OPPORTUNISTIC PROTOZOAN PARASITIC INFECTIONS OF THE GASTROINTESTINAL TRACT

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ABSTRACT

Opportunistic infections of the gastrointestinal tract have played a critical role in determining symptomatic illness in immunocompromised individuals. Protozoan parasites that cause mild or self-limited disease in immunocompetent individuals can cause protracted and severe diarrhoea in immunocompromised patients. Among the protozoans that cause intestinal infections in the immunocompromised, Cryptosporidium species, Isospora and microsporidia are the most common organisms encountered. In addition, infections due to Entamoeba histolytica, Cyclospora cayatenensis and Giardia are also seen. Treatment of these infections is available for Isospora, some microsporidia, E. histolytica, Cyclospora and Giardia. In addition, it has been shown recently that antiretroviral therapy can resolve diarrhoea due to Cryptosporidium and microsporidia.

KEY WORDS: AIDS, immunocompromised, parasitic infections, protozoa.

Introduction

Parasitic infections of the gastrointestinal (GI) tract are a major cause of morbidity in developing countries and are increasingly important in certain populations from developed countries, particularly in patients with the acquired immunodeficiency syndrome (AIDS)1. Among the parasitic infections that affect the GI tract, infections due to helminths have been known to be a major cause of morbidity since these were first described in the 17th century. However, several trends in modern clinical medicine have placed protozoal infections in a position of increasing importance. These include the pandemic of human immunodeficiency virus with associated opportunistic infections and the increasing use of powerful chemotherapeutic and immunosuppressive agents to prevent rejection of transplanted tissue in human allograft recipients2.

Opportunistic infections occur in patients with impaired host defenses and are caused by infectious agents that do not ordinarily produce disease in healthy individuals. The term compromised host refers to an individual who has one or more defects in the body’s natural defense mechanism, sufficiently significant that the individual is rendered predisposed to severe, often life-threatening infection3. Causes of deficient host defence include inherited disorders, coexisting disease or malignancies, alcoholism/malnutrition and drug therapies. Drugs that most often cause deficient host defenses are steroids and cytotoxic agents which are administered to treat malignant neoplasms, collagen vascular disorders and organ transplants.

The probability that an immunocompromised host will develop opportunistic infection is influenced by several factors, including the degree of immunodeficiency, exposure to potential pathogens, relative virulence of a potential pathogen and the usage of chemoprophylaxis.

In contrast to bacterial and viral infections, parasitic infections of the GI tract may be diagnosed rapidly by direct microscopic examination of clinical specimens4. However, the diagnosis of parasitic infections continues to present multiple challenges to the clinician and the laboratory. First, excretions of parasitic pathogens in stool may be intermittent, requiring examination of several specimens collected at different times. Second, identification of various morphological stages of parasites and differentiation between potential pathogens and commensal parasites requires expertise, which may be insufficient in laboratories that handle small numbers of specimens. Third, the presence of multiple parasites in a single specimen may make it difficult to determine whether disease is caused by one or more than one pathogen. Finally, the correlation between the presence of parasitic
pathogens in stool and clinical disease is not always straightforward, particularly in persons from developing countries who may harbor potential pathogens asymptomatically for prolonged periods.

Protozoan pathogens that commonly cause opportunistic infections of the gut are *Cryptosporidium parvum*, *Isospora belli* and microsporida\(^5\). Additionally, protracted diarrhoea is seen in HIV infected patients infected with *Cyclospora cayatensis*\(^6\) although these patients are not more susceptible to infection than immunocompetent individuals. HIV infected patients also develop infection with *Giardia lamblia* and *Entamoeba histolytica*\(^7\). However, giardiasis is not more frequent or severe than in HIV negative individuals and responds to metronidazole or tinidazole\(^7\). *Entamoeba histolytica* in the immunocompromised patient is believed to be usually a commensal, belonging to nonpathogenic zymodesmes\(^8\) or *Entamoeba dispar*. Recently it has been reported that invasive amoebiasis is seen increasingly in patients infected with HIV in Taiwan\(^9\). Isolates of *E.histolytica* from patients with invasive amoebiasis have unique isoenzymes, surface antigens, DNA markers, and virulence properties and now are classified as a separate species from the noninvasive species *Entamoeba dispar*\(^10\). *Blastocystis hominis* is also frequently identified in human stools, but the role of this anaerobic protozoan as a human pathogen is controversial, and as yet unresolved\(^11\).

