Cyclosporiasis: an emerging public health concern around the world and in Africa

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Background: Cyclosporiasis is an emerging gastro-enteric disease caused by the coccidia protozoan *Cyclospora cayetanensis*. It is associated with diarrhoea among children in developing countries, in the Americas where *C. cayetanensis* is endemic, traveller's diarrhoea and/or food and waterborne outbreaks in the developed countries.
Objectives: The aim of this review is to highlight cyclosporiasis and its relevance to public health in East Africa and Africa at large.
Methods: All literature on *Cyclospora*, *C. cayetanensis*, cyclosporiasis in Africa, and endemic cyclosporiasis was searched from libraries, colleagues and internet but only literature on its history, clinical presentation, epidemiology in endemic settings, and occurrence in Africa were scrutinised.
Results: In Sub Saharan Africa, cyclosporiasis has been reported in at least 3 countries, including Tanzania, in East Africa, occurring in both immunocompromised and immunocompetent patients. Zoonotic species of *Cyclospora* have also been identified in East African primates, indicating likely endemicity of this little reported disease in the region. This can be attributed to lack of awareness in the public and medical profession concerning the disease, and therefore not routinely checked at the health centres. Cyclosporiasis is characterized by intermittent diarrhoea, and secondary conditions or sequelae such as reactive arthritis syndrome (Reiter's syndrome), have been associated with progression of the disease. Its management is based on antibiotics, an unusual scenario for a protozoa.
Conclusions: Although many aspects of this disease and its transmission remain an enigma, the situation has been rapidly changing since the disease first came to medical attention in the 1970s.
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Introduction

Cyclosporiasis and Africa – should we be concerned?

Cyclosporiasis is a diarrhoeal disease caused by the protozoan pathogen *Cyclospora cayetanensis*[1]. It is considered an emerging disease of public health concern primarily in the developed countries where it has been identified as the cause of several outbreaks in North America and Europe, and with traveller's diarrhoea[2-6]. In these countries, cyclosporiasis transmission has primarily been linked to foods imported from developing countries adding impetus to the review of food import regulations with far reaching implications on developing country economies that depend on North-South trade in agricultural produce[4-9]. The transmission of the disease in the developing South where *C. cayetanensis* is endemic, remains little understood but has been associated with water and sanitation in Nepal, Guatemala, Nigeria and Egypt[7, 10, 11].

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Ashford is credited with having first identified *C. cayetanensis* as a human pathogen in 1979 in Papua New Guinea[12]. Thereafter, the parasite remained largely uninvestigated and it was not until 1994, when the first detailed morphological description and naming of *Cyclospora cayetanensis* was done[1]. Few studies have been carried out since then to determine the transmission and epidemiology of cyclosporiasis in Africa and its public health impact on the continent. The disease has been reported in Nigeria, Tanzania and Egypt[13-15]. Although it is thought to be self-limiting in immuno-competent individuals, cyclosporiasis can cause prolonged diarrhoea that could be life threatening in immuno-compromised patients. Moreover, like other coccidian parasites, *C. cayetanensis* has been associated with various sequelae including biliary disease, aculculous cholecystitis, Guillain-Barré syndrome and reactive arthritis syndrome following prolonged infection[16-19].

The study of cyclosporiasis is rife with difficulties that have largely contributed to its underreporting in Africa and little public awareness[20]. A key contributor to this is the specialised staining methods for diagnosis that are not routinely carried out in clinical

laboratories[20-22]. Additionally, efforts to elucidate its transmission and biology in endemic areas is impeded by lack of sensitive and specific methods for *Cyclospora* detection in the environment, agricultural produce and water bodies and further compounded by the lack of a suitable animal-model[23-26]. Cyclosporiasis has therefore remained a little known disease albeit with potentially far reaching impact on the health and economy of developing countries due to its impact on the horticulture industry as a food borne pathogen. Several publications pertaining to the methods of diagnosis and detection exist and have been ably reviewed by others[9]. Our aim is to bring this disease to the attention of African health researchers and to highlight potential areas of study in our likely endemic setting.

History of Cyclosporiasis

According to Lainson[27], it was Eimer who as early as 1870 first described an organism in intestines of the mole *Talpa europaea* that was eventually characterized and named *Cyclospora caryolytica* by Schaudinn in 1902[28]. But *C. glomericola,* observed in the millipede, was the first species to be classified as *Cyclospora* by Schneider who established the genus in 1881[29]. So far *C. glomericola* is the only species out of a total of 19 *Cyclospora* species identified to date to be encountered in an invertebrate host, the rest being found in reptiles (snakes) and mammals (rodents and non-human primates)[27].

