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section 8 Infectious diseases 1440 Treatment There is no specific therapy for sarcocystosis in humans or ani- mals, although various compounds, including albendazole, metronidazole, cotrimoxazole, and corticosteroids, have been used empirically in both humans and animals. Experimentally infected calves and lambs appeared to respond to amprolium and salinomycin. Ponazuril prevented infection of the central ner- vous system of mice experimentally given sporocysts of S. neu rona and is currently used in the New World for the treatment of equine protozoal myeloencephalitis in horses. When humans are the final host, symptomatic and supportive treatment is indicated. Prevention Sarcocystosis can be best prevented by not eating raw or under- cooked meat from any animal and by improvements to food hygiene, especially in poorer countries. Experimental vaccines have been shown to produce cellular immunity to Sarcocystis species in horses and studies on experimentally inoculated pigs demonstrated a persistent immunity to further infection. FURTHER READING AbuBakar S, et al. (2013). Outbreak of human infection with Sarcocystis nesbitti, Malaysia 2012. Emerg Infect Dis, 19, 1989–91. Arness MK, et al. (1999). An outbreak of acute eosinophilic myositis attrib- uted to human Sarcocystis parasitism. Am J Trop Med Hyg, 61, 548–53. Bunyaratvej S, Bunyawongwiroj P, Nitiyanant P (1982). Human intestinal sarcosporidiosis: report of six cases. Am J Trop Med Hyg, 31, 36–41. Dubey JP, et al. (2015). Sarcocystosis of animals and humans, 2nd edi- tion. CRC Press, Boca Raton, FL. Fayer R (2004). Sarcocystis spp. in human infections. Clin Microbiol Rev, 17, 894–902. Hilali M, et al. (1995). Isolation of tissue cysts of Toxoplasma, Isospora, Hammomdia and Sarcocystis from camel (Camelus dromedarius) meat in Saudi Arabia. Veterinary Parasitology, 58, 353–6. Hofmann P, et al. (1999). Sarcocystosis and Malassezia infection in an im- munodeficient rhesus macaque—a case report. Primate Report, 55, 19–24. Marsh AE, et al. (2004). Evaluation of immune responses in horses im- munized using a killed Sarcocystis neurona vaccine. Vet Ther, 5, 34–42. Mehrotra R, et al. (1996). Diagnosis of human Sarcocystis infection from biopsies of the skeletal muscle. Pathology, 28, 281–2. Mohammed OB, et al. (2000). Sarcocystis infections in gazelles at the King Khalid Wildlife Research Centre, Saudi Arabia. Veterinary Record, 146, 218–21. Palmer SR, et al. (2011). Oxford textbook of zoonoses, 2nd edition. Oxford University Press, Oxford. Slapeta JR, et al. (2003). Evolutionary relationships among cyst- forming coccidia Sarcocystis spp.

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8.8.9 Giardiasis and balantidiasis 1441 food, or after contact with faecal material from infected individuals. Strains of the parasite that can potentially infect humans are also har- boured by various mammals, including dogs and cattle. Clinical features—manifestations include watery diarrhoea, abdom- inal discomfort, distension and pain, nausea and vomiting, weight loss, and malabsorption, with the infection typically being persistent and severe in individuals with certain immunodeficiencies. Chronic G. intestinalis infection can lead to micronutrient deficiencies, and im- pairment of growth and cognitive development in children. Diagnosis and treatment—diagnosis is by faecal examination for evi- dence of G. intestinalis infection, including (1) by light microscopy of fresh or stained smears to detect cysts (ova and parasites test; a simple historic and still common approach that lacks sensitivity); (2) by immunofluores- cence microscopy of samples stained with direct fluorescent antibodies; or (3) by nucleic acid amplification techniques, such as the polymerase chain reaction, to detect parasite DNA or RNA. Treatment is primarily with metronidazole or tinidazole, and less commonly with nitazoxanide, albendazole, paromomycin, or quinacrine. Resistance to all common drugs has been reported, but can presently be overcome by switching antimicrobial classes or with combination therapies. Prevention—cysts of G. intestinalis are relatively resistant to several common disinfectants, including chlorine- and iodine-based chem- icals, but can be killed by boiling or exposure to ultraviolet light, or removed by filtration. A vaccine is available and moderately effective in animals, but a human vaccine has not been developed. Balantidiasis Balantidium coli is a relatively rare ciliate protozoan that invades the colonic mucosa. Infection—which may or may not be acquired from pigs or other animals—might be asymptomatic or cause diarrhoea that can be watery or contain blood and mucus. Perforation of the colon can occur, leading to peritonitis, and the parasite can also spread to the liver, lungs, and spine. Diagnosis is by recognition of the parasite on microscopic examination of freshly obtained diarrhoeal stools, co- lonic mucus, or rectal biopsies. Aside from supportive care, treatment with metronidazole or tetracycline has reportedly eradicated infection. Prevention is by filtration or boiling of drinking water, hand washing before handling food, and careful cleaning and cooking of food. Introduction The two organisms covered in this chapter, Giardia and Balantidium, are protozoa that can cause diarrheal disease. Transmission of both occurs through ingestion of environmentally resistant life cycle stages of the parasites. Giardia is one of the two most common