**Cryptosporidium**

This small, coccidian parasite was considered a veterinary pathogen but not an important human pathogen until the early 1980s, when it was recognized in patients with AIDS\(^12\). Subsequently, it has become recognized as a major cause of diarrhoeal illness both in immunocompromised and immunocompetent individuals. To date, the only species considered pathogenic to humans is *C. parvum*, although there is one case report of infection with *C. baileyi*. However molecular studies have now shown that there are two distinct genetic groups of *C. parvum* which infect humans and animals respectively\(^13\). Further epidemiological studies are required to assess the frequency of interspecies transfer of *C. parvum*.

*C. parvum* causes an acute watery diarrhoea in immunocompetent individuals and a protracted life-threatening diarrhoea in immunodeficient individuals. This is considered an AIDS-defining illness. Recently, following multiple outbreaks of diarrhoea, including one affecting over 400,000 people\(^14\) following contamination of water supplies, it is also described as a waterborne pathogen, which is difficult to eradicate. This coccidian has a worldwide geographical distribution, with increased incidence in developing countries. Prevalence rates of over 12% are seen in asymptomatic individuals, including those infected with HIV\(^15,16\). Transmission can be by the faeco-oral route, waterborne, due to contact with farm animals and by drinking unpasteurised milk.

The incubation period ranges from 1 to 14 days. Infection results in a self-limiting (3–14 days), watery, foul smelling diarrhoea in immunocompetent hosts, which is associated with abdominal pain, nausea and vomiting, rarely with fever. Respiratory symptoms are common in children. In immunocompromised hosts there may be a severe, prolonged cholera-like illness, weight loss, dehydration and malabsorption\(^17\).

Infection is acquired by ingestion of mature oocysts which release sporozoites by partial digestion of oocysts. These then develop into trophozoites that enter into the enterocytes and develop into meronts in an extracytoplasmic vacuole. The meronts undergo division into merozoites which are released from the enterocyte to reinfect other cells to give rise to the second generation of meronts\(^18\). These develop further into microgamonts and macrogamonts which fuse to give rise to oocysts. The cyst wall has a central electron lucent layer with outer electron dense layers. In immunocompetent hosts, parasite replication is confined to the apical border of enterocytes in the lower jejunum and ileum. In immunocompromised hosts, the entire GI tract, extraintestinal sites such as the biliary and pancreatic ducts and parts of the respiratory tracts become infected\(^19\). Mild to severe villous atrophy, with stunting and broadening of villi is seen. Cysts enlarge and become hyperplastic. Infected cells lack microvilli at sites of attachment and the cytoplasm is vacuolated.

Diarrhoea is due to impairment of glucose-stimulated sodium and water absorption, with bacterial overgrowth, osmotic pressure changes across the gut wall and release of fluid into the lumen\(^20\).

The stage seen in faeces is the oocyst which measures 4–6 \(\mu\)m, and contains 4 fully developed and infectious sporozoites, 80% are thick-walled and 20% thin-walled. In a wet mount under the light microscope, the oocysts are pink tinged, spherical, translucent bodies. Under phase contrast, the oocysts are bright, birefringent, containing upto 4 sporozoites and dark granules, the oocyst residual body.