It was not until the 1970s that cyclosporiasis first came to medical attention when an un-described coccidian was associated with diarrhoea in Papua New Guinea[4, 12]. Thereafter, endemic cases were also reported in Haiti, Nepal, Peru and linked to Travellers Diarrhoea in the 1980s, where it was reported variously as *Cryptosporidium muris*-like, a flagellate, an unsporulated coccidian, a large *Cryptosporidium*, a blue-green alga (cyanobacterium-like body), or a coccidian-like body[4, 30]. By 1990 the new pathogen was associated with chronic diarrhoea in acquired immunodeficiency syndrome patients[31]. Nevertheless, it was not until the 1990s that food and water-borne outbreaks in North America grabbed public and scientific attention to the emergence of the disease, after a food or waterborne outbreak of 21 was reported in a Chicago teaching hospital in 1990[32].

This fuelled efforts to definitively identify and characterise the pathogen, with the first breakthrough occurring in 1992 when Ortega *et al.,* reported they had sporulated and excysted the oocysts, thus ending speculation on the classification of the parasite and placing it in the genus *Cyclospora*[33]. The same group finally identified and characterised the causative agent as the coccidian *Cyclospora cayetanensis* (Apicomplexa: Eimeriidae) using classical morphological methods[1, 30]. However, molecular techniques have placed the pathogen closer to *Eimeria* species, suggesting its classification as such[34, 35]. Today, *C. cayetanensis* transmission in developed countries through food and water borne outbreaks has been well documented and understood, however the modes of transmission and risk factors in endemic areas remain poorly understood[4, 36, 37].

Life cycle and Biology

Cyclospora cayetanensis is an obligate intracellular parasite that is normally found in the jejunum. Oocysts found in the stool can be mistaken for the common *Cryptosporidium* spp. infecting humans, but can easily be distinguished by their larger size (8-10 μm) using an ocular micrometer[38]. The life cycle begins with the ingestion of the sporulated oocyst in contaminated water or food. Unlike *Cryptosporidium*, this oocyst when freshly passed in the stool is not sporulated and therefore not infective[1, 30]. Oocysts require a few days to weeks, depending on climatic factors, to develop and mature in the environment into the infective sporulated oocyst, thus precluding direct fecal-oral transmission[1, 38]. Temperatures ranging between 25-30°C are most suitable for sporulation. Upon being ingested, the oocysts excyst in the gut, releasing the sporozoites, which proceed to invade the epithelial cells of the small intestine[39, 40]. The sporozoites undergo two generations of asexual reproduction in the cell, whereby they form meronts that contain numerous merozoites[40]. The first generation meronts have 8-12 merozoites, whilst the second have only 4 merozoites, which penetrate new cells to form gametes. Some of these gametes enlarge to form the female macrogametes and some microgametes that undergo meiosis to form numerous flagellated sperm-like microgametes. The mature microgametes leave the microgametocyte and migrate to fertilize the macrogamete. The development of a resilient oocyst wall around the zygote, which contains 2 sporocysts or small oocysts, makes up the unsporulated oocyst. Eventually the oocyst is passed out with stool to begin the process of sporulation. Sporogyny commences in the presence of higher atmospheric oxygen concentrations and is complete between 7-12days[40-43].

Clinical aspects of C. cayetanensis infection

Presentation:

The clinical presentation of *C. cayetanensis* may include gastrointestinal (GI) symptoms such as loose or watery diarrhoea, nausea, vomiting, abdominal cramps, loss of appetite, or unintentional weight loss; or constitutional symptoms such as fever, chills, muscle

aches, joint aches, generalized body aches, headache, or fatigue[8]. Although asymptomatic infections are known to occur, the onset of symptoms in naïve populations observed in outbreaks is 1-14 days post exposure and is often accompanied by a characteristic waxing and waning of symptoms[6, 40, 41, 44]. In endemic countries, symptoms begin approximately 5-8 days after and may persist for a month or more. However, infection without watery diarrhoea is a common occurrence and has been observed in Haiti among other areas[45].