protozoan causes (along with Cryptosporidium) of diarrheal disease world- wide, while infections with Balantidium are less common and occur primarily in tropical and subtropical countries. Other important protozoan causes of diarrheal disease, including Cryptosporidium, Entamoeba, and Cyclospora, are discussed in separate chapters. Giardiasis Aetiology Giardia, the causative agent of giardiasis, was first discovered in 1681, when van Leeuwenhoek undertook a microscopic examination of his own diarrhoeal stool. Historically, the literature on human giardiasis had emphasized a relationship between drinking unfiltered water in wilderness areas and acquiring this infection, as well as occurrence of Giardia species in muskrats and beavers (which led to the now out- dated term ‘beaver fever’ for giardiasis). Although it is possible that faeces from these animals were sources of Giardia cysts that could infect humans, it is also possible that cysts of human origin could have infected the amphibious mammals. The host range of organ- isms morphologically classifiable as Giardia is wide and include birds and various terrestrial and marine mammals. Giardia organisms have been divided into eight genetic assemblages (designated A-H), on the basis of their DNA sequences. Assemblages A and B cause human in- fection; the other assemblages infect nonhuman hosts. Besides human subjects, hosts for assemblages A and B include domestic pets (dogs, cats, rabbits, chinchillas, and ferrets), and livestock (cattle, sheep, and pigs). Proving that an assemblage A or B organism from a nonhuman host can cause human infection is difficult; although giardiasis is clas- sified as a zoonosis, evidence of animal-to-human (and vice versa) transmission is largely circumstantial. In this chapter, the terms ‘as- semblage’ and ‘genotype’, as applied to Giardia, are used interchange- ably. Because they lack mitochondria and some other features of ‘higher’ eukaryotic (nucleated) cells, Giardia was formerly considered to be primitive. Following the discovery of gene sequences homolo- gous with mitochondrial DNA and of organelles (mitosomes) that ap- pear to be derived from mitochondria, the organism is now regarded as highly specialized rather than primitive. The apparently primitive features are almost certainly adaptations to the parasitic lifestyle, re- flecting the colonization of a microaerophilic or anaerobic niche (the vertebrate intestinal lumen) by Giardia species. Giardia intestinalis (synonyms G. lamblia and G. duodenalis) is a flagellated parasite that belongs to the order of diplomonads in the phylum of metamonads, a large group of flagellate amitochondriate protozoa. The parasite has a two-stage life cycle (Fig. 8.8.9.1), which generally occurs in a single host (monoxenous) and involves an in- fectious thick-walled cyst and a motile trophozoite. Hosts become infected by ingesting G. intestinalis cysts, from which trophozoites emerge (excystation) in the small intestine upon prior exposure to gastric acid in the stomach. Trophozoites are the vegetative, replicating forms of the parasite. They are pear-shaped, dorsoven- trally flattened organisms with dimensions of 12–15 µm in length and 5–9 µm in width (Fig. 8.8.9.2). Four pairs of flagella located at the anterior, posterior, caudal and ventral sides of the organism confer motility, and together with a ventral adhesive disc enable attachment to the luminal surface of intestinal epithelial cells. The parasite resides in the intestinal lumen and in close proximity to the epithelium, but does not invade the mucosa or spread systemically. It replicates in its luminal niche and ultimately forms cysts that are shed in the faeces for transmission to other hosts. The genome sequences of multiple G. intestinalis isolates have been determined and analysed. The compact haploid genome of G. intestinalis comprises c.12 Mb of DNA encoding c.5000 genes. Trophozoites have two morphologically similar nuclei, which each carry diploid genomes (making the trophozoite genome overall tetraploid) and are both transcriptionally active. Although the polyploid genome sequences have generally been considered to be identical, some evidence exists for allelic sequence heterozygosity of specific genes within single parasites. Genome comparison of isolates from the human-pathogenic assemblages A and B shows only 77% nucleotide and 78% amino acid identity

