological characteristics, clinical features, epidemiology, detection methods and treatment of *C. cayetanensis*, and assess some risk factors for human infection with this foodborne pathogen.

Biological characteristics

History of discovery and research

The genus *Cyclospora*, created by Schneider in 1881, was first described by Eimer in 1870 (Ortega and Sanchez, 2010). Until the 1990s, the genus only included species that infect animals, such as rodents, insectivores and reptiles (Casemore, 1994). The earliest description of human infection with *Cyclospora* was from Papua New Guinea in 1979 (Ashford, 1979).

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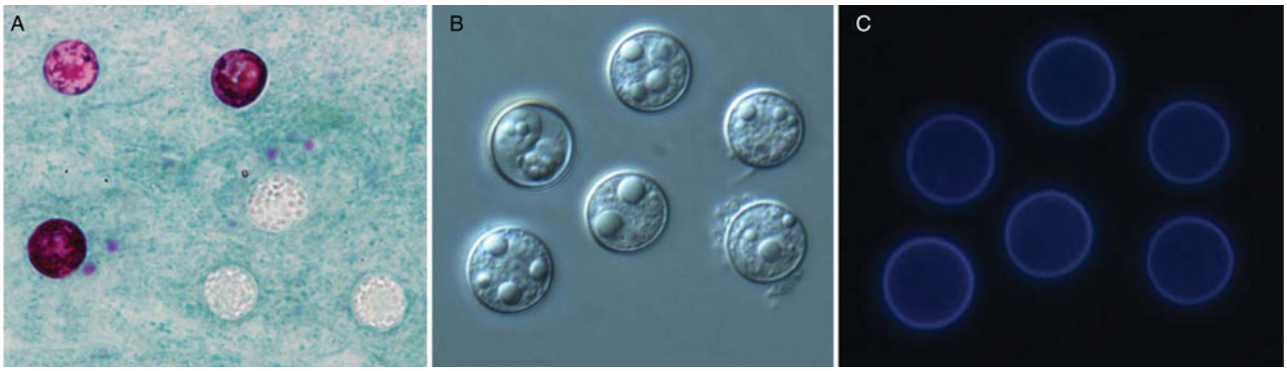

Fig. 1. Morphology of *C. cayetanensis* oocysts under microscopy. Oocysts in stool smears stained with modified acid-fast stain under light microscopy; two oocysts are stained with different intensities (A); differential interference contrast microscopy of wet mounts, a partially sporulated oocyst can be seen (B); epifluorescence microscopy with a 330–380 nm UV excitation filter (C).

Oocysts were subsequently observed in the faeces of patients from Haiti and Peru in 1983–1985, American travelers returning from Haiti and Mexico in 1986, British travelers who became ill in Nepal in 1989 and travelers and foreign residents in Nepal in 1993 (Herwaldt, 2000), although the identity of the pathogen was uncertain at that time. In 1994, Ortega *et al.* named this human causative organism *C. cayetanensis* (Ortega and Sanchez, 2010).

Cyclospora cayetanensis has received further attention since the first outbreak of *Cyclospora*-associated diarrheal illness in the USA in 1990 (Huang *et al.*, 1995). In 1996, more than 1400 cases of cyclosporiasis were reported in the USA and Canada (Herwaldt and Ackers, 1997). Since then, very large studies of *Cyclospora* infection among travelers, immunodeficient patients, diarrheal patients and asymptomatic individuals have been reported, as have studies of detection methods and treatment measures for *Cyclospora*.

Morphology and taxonomy

Cyclospora cayetanensis is the only documented *Cyclospora* species infecting humans, and it is widely accepted that among common mammals, only humans are susceptible to infection by this microbe (Ortega and Sanchez, 2010).

Under light microscopy, *C cayetanensis* oocysts have a spheroid shape, 8–10 μm in diameter, with indistinguishable protoplasm (Fig. 1). When sporulated, each oocyst contains two ovoid sporocysts that, in turn, contain two sporozoites each (Ortega and Sanchez, 2010). *Cyclospora* oocysts are modified with Ziehl–Neelsen acid-fast stain in different ways: some stain dark red with a mottled appearance, some stain pink, whereas others do not stain all and appear as non-refractile glassy spheres against the blue-green background (Clarke and McIntyre, 1996; Zhou *et al.*, 2011). Their autofluorescence makes *C. cayetanensis* oocysts readily visible in clinical samples with epifluorescence microscopy under a 330–380 nm ultraviolet (UV) filter (Zhou *et al.*, 2011).