Diagnosis

Detection is based on the identification of oocysts in stool specimens using modified acid fast staining, or hot safranin test[21]. Where cyclosporiasis is suspected, up to 3 stool specimens taken 2 days apart should be tested to rule out the parasite. A rapid method of identification is possible on wet mounts using fluorescent microscopy employing a filter with a wavelength in the range of 340-380 nm, which reveals the bright, pale blue oocysts glow[30]. Polymerase chain methods (PCR) have also been developed for diagnosis and detection in the environment, but the primers appears to cross react with *Eimeria* spp.[26, 35, 46].

Differential diagnosis of cyclosporiasis includes all other causes of diarrhoea. Infections with *Giardia*, *Cryptosporidium, Isospora, Toxoplasma* and *Microsporidia* should be suspected in cases of persistent diarrhoea that does not respond to the usual treatment[6, 20, 31, 36, 41, 47]. Cyclosporiasis can also present symptoms closely resembling celiac disease and irritable bowel syndrome[48].

Sequelae

Like cryptosporidiosis, *C. cayetanensis* infection has also been associated with the onset of various extra-intestinal complications that may accompany prolonged infection, especially in the HIV immunocompromised patients. These include acalculous cholecystitis[19]; biliary disease[18], Guillian-Barré syndrome[17], and Reiter or reactive arthritis syndrome[16]. One case of *C. cayetanensis* oocysts found in the sputum of 60-year-old HIV negative male with a history of successfully treated TB has been reported in Argentina[49], and a similar case has also been reported in Egypt where a 45 year-old HIV negative male with TB history also presented oocysts in the sputum accompanied with active TB infection[50]. The latter has prompted calls for the inclusion of cyclosporiasis as a new causative agent of respiratory disease and as a differential diagnosis for TB. In both TB negative and positive cases, the patients presented with loss of weight, cough with expectoration of purulent sputum and dyspnea. The implications of pulmonary infection to the biology, life cycle and transmission of *C. cayetanensis* remains to be evaluated, as does its interaction with TB, a disease currently on the increase in Africa due to the HIV/AIDS pandemic[51].

Treatment

Symptoms resolve with the administration of Trimethoprim-Sulfamethoxazole, the standard treatment for cyclosporiasis at 160/800 mg oral dose taken twice a day for 7 days or 160/800 mg oral dose taken 4 times a day for 10 days in immunocompromised patients with AIDS often with symptoms resolving and passage of oocysts in the stool ceasing within 24-48 hr[52, 53].

Epidemiology in endemic setting

The first epidemiological studies conducted in an area endemic for *C. cayetanensis,* were carried out in 1997-1998 in Guatemala where raspberry exports to USA were linked to cyclosporiasis outbreaks[10]. Children of age groups 1.5 to 4 years and 5 to 9 years were found to be 5 times more likely to be positive for *C. cayetanensis* in stool samples than adults. Overall infection did not differ significantly by sex. The infection rates were also found to fluctuate seasonally, with prevalence peaking in the rainy season (6.7%) and falling to undetectable levels in the dry seasons. Drinking of untreated water or swimming in rivers or springs, having a septic tank as opposed to municipal sewage, direct contact with the soil and ownership of dogs, chicken or other fowls, were all found to significantly increase risk of infection although cats and pigs did not[10].

Subsequent epidemiological studies in endemics areas of Haiti, Nepal and Peru have corroborated higher infection rates in children, seasonal fluctuation of prevalence, and risk associated with water source and ownership of domestic animals, particularly fowl, guinea pigs and rabbits[37, 54]. However no oocysts were detected in a survey for *C. cayetanensis* in domestic animals carried out for 1.5 years in an endemic area in Haiti, ruling out domestic animals as a reservoir[24]. Studies in Peru have also shown that previous infection offers significant protection against subsequent exposures[54]. This consolation is however short lived as clinical sequale may still be linked with asymptomatic infection. Humans are therefore the only known host for *C. cayetanensis*, but the eating of raw or insufficiently cooked bivalves, which are filter feeders that concentrate pathogens from waters, has been shown to be a risk factor[55].

Reports of *Cyclospora* spp. infecting non-human primates in the East African countries of Uganda, Kenya, Tanzania and Ethiopia have led to efforts to conduct

studies of non-human primates in order to better understand the epidemiology and ecology of endemic cyclosporiasis[25, 56-59]. Host specificity of *Cyclospora* species in the different non-human primates, geographic overlaps of both monkey and parasite species notwithstanding, corroborated the likelihood of humans as the sole definitive host of *C. cayetanensis*[58]. However the collection of oocyst positive stools from the studied troops of vervet monkeys, baboons and colobus monkeys at all times of the year in Kenya, which is notable for extreme weather patterns, demonstrated a lack of seasonality in infection contrary to expectations[58].