The oocysts stain with modified acid-fast and auramine-phenol stains, appearing bright orange-red or fluorescing golden yellow under UV illumination\(^21\). The acid-fast stain of stool smear can also be done after concentration. The red color may be unevenly distributed due to variable uptake by the oocyst wall especially in rapidly shed young oocysts. Other stains used on include methenamine-silver, Giemsa and periodic acid-Schiff stains. Polyclonal and monoclonal antibody conjugates with fluorescein isothiocyanate for direct identification of oocysts in stool are also available commercially. Identification of antibodies to *Cryptosporidium* measures exposure of the
population to this parasite, but is not useful in diagnosis21.

A large number of studies on treatment of this infection have not identified any drugs that are useful in a majority of cases. The therapy currently recommended is the use of anti-motility agents. Other drugs that may be useful include paromomycin, spiramycin and azithromycin5,22.

Isospora belli

First described in 1860 by Virchow, this diarrhoeal disease pathogen is a host specific coccidian protozoan parasite23. There is no evidence that the human Isospora species is capable of infecting nonhuman hosts or that animal species affect humans. Infection with this opportunistic parasite is considered an AIDS defining illness if present for over 4 weeks24.

It has a worldwide geographical distribution. Transmission may be by the faeco-oral, water borne or sexual routes. The incubation period ranges from 1 to 4 days and can be up to 1 week. The clinical features of infection with Isospora belli are diarrhoea, malabsorption, weight loss and abdominal pain25.

Human infection follows ingestion of mature sporulated oocysts containing 2 sporocysts each. Liberated sporozoites invade small bowel enterocytes and initiate asexual reproduction. Sexual reproduction follows and produces non-infectious oocysts which are excrated. Oocysts are elongated, measure 20–33 μm X 10–19 μm, narrow at 1 pole, unsporulated when excrated, mature after 48 hours developing into sporoblasts and then into sporocysts, each of which has 4 sporozoites, and 1-2 crystalloid bodies25.

In the epithelial cells of the duodenum and jejunum developmental stages of I. belli are seen along with villous atrophy and inflammatory cells in the lamina propria in these areas. In experimental studies, focal to widespread mucosal changes that varied from severe flattened villi to mild nonspecific alteration and increased inflammatory cells in the lamina propria have been described. The changes seem to correlate with severity of symptoms.

Diagnosis of infection is by examination of stool samples. Oocysts can be demonstrated in wet preparations and stained with modified acid-fast stains and direct fluorescent stains26. Duodenal aspirates can also be examined. Small bowel biopsies may show developmental stages of the parasite.

Treatment with trimethoprim and sulfamethoxazole for 10 days have been shown to eradicate infection, although there is a 50% risk of relapse if treatment is not completed. Pyrimethamine can also be used11,24.

Cyclospora cayatenensis

This organism was first described in humans in Papua New Guinea in 1979. Initially there was much confusion about the identity of this parasite and it has been referred to as a blue green algae (cyanobacterium like body), a fungal spore, a coccidian like body and a large cryptosporidium. However, ultrastructural and molecular studies indicate that it is a coccidian apicomplexan of the genus Cyclospora6,27.

This parasite has been described from South America, North America and Nepal, and appears to have a worldwide distribution. Transmission by contaminated water supplies has been shown to be responsible for outbreaks in many settings, and it likely that this is also transmitted by the faeco-oral route6.

Ingestion of the mature oocyst results in watery diarrhoea with weight loss, abdominal pain, fatigue, anorexia, bloating, flatulence and fever. The illness may be prolonged, lasting 2–6 weeks. It is chronic and severe if untreated, especially in patients with AIDS28.

The life cycle is yet to be completely described. The unsporulated oocyst is shed in the faeces and up to 40% of these sporulate in 1–2 weeks. The oocyst is generally round, 8–9 μm in diameter, similar to Cryptosporidium but larger, with a central refractile morula like structure composed of a cluster of globules enclosed within a cell membrane. When examined under UV illumination, Cyclospora oocysts exhibit a blue fluorescence. Each sporulated oocyst has 2 ovoid sporocysts with 2 sporozoites each27.