Cyclospora cayetanensis belongs to the subphylum Apicomplexa, subclass Coccidiasina, family Eimeriidae and genus *Cyclospora* (Ortega and Sanchez, 2010). Phylogenetic analyses have shown that human-associated *Cyclospora* is closely related to members of the genus *Eimeria* (Fig. 2) (Relman *et al.*, 1996; Liu *et al.*, 2016). *Cyclospora cercopitheci* in vervet monkeys (*Cercopithecus aethiops*), *C. colobi* in colobus monkeys (*Colobus guereza*) and *C. papionis* in olive baboons (*Papio anubis*) were characterized in 1999 (Eberhard *et al.*, 1999); *C. macacae* was described in rhesus monkeys (*Macaca mulatta*) in 2015 (Li *et al.*, 2015); and *C. duszynskii* and *C. yatesi* were characterized in moles (*Scalopus aquaticus*) in 2018 (McAllister *et al.*, 2018). A total of 22 *Cyclospora* species have so far been described in vipers, moles, myriapodes, rodents, monkeys and humans (Lainson, 2005; Li *et al.*, 2015; McAllister *et al.*, 2018). However, *Cyclospora*-like organisms have also been described in dogs, cattle, chickens, rats, house mice, birds, monkeys, shellfish, etc., and even in environmental samples (Sherchand and Cross, 2001; Chu *et al.*, 2004; Li *et al.*, 2007; Cordón *et al.*, 2008; Aksoy *et al.*, 2014; Helenbrook *et al.*, 2015; Ghozzi *et al.*, 2017).

Life cycle of *C. cayetanensis*

Infections of *C. cayetanensis* mainly occur *via* the faecal–oral transmission route. Fresh (unsporulated) oocysts are excreted in stools. Oocysts are spheroid, 8–10 μm in diameter, and contain indistinguishable protoplasm (Brown and Rotschafer, 1999). In the environment outside the host, freshly excreted oocysts are not infectious until their sporulation is complete, which occurs within a few days to weeks (at maximum) at temperatures between 22 and 30 °C. Storage at either 4 or 37 °C retards sporulation (Smith *et al.*, 1997). The sporulation of the oocysts occurs irrespective of whether they are stored in deionized water or potassium dichromate solution, and results in the division of the sporont into two sporocysts, each containing two elongated sporozoites (Smith *et al.*, 1997). During this time, food or water can act as the vehicle for *Cyclospora* transmission. Once the sporulated oocysts in food, water or soil are ingested by a new host, the mature oocysts usually excyst in the small bowel, and sporozoites are released to invade the epithelial cells of the upper small intestine (duodenum or jejunum) (Ortega and Sanchez, 2010).

The presence of asexual and sexual stages in the same host suggests that the life cycle of this microorganism can be completed within one host (Ortega *et al.*, 1997). The intracellular developmental stages begin with the formation of intracytoplasmic parasitophorous vacuoles in the intestinal epithelium cells (Sun *et al.*, 1996; Ortega and Sanchez, 2010), which are sometimes also observed in biliary epithelium cells (Zar *et al.*, 2001). Asexual multiplication results in type I and II meronts (Ortega *et al.*, 1997). Type I meronts give rise to 8–12 merozoites that then infect neighbouring epithelial cells, and this type of asexual reproduction is often quite prolific. Type II meronts form later, releasing four merozoites to invade neighbouring cells. Some of these meronts form macrogametes, whereas others undergo multiple fission events to form microgametocytes containing flagellated microgametes (Ortega *et al.*, 1997). The macrogametocyte is fertilized by the microgametocyte, producing a zygote, in the sexual stages. Once fertilization occurs, an environmentally resistant wall is formed, and the oocyst is excreted from the host into the