Cyclospora the enigma

It is clear that the epidemiology of cyclosporiasis as we know it today is a study in contradictions, as the causative agent itself remains shrouded in mystery. How can a pathogen incapable of direct faecal-oral infection and lacking a zoonotic reservoir or ubiquitous presence in the environment[38], be responsible for so many widespread outbreaks of gastroenteritis all over the world? Trace amounts of oocyst contamination in food products indicate that the infective dose of *C. cayetanensis* is likely to be very low[38]. However attempts at experimental infection in both humans and animals have all proved futile[25, 60]. The lack of an animal model has therefore made it hard to determine the viability of oocyst exposed to varying environment conditions that may impact its transmission[38]. While most outbreaks have been linked to food sources, just as many unexplained sporadic cases linked to water/sewage transmission have been reported in the developed countries[32, 38, 61]. These cases may signify ubiquitous endemicity, remaining undetected due to seasonality of infection, but by what means the parasite survives from one infection season to the next remains unknown.

Even more puzzling is the dearth of *C. cayetanensis* reports in African populations and as a cause of traveller's diarrhoea in visitors returning from the continent. The screening of over 4,800 samples using modified acid fast staining, and other concerted efforts in previous years have all failed to determine the presence of *C. cayetanensis* in Kenya[62]. In addition to economic restraints for effective routine diagnosis in health centres and national/regional surveillance, it has been postulated that the amount of sulpha drugs administered in sub Saharan Africa as drugs of choice for malaria and other infectious diseases may suppress the parasite, humans being the only reservoir[36]. Could sulfadoxine-pyrimethamine (SP), the now largely ineffective but still affordable treatment of malaria, be our unlikely saviour? Or is it the use of trimethoprim (Septrin), which is widely used as a first line of antibiotics and in management of HIV before ARVs? Significantly higher HIV and malaria prevalence in sub-Saharan Africa may account for a higher drug pressure than other parts of the world, however, the withdrawal of SP as a first line antimalarial due to resistance and similar use of Septrin in *C. cayetanensis* endemic Peru and Haiti where 5-11% HIV prevalence have been documented, makes the dearth of reported African cyclosporiasis all the more mysterious.

The controversy and questions surrounding *C. cayetanensis* are no less intriguing. Whereas there is compelling molecular evidence for its reclassification as a mammalian *Eimeria* species, only *C. cayetanensis* DNA sequences and *C. colobi, C. cercopitheci* and *C. papionis* from East African non-human primates are available[27, 34, 36, 57]. The way forward on the classification of this parasite in the light of these new developments remains uncharted waters. The biology of *C. cayetanensis* in the host is no less intriguing. The presence of oocysts in HIV negative TB patients strongly suggests the existence of an extra-intestinal parasite lifecycle and colonization in humans that is yet unknown[49, 50]. Moreover, more studies are required to determine whether *C. cayetanensis* interacts with TB or has only been observed in TB patients because it is an acid-fast staining organism like the TB bacillum. Extra-intestinal stages in the life cycle of *Cyclospora talpae* in European moles have been demonstrated in the liver where sexual stages develop in the bile duct epithelial cells[63, 64]. *C. cayetanensis* has similarly been shown to cause biliary disease in HIV/AIDS patients suggesting possible colonization of the liver[18, 36]. Unfortunately, the lack of animal/experimental models remains an impediment to resolving these questions.

Conclusions and recommendations

Cyclosporiasis therefore presents an ever-broadening frontier cutting across multiple disciplines of research including clinicians, epidemiologists, parasitologists, veterinarians, environmental scientists among others. As a food-borne disease that impacts on horticulture (the backbone of most developing country economies) and primarily infects children and HIV/AIDS patients, routine surveillance in both the population and environment should be carried out. Surveillance in populations should include sputum samples regardless of TB or HIV status. The role of its interaction with TB and/or HIV should also be elucidated. Use of non-pathogen *Cyclospora* spp. such as non-human primates cyclosporiasis and *C. talpae* and mole hosts should be developed as alternative animal models for the study of extra-intestinal colonization and disease. Additionally,

the possible role of sulphur drugs in control of cyclosporiasis should be evaluated, in order to address the impact of the disease in Africa.

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