Diagnosis of infection is by identification of the oocysts in wet preparations, and by staining with modified acid fast stains as for Cryptosporidium and Isospora. Cyclospora does not stain with monoclonal antibodies specific for Cryptosporidium6. These organisms have also been observed in duodenal aspirates and in small bowel biopsies by electronmicroscopy. Intestinal biopsies have revealed evidence of small bowel injury29.

Microsporidia

These are obligate intracellular spore forming protozoan parasites that have been reported to infect every major animal group. They have true nuclei, but lack mitochondria and the ribosomal RNA is of prokaryotic size. They were first identified in silkworms in 1857 and the first human case was reported in 1959. Five species affect humans (Enterocytozoon, Encephalitozoon, Microsporium, Pleistophora and Nosema) and are mainly seen in immunocompromised hosts, although some occasionally cause self-limiting diarrhoea in travellers6.

Enterocytozoon localizes in the small intestine enterocytes and occasionally in epithelial cells of the biliary tree, liver, pancreas and respiratory system. Encephalitozoon localizes in macrophages and epithelial cells of cornea, conjunctiva and respiratory and urinary systems. Septata (now known as Encephalitozoon intestinalis) is found in enterocytes and lamina propria...
macrophages, also in epithelial cells of the biliary tree, tubular kidney cells and bronchial epithelium. *Pleistophora* has been found in muscles of patients with myositis (these spores can measure up to 3.4 μm). *Nosema* has been found in corneal ulcers as well as a variety of other tissue in disseminated infection. *Microsporium* also causes corneal ulcers.

Infection with microsporidia may be i) latent asymptomatic, or chronic mild symptomatic in adults with normal immunity, ii) acute, potentially fatal, in neonates, iii) proliferative disease in the absence of competent host defences and iv) symptomatic disease in immunocompetent hosts, usually localised.

The route of transmission is not known, although infection by aerosols or ingestion, from animals and sexual partners have been proposed as potential routes of acquisition of microsporidiosis. The infective spore is less than 2 μm in size and has a 3 layered spore wall (exosporium, endosporium and unit membrane), the sporoplasm is uninucleate or binucleate, and has a unique extrusion apparatus (the polar tubule) for injecting the sporoplasm into new host cells.

The infective spore extrudes the polar tubule and injects sporoplasm into the host cell. Merogyne, the proliferative vegetative stage follows with meronts developing from infective spores. These are structurally simple cells with little cytoplasmic differentiation and multiply by binary and multiple fission resulting in rounded multinucleate plasmodial forms (e.g. *Enterocytozoon*) or ribbonlike multinucleate cells (e.g. *Septata*). Meronts develop finally into sporonts with the formation of dense amorphous surface coat around the cell. Development may take place directly in contact with the cytoplasm or within a parasitophorous vacuole limited by a host-derived membrane.

Laboratory diagnosis of these infections is by demonstration of microsporidia in infected tissue or body fluids. Stool specimens can be examined by the modified trichrome stain, chemofluorescent stains or the Giemsa stain on concentrated specimens. Cytological examination of sediments of duodenal aspirates, bile, urine, bronchoalveolar lavage fluid and CSF stained by Giemsa can be done. Tissue biopsies for histological examination are stained by Giemsa stain, Gram stain or the toluidine blue stain. Microsporidia can also be identified in tissue specimens by electron microscopy. Immunofluorescence using polyclonal antibodies has also been done. ELISA and IIF have been shown to be useful in animals for antibody detection.

Treatment with fumagillin, sinfungin, albendazole, and metronidazole has been attempted and of these, albendazole is the most promising agent. Prophylaxis is indicated in any HIV infected patients with CD4 counts less than 200 cells/cu.mm.

**Conclusions**

In HIV, the gastrointestinal tract is a major target organ and diarrhoea is a common problem in 30–60% of AIDS patients in industrialised countries and upto 90% in the underdeveloped world. Specific pathogens can be identified in 44–88% of patients with AIDS and chronic diarrhoea, but this requires awareness of potential opportunistic pathogens on the part of the clinician as well as a competent laboratory capable of identifying these organisms.

**REFERENCES**


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