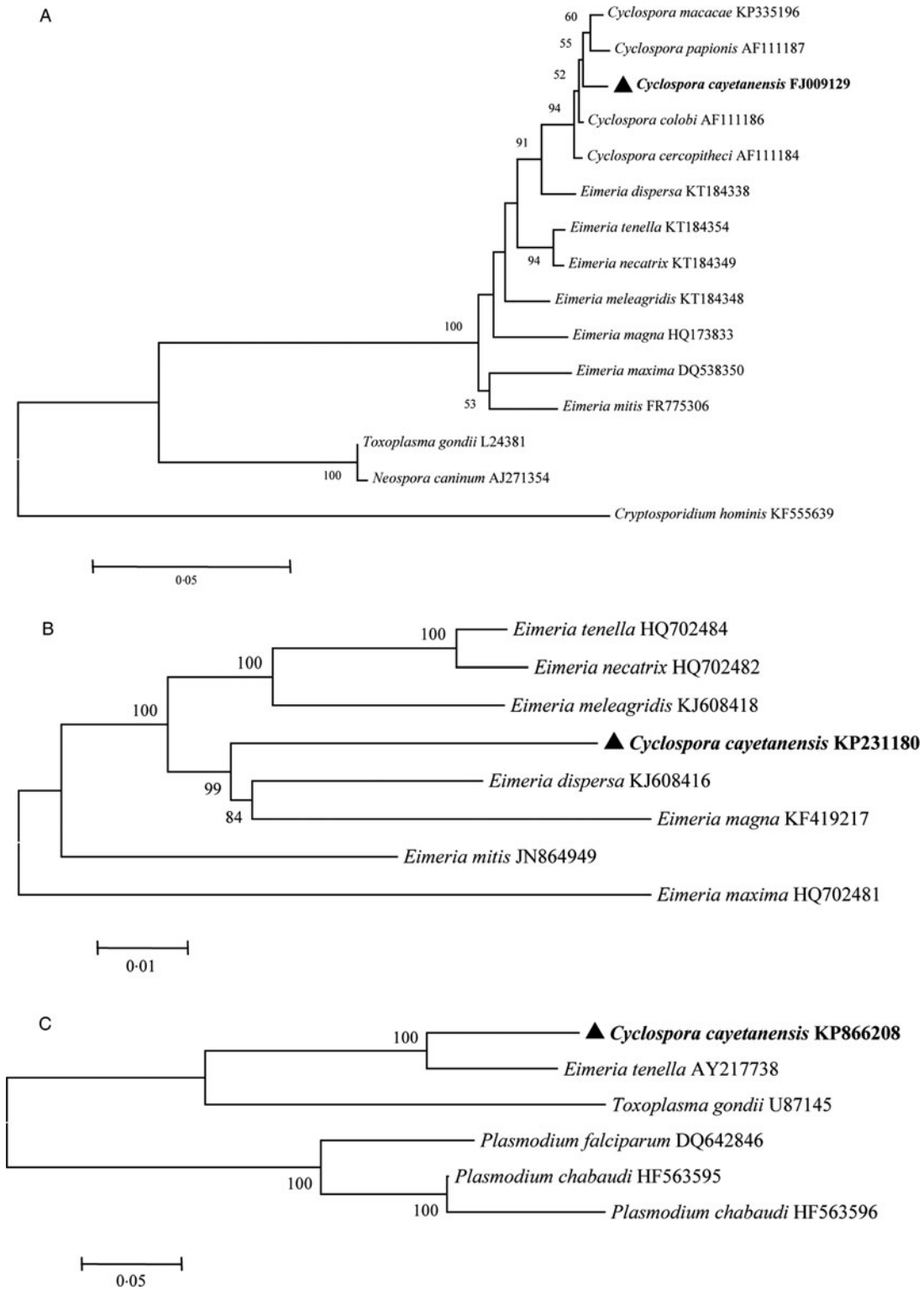

Fig. 2. Phylogenetic relationships of *C. cayetanensis* and other apicomplexan protozoa. Phylogeny inferred with a neighbour-joining analysis of small-subunit ribosomal RNA gene sequences (A) reported by Li *et al.* (2017); mitochondrial genomes (B) reported by Cinar *et al.* (2015) and apicoplast genomes (C) reported by Tang *et al.* (2015), based on distances calculated with the Kimura 2-parameter model. Bootstrap values >50% from 1000 replicates are shown at the nodes. Scale bars indicate estimated substitutions per site.

environment as an unsporulated oocyst in the faeces (Shields and Olson, 2003; Ortega and Sanchez, 2010).

Molecular characteristics

The characteristics of the polymorphic regions of the *Cyclospora* genome have been studied to better understand the microorganism's mode of infection and epidemiology. Small subunit ribosomal RNA (SSU rRNA) gene sequences show minimal genetic diversity among *C. cayetanensis* isolates from around the world (Sulaiman *et al.*, 2014), and a phylogenetic analysis showed that *C. cayetanensis* is genetically related to members of the genus *Eimeria* (Fig. 2A) (Relman *et al.*, 1996).

However, the internal transcribed spacer (ITS) sequences in *C. cayetanensis* are highly variable within and between samples, and this variability does not correlate with the geographic origins

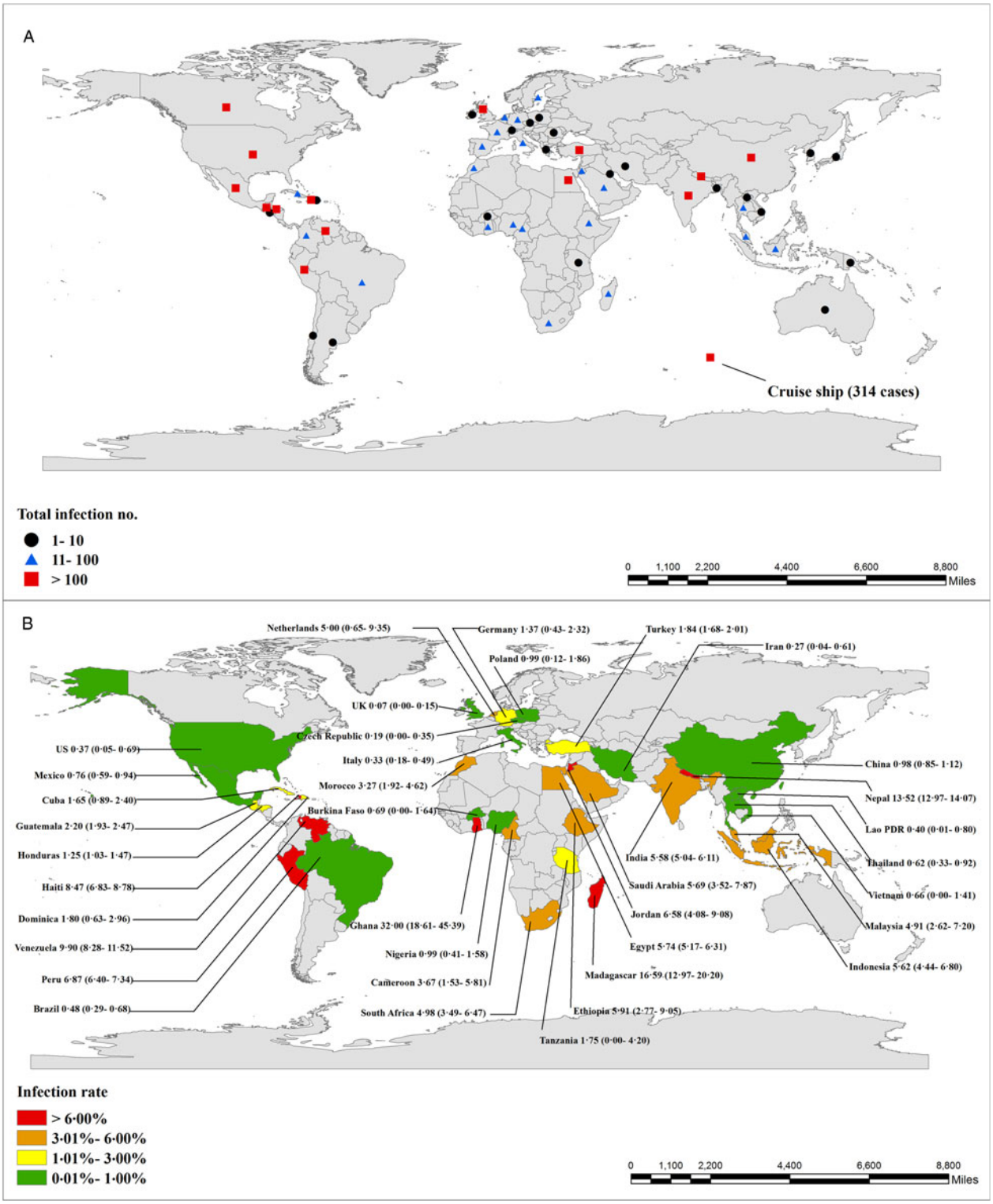

Fig. 3. Number of documented human *C. cayetanensis* infections and prevalence worldwide. Number of documented infection cases (A) and prevalence (B) worldwide (95% confidence intervals are shown in brackets).

of the samples (Olivier *et al.*, 2001). It has been demonstrated that this ITS sequence variability occurs at the individual-genome level and approaches or exceeds the variability observed among oocysts (Riner *et al.*, 2010).

No genetic polymorphism has been observed in regions of the 70 kilodalton heat shock protein (HSP70) locus characterized in a previous study (Sulaiman *et al.*, 2013). These results also support the lack of geographic segregation and the existence of genetically homogeneous population of *C. cayetanensis* parasites at this genetic locus (Sulaiman *et al.*, 2014).

Genome characteristics

Tracing the source of infection is facilitated by the genomic comparison of isolates. *Cyclospora* can also be clearly identified and differentiated from other protozoan parasites involved in foodborne or waterborne outbreaks by their genomic differences. The mitochondrial genome of *C. cayetanensis* is ~6200 bp in length, with 33% GC content (Cinar *et al.*, 2015; Ogedengbe *et al.*, 2015; Tang *et al.*, 2015). It contains three protein-coding genes (*cytb*, *cox1* and *cox3*) and 14 large subunit (LSU) and

nine SSU fragmented rRNA genes (Cinar *et al.*, 2015; Ogedengbe *et al.*, 2015). The mitochondrial genome of *C. cayetanensis* has a linear concatemeric or circular mapping topology (Tang *et al.*, 2015). A comparative genomic analysis showed strong similarity between the *C. cayetanensis* and *E. tenella* genomes, with 90.4% nucleotide sequence similarity and complete synteny in gene organization (Tang *et al.*, 2015). Phylogenetic analyses of the mitochondrial genomic sequences have confirmed the genetic similarities between avian *Eimeria* spp. and *C. cayetanensis* (Fig. 2B).

The apicoplast genome of *C. cayetanensis* is ~34 000 bp in size and encodes ~65 genes, with 22% GC content (Tang *et al.*, 2015; Liu *et al.*, 2016). The apicoplast genome is circular, encodes the complete machinery for protein biosynthesis and contains two inverted repeats that differ slightly in the LSU rRNA gene sequences (Tang *et al.*, 2015). A comparative genomic analysis revealed high-nucleotide sequence similarity (85.6%) between *C. cayetanensis* and *E. tenella*, and a phylogenetic analysis of apicoplast genomic sequences also confirmed the genetic similarities between avian *Eimeria* spp. and *C. cayetanensis* (Fig. 2C).

The whole genome of *C. cayetanensis* is estimated to have a total length of 44 Mbp, with 52% GC content and ~7500 gene (Liu *et al.*, 2016). A comparative genomic analysis indicated that *C. cayetanensis* shares a coccidia-like metabolism and invasion components, but has unique surface antigens (Liu *et al.*, 2016). There are also some major differences in the amino acid metabolism and the posttranslational modification of proteins between *C. cayetanensis* and other apicomplexans (Liu *et al.*, 2016). A multilocus sequence typing tool for *C. cayetanensis* has been developed based on its whole genome, which involves five microsatellite loci (Guo *et al.*, 2016). Noticeable geographic clustering has been observed in human *C. cayetanensis* isolates from around the world (Li *et al.*, 2017). Quantitative polymerase chain reaction (PCR) (Guo *et al.*, 2019) and PCR assays (Nascimento *et al.*, 2019), both targeting the polymorphic region in the mitochondrial genome, have been developed to genotype *C. cayetanensis* isolates. Two novel similarity-based classification algorithms for *C. cayetanensis* have been developed, including a Bayesian and heuristic component that infer the relatedness of pathogen isolates (Barratt *et al.*, 2019). These useful genotyping tools should be helpful in initial source-tracking studies and in distinguishing different case clusters, especially during cyclosporiasis outbreaks.

Clinical features

The clinical symptoms of cyclosporiasis in humans typically manifest as periodic profuse watery diarrhea, together with malaise, nausea, anorexia, cramping and periods of apparent remission (Shields and Olson, 2003). Mild-to-moderate self-limiting diarrhea is common among healthy individuals who have ingested sporulated oocysts (Mansfield and Gajadhar, 2004). However, patients with immune dysfunction can experience severe intestinal injury and prolonged diarrhea (Shields and Olson, 2003; Mansfield and Gajadhar, 2004). In some cases, low-grade fever and the malabsorption of D-xylose may be present (Shields and Olson, 2003). Asymptomatic infections also occur frequently in disease-endemic areas.

Striking intestinal histological changes are observed during *C. cayetanensis* infection, including acute or chronic inflammation, disruption of the surface epithelium, villous atrophy, crypt hyperplasia (Connor *et al.*, 1993) and intense lymphocytic infiltration within the lamina propria and epithelial cells (Ortega *et al.*, 1997; Wiwanitkit, 2006). The inflammatory changes associated with *C. cayetanensis* infection may persist beyond the eradication of the parasite (Connor *et al.*, 1999). Reactive hyperaemia with vascular dilatation and congestion of the villous capillaries has also been observed (Ortega *et al.*, 1997).

In addition to gastrointestinal symptoms, *C. cayetanensis* can infect the biliary tract (Sifuentes-Osornio *et al.*, 1995), resulting in acalculous cholecystitis in people with acquired immunodeficiency syndrome (AIDS), and the presence of oocysts in gallbladder epithelial cells (Zar *et al.*, 2001). Although no *C. cayetanensis* respiratory infection has yet been identified, *C. cayetanensis* oocysts were detected in the sputum of two patients with tuberculosis (Di Gliullo *et al.*, 2000; Hussein *et al.*, 2005). *Cyclospora cayetanensis* infection has been associated with a variety of sequelae, including reactive arthritis syndrome, Reiter syndrome and Guillain-Barre syndrome (Connor *et al.*, 2001; Shields and Olson, 2003; Abanyie *et al.*, 2015).

Epidemiology

Outbreaks of human cyclosporiasis

Cyclospora cayetanensis infections in humans have been documented in over 56 countries worldwide, distributed across all five human-inhabited continents (Fig. 3; Table 1S). The first recorded outbreak of *C. cayetanensis* infection (called an 'alga-like organism' at the time) occurred among 55 British expatriates with prolonged diarrhea in Nepal between June and November, 1989 (Shlim *et al.*, 1991). The first reported outbreak of diarrheal illness associated with *Cyclospora* infection in the USA was in 1990 (Huang *et al.*, 1995).

Up to 1996, more than 1400 cases of cyclosporiasis were recorded in multistate outbreaks in the USA and Canada (Herwaldt and Ackers, 1997). The most recent large outbreaks were documented in 2013 and 2018 concerning multistate outbreaks in the USA (Abanyie *et al.*, 2015; Casillas *et al.*, 2018). Up to December 2018, at least 13 countries documented cyclosporiasis outbreaks, involving ~6557 cases (Table 2S). Among these countries, cyclosporiasis has mainly been documented in the Americas and Europe, including Peru, Mexico, the USA, Canada and the United Kingdom (Table 2S).

Prevalence and case reports of C. cayetanensis in humans

A total of 13 845 *C. cayetanensis* cases have been recorded in humans, either during epidemiological studies (5478), during outbreak investigations (6557), or in case reports (1810) (Table 2S; Table 3S; Table 4S). The overall prevalence of *C. cayetanensis* among humans worldwide is 3.55% (5478/1 54 410). Asia (5.63%, 2771/49 254) and Africa (5.33%, 554/10 401) have shown greater prevalence than the Americas (3.03%, 1625/53 775) and Europe (1.28%, 528/41 186). A high prevalence of *C. cayetanensis* and large numbers of cases have been recorded in Nepal (13.68%) and India (5.58%) in Asia; Madagascar (16.59%) and Egypt (5.74%) in Africa and Venezuela (9.90%), Peru (6.87%) and Haiti (8.47%) in the Americas (Fig. 3).

Transmission risk factor assessment

A marked seasonality (rainy season or summer) has been observed in human *C. cayetanensis* infections in the northern hemisphere, including in China (Zhou *et al.*, 2011; Jiang *et al.*, 2018), Nepal (Sherchand and Cross, 2001; Kimura *et al.*, 2005; Bhandari *et al.*, 2015), Turkey (Ozdamar *et al.*, 2010), Honduras (Kaminsky *et al.*, 2016) and Mexico (Orozco-Mosqueda *et al.*, 2014). The consistent pattern of the seasonal distribution of *C. cayetanensis* infections probably reflects the optimal environmental conditions (temperature and humidity) that are required for oocysts to sporulate. The major risk factors for *Cyclospora*

transmission are probably the consumption of or contact with oocysts in contaminated food, water or soil; contact with animals and poor sanitation. These findings are typically documented in Peru (contaminated water sources) (Burstein Alva, 2005), Nepal (contaminated drinking water) (Bhattachan *et al.*, 2017), Venezuela (contact with soil contaminated with human faeces) (Chacín-Bonilla *et al.*, 2007), Nepal (livestock kept near households and the consumption of raw vegetables and fruits) (Bhandari *et al.*, 2015) and Turkey (consumption of tap water or eating in unsanitary establishments) (Erdogan *et al.*, 2012), among others. In summary, the epidemiological determinants and risk factors for human cyclosporiasis are shown in Table 1.

Table 1. Epidemiological determinants and risk factors for human cyclosporiasis

Factors	Main points
Sources of transmission: infection oocysts	Suitable environmental temperature and humidity (rainy or summer season) Infectious (sporulated) *Cyclospora cayetanensis* oocysts
Routes of transmission: Biology vectors or mechanical vehicle	Produce (fresh vegetables or fruits) as the vehicle Travel to or residence in endemic areas Water/soil as the vehicle Poor sanitary conditions
Susceptible human populations: clinical symptoms and immune status	Residents in low-income communities or endemic areas Patients with diarrhea or gastroenteritis symptoms Immunodeficient patients with diarrhea Immunodeficient patients

Susceptible populations and risk factors

Cyclospora cayetanensis is recognized as an opportunistic protozoan pathogen of humans (Wiwanitkit, 2006). Immunodeficiency and diarrhea in the host are two major risk factors for *C. cayetanensis* infection. Notable distributions of infection have been documented in Nigeria (human immunodeficiency virus (HIV) patients with diarrhea) (Alakpa *et al.*, 2002), Mexico (patients with diarrhea) (Jiménez-González *et al.*, 2012), Honduras (patients with diarrhea or liquid stools) (Kaminsky *et al.*, 2016) and Turkey (immunosuppressed patients) (Karaman *et al.*, 2015), among others.

The statistics for *C. cayetanensis* infection in different human populations demonstrate that diarrhea is a major risk factor for *Cyclospora* infection: immunocompromised and immunocompetent individuals with diarrhea (7.38 *vs* 9.14%, respectively) both had a significantly higher prevalence of infection than patients with other symptoms (4.91 *vs* 2.09%, respectively; $P = 0.0001$).

Poor sanitation conditions are another risk factor for infection with *C. cayetanensis*. It should be noted that people from low-income communities living in areas with poor sanitation have the highest prevalence of infection. Remarkably high-prevalence rates have been reported in Peru (54.88 and 41.58%), Venezuela (24.20%) and India (22.27%), together with poor sanitary conditions (Burstein Alva, 2005; Nundy *et al.*, 2011; Cazorla *et al.*, 2012; Jeevitha *et al.*, 2014). In one study in Nepal, the members of a family that kept livestock at home had higher *Cyclospora* infection rates than families who did not (Bhandari *et al.*, 2015).

Age may be another factor that affects the occurrence of cyclosporiasis in humans. Many studies have reported that children show a higher prevalence of *C. cayetanensis* infection than the general populations, including in Guatemala, Nepal, Turkey and Honduras, among others (Bern *et al.*, 1999; Kimura *et al.*, 2005; Erdogan *et al.*, 2012; Bhandari *et al.*, 2015; Kaminsky *et al.*, 2016). However, unexpectedly, children had a lower infection rate than the general population (4.90 *vs* 9.36%, respectively) of immunocompetent individuals with diarrhea, according to epidemiological statistics ($P < 0.0001$) (Table 2). This may be because the general population has more opportunity to consume raw produce than children.

Cyclospora cayetanensis is also an important pathogen causing traveler's diarrhea, especially in industrialized regions (Shields and Olson, 2003; Mansfield and Gajadhar, 2004). International travel or expatriate relocation to developing countries with disease-endemic areas or poor sanitation might be a risk factor for cyclosporiasis in humans (Fryauff *et al.*, 1999; Pandey *et al.*, 2011; Kłudkowska *et al.*, 2017).

Animal reservoirs

Several *Cyclospora* species or *Cyclospora*-like organisms have been reported in various animals (Table 5S), including five *Cyclospora* species identified in primates (Eberhard *et al.*, 1999, 2001; Ortega and Sanchez, 2010; Li *et al.*, 2015). *Cyclospora*-like organisms have been documented in dogs, cattle, chickens, rats, house mice, birds and even shellfish. The Asian freshwater clam (*Corbicula fluminea*) can recover the oocysts of *C. cayetanensis* during artificial contamination, and could therefore be used as a biological indicator of water contaminated with oocysts (Graczyk *et al.*, 1998).

Another study attempted to develop an animal model of *C. cayetanensis* in which to study human cyclosporiasis. Various types of animals (various strains of mice, rats, sand rats, chickens, ducks, rabbits, birds, hamsters, ferrets, pigs, dogs, owl monkeys, rhesus monkeys and cynomolgus monkeys) were inoculated with human *C. cayetanensis* oocysts by gavage. None of the animals had developed patent infection or signs of infection 4–6 weeks after inoculation. It was concluded that none of the mammals tested are susceptible to infection by *C. cayetanensis* (Graczyk *et al.*, 1998). Combined with the unpublished observation and personal communication data, great efforts had been made to attempts to infect various animals, the animal models of *C. cayetanensis* infections were still unsuccessfully.

A pilot study sought to infect human volunteers with *C. cayetanensis*, but no oocysts were detected in any stool sample from any of the seven volunteers during the 16-week trial (Alfano-Sobsey *et al.*, 2004). These results suggest that the conditions necessary for *Cyclospora* to become infectious were not maintained during the preparation or storage of the oocysts. Future studies are required to assess the effects of temperature, humidity, storage conditions and disinfection on the survival, viability and infectivity of stored *Cyclospora* oocysts.

Food, water and soil sample contamination

In industrialized countries or regions, cyclosporiasis is most often linked to foodborne outbreaks (Rose and Slifko, 1999). In developing countries or disease-endemic areas, recorded *C. cayetanensis* infections have been associated with contact with contaminated food, water or soil (Burstein Alva, 2005; Chacín-Bonilla, 2008; Bhandari *et al.*, 2015). In a community in Venezuela, a strong association between environmental contact with faecal-contaminated soil and the occurrence of cyclosporiasis was detected, suggesting that contact with soil may be an important mode of transmission (Chacín-Bonilla, 2008).

There are many records of vegetables, fruits, water and soil contaminated with *Cyclospora* oocysts in countries as diverse as

Table 2. *Cyclospora cayetanensis* prevalence in different human population groups

Population groups	Number of investigation samples	Number of positive	Prevalence (95 CI)
HIV/AIDS or immunodeficient patients with diarrhea	3863	285	7.38% (6.55–8.20)
Children	0	0	0
General	3863	285	7.38% (6.55–8.20)
HIV/AIDS or immunodeficient patients without diarrhea	5661	278	4.91% (4.35–5.47)
Children	364	17	4.67% (2.49–6.85)
General	5297	261	4.93% (4.34–5.51)
Individuals with diarrhea	26 852	2453	9.14% (8.79–9.48)
Children	1347	66	4.90% (3.75–6.05)
General	25 505	2387	9.36% (9.00–9.72)
Individuals without diarrhea	118 034	2462	2.09% (2.00–2.17)
Children	25 077	439	1.75% (1.59–1.91)
General	92 957	2023	2.18% (2.08–2.27)
Total	154 410	5478	3.55% (3.46–3.64)

Note: Summarized in 'Table 3S: Epidemiology investigation of *Cyclospora cayetanensis* prevalence in humans'.

Italy (Giangaspero *et al.*, 2015), Malaysia (Bilung *et al.*, 2017), Peru (Sturbaum *et al.*, 1998), Nepal (Sherchand and Cross, 2001) and Vietnam (Tram *et al.*, 2008), among others (Table 6S). Numerous methods have been developed for the recovery and analysis of *Cyclospora* oocysts in contaminated food, water and soil samples (Robertson *et al.*, 2000; Shields *et al.*, 2012).

Detection methods

A laboratory diagnosis of *C. cayetanensis* infection can be made simply by examining wet-mount preparations of faeces under light microscopy or by the autofluorescence of oocysts under UV epifluorescence microscopy. A more-automated flow-cytometric detection assay for *C. cayetanensis* in human faecal specimens was developed based on the morphology and auto-fluorescence characteristics of oocysts (Dixon *et al.*, 2005). Modified Ziehl–Neelsen acid-fast staining is recommended for the detection of *Cyclospora* oocysts (Brennan *et al.*, 1996; Clarke and McIntyre, 1996). Some other staining methods, such as (modified) Kinyoun acid-fast staining (Gonçalves *et al.*, 2005; Hussein, 2007; Behera *et al.*, 2008; Dillingham *et al.*, 2009; Bhandari *et al.*, 2015), trichrome staining (Turgay *et al.*, 2007; Al-Megrin, 2010), carbol fuchsin staining (Alakpa *et al.*, 2002; Chacín-Bonilla *et al.*, 2007), (modified) safranin staining (Visvesvara *et al.*, 1997) and lactophenol cotton blue staining (Parija *et al.*, 2003), have been used in the past to identify *Cyclospora* oocysts in faecal smears, with variable degree of sensitivity and specificity. However, these morphology-based detection methods need more parasites burden, and may lead to frequent false positive results or false negatives. There are large differences in the performance between the different microscopy techniques. Direct detection using epifluorescence is actually the very best option, followed by the safranin-stain. In practice, two or more techniques could be used together to detect the presence of parasites.

Several PCR-based detection methods that amplify specific genes of *C. cayetanensis* have been developed. The first PCR method used for the clinical identification of *C. cayetanensis*, based on SSU rRNA gene sequences, was developed by Relman *et al.* (1996). Many other different PCR assays have since been developed. The real-time PCR based on the SSU rRNA gene has been optimized to specifically detect DNA from as few as one *C. cayetanensis* oocyst (Varma *et al.*, 2003; Verweij *et al.*, 2003). Another method uses the real-time quantitative PCR with a melting curve analysis to detect, identify and differentiate *C. cayetanensis* from other coccidian species of concern in animal health, zoonotic diseases and food safety (Lalonde and Gajadhar, 2011). Several other assays have been developed based on sequences other than the SSU rRNA gene, such as a PCR-based ITS assay, which is highly sensitive in oocyst detection (Olivier *et al.*, 2001; Lalonde and Gajadhar, 2008), and an *hsp70*-gene-based nested PCR protocol for the detection of *C. cayetanensis*, which was developed in 2013 (Sulaiman *et al.*, 2013). Many molecular methods have also been used to recover and detect *Cyclospora* oocysts in environmental water samples and agricultural products (Quintero-Betancourt *et al.*, 2002; Steele *et al.*, 2003; Murphy *et al.*, 2018). Generally speaking, molecular-based detection methods can reliably detect a smaller parasites burden than other methods, even a single oocyst, and they thus overcome many of the limitations of microscopic diagnoses (Lalonde and Gajadhar, 2008).

Serological screening tests for *Cyclospora* would support epidemiological studies, and would be especially useful in the investigation of outbreaks (Ortega and Sanchez, 2010). However, no serological assays to determine human exposure to *Cyclospora* are yet available. Specific antibodies for the diagnosis of *C. cayetanensis* infection are not easily obtained, which greatly restricts immunological testing. Another serious limitation of serological assays is the lack of a laboratory culture method with which *Cyclospora* can be propagated *in vitro* (Eberhard *et al.*, 2000; Cinar *et al.*, 2015).

Treatment

Treatment with trimethoprim–sulfamethoxazole (TMP–SMX) (160 mg trimethoprim, 800 mg sulfamethoxazole) twice daily for 7–10 days is reported to be effective in curing *Cyclospora* infection (Hoge *et al.*, 1995; Escobedo *et al.*, 2009). This is also an effective therapy for *Cyclospora* infections in HIV patients (Pape *et al.*, 1994; Verdier *et al.*, 2000) and AIDS patients with biliary disease (Sifuentes-Osornio *et al.*, 1995). TMP–SMX (also known as co-trimoxazole) is an effective treatment, and a low recurrence rate has been reported in many studies (Hoge *et al.*, 1995; Madico *et al.*, 1997; Goldberg and Bishara, 2012).

Ciprofloxacin is less effective than TMP–SMX, but is suitable for patients who are intolerant of sulfonamide drugs (Verdier *et al.*, 2000). Successful treatment of *C. cayetanensis* infections with nitazoxanide has only been reported in a small number of patients (Diaz *et al.*, 2003). However, nitazoxanide is an important treatment option for patients with a sulfa allergy or for whom treatment with sulfa or ciprofloxacin has failed (Zimmer *et al.*, 2007). However, norfloxacin, metronidazole, tinidazole and quinacrine have been shown to be ineffective in several studies of human cyclosporiasis (Escobedo *et al.*, 2009).

Conclusions

Since the earliest reported cases of human *Cyclospora* infection in Papua New Guinea in 1979, at least 54 countries have documented *C. cayetanensis* infections (involving 13 845 cases) up to December 2018. Of these countries, more than 13 have recorded cyclosporiasis outbreaks (including 6557 cases). The overall *C. cayetanensis* prevalence in humans worldwide is 3.55% (5478/154 410). *Cyclospora cayetanensis* infections are commonly reported in developing countries with low-socioeconomic levels or disease-endemic areas, such as Madagascar, Nepal, Indonesia, Peru and Haiti, among others. However, large outbreaks have also been documented in developed countries in Europe and the Americas, and among travelers from these countries and those returning from tropical endemic areas. Among susceptible populations, the highest prevalence has been documented in immunocompetent individuals with diarrhea. The marked seasonality of *C. cayetanensis* infection, which occurs predominantly during the rainy season or summer, is well documented. Infection with *C. cayetanensis* is mainly transmitted through the ingestion of food contaminated with oocysts. *Cyclospora cayetanensis* or *Cyclospora*-like organisms have also been detected in food, water, soil and faecal material from some animals. Detection methods based on oocyst morphology, staining and molecular testing have been developed. Treatment with TMP–SMX effectively cures *C. cayetanensis* infection. Ciprofloxacin is less effective than TMP–SMX, but is suitable for patients who cannot tolerate co-trimoxazole.

Despite many recent advances in research, our understanding of human cyclosporiasis is hampered by several technical difficulties. It will be necessary to establish an *in vitro* or animal model of *C. cayetanensis* in the near future, in which to study human cyclosporiasis. Rapid, convenient, precise and economic detection methods for its diagnosis and genotype in humans, and effective tracing methods, must also be developed to monitor the transmission of *C. cayetanensis*. More importantly, the proper disposal of faeces to avoid the contamination of soil and food, boiling and filtering drinking water and improved personal hygiene will go a long way toward preventing enteric parasitic infections.

Search strategy and selection criteria

We searched PubMed, Web of Science, ScienceDirect, Wangfang and the China National Knowledge Infrastructure, with no language restriction, using the following search terms to screen for relevant articles: '*Cyclospora*' or '*Cyclospora*-like organisms' or 'cyclosporiasis' or 'cyanobacterium-like body' or 'alga-like organism'. For articles without the full text or published in other languages, the titles and abstracts in English were screened for mention of *Cyclospora* infection. We included articles published up to 31 December 2018, when calculating the epidemiology data and summarizing the cases of infection. Articles published in English, Spanish, Portuguese, French, Turkish, Chinese, Czech, Dutch, Japanese, Rumanian and German were included.

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Conflict of interest. The authors declare that they have no conflicts of interest.

Ethical standards. Not applicable.

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