in protein-coding

section 8 Infectious diseases 1442 regions, indicating that the two assemblages truly represent different Giardia species, despite the historic convention of a single species name. The metabolism of G. intestinalis is fermentative, and electron transport proceeds in the absence of mitochondrial oxidative phos- phorylation. However, the parasite is microaerotolerant and can re- duce molecular oxygen and thus protect the highly oxygen-sensitive, central metabolic enzyme, pyruvate:ferredoxin oxidoreductase (PFOR), and iron-containing ferredoxins. PFOR decarboxylates pyruvate and donates electrons to ferredoxin, which in turn reduces other components in the electron transport chain and leads to ad- enosine triphosphate generation. Epidemiology G. intestinalis is a major cause of diarrheal disease with c.180 mil- lion annual cases worldwide and prevalence rates ranging from 1 to 20% in different countries and regions. Infections are more frequent and severe in young children, particularly in day-care centres, and persons changing their diapers, as well as among individuals with a history of foreign travel, use of swimming pools, hiking, and keeping a dog. A slight predominance of males (60%) over females (40%) has been reported in giardiasis cases in Austria, but the underlying reasons are unclear. The infection is acquired by ingestion of water or, less commonly, food contaminated with cysts, or by direct faecal–oral transmission of cysts, particularly among young children. Waterborne giardiasis occurs as a result of drinking contaminated water from streams and lakes containing G. intestinalis cysts. Swimming in (and inad- vertently drinking) water in lakes and rivers containing the cysts is also a risk factor for giardiasis. Outbreaks of this infection have re- sulted from the unintended presence of G. intestinalis cysts in public Fig. 8.8.9.1 Life cycle of Giardia intestinalis in humans. Transmission occurs by ingestion of cysts from contaminated water or food (step 2). Once ingested, trophozoites emerge from the cysts in the small intestine, where they reside and replicate (steps 3 and 4) in the lumen or in close proximity to the epithelial surface. Trophozoites differentiate into infective cysts in the small intestine (step 5), and are passed in the stool (step 1). Reprinted from the Public Health Image Library of the Centers for Disease Control and Prevention, Atlanta, Georgia, USA; image generated by Dr Alexander J. da Silva and Melanie Moser.

8.8.9 Giardiasis and balantidiasis 1443 drinking water supplies and in swimming pools due to inadequate disinfection. Giardiasis is one of several parasitic and bacterial diseases that are potentially or actually transmitted by eating raw vegetables grown on fields irrigated or contaminated with untreated human sewage or animal manure. From the 1990s onwards, numerous comparisons have been made of genome sequences of G. intestinalis isolated from human and nonhuman hosts. Close similarity between DNA sequences of G. intestinalis from different host species suggests, rather than proves, interspecies transmissibility of the parasite. Genotyping has revealed genetic similarity between Giardia isolates from people and from dogs occupying the same households in India, a finding that suggests transmission of G. intestinalis between dogs and people. Approximately 10% of Giardia isolates from cattle belong to geno- types that can potentially cause human infection. Flies that feed on garbage and sewage are able to carry Giardia cysts on their exoskel- etons and in their alimentary tracts and may therefore contaminate human food with viable cysts. Immunodeficiency predisposes to the occurrence of severe and persistent giardiasis. Human immunodeficiency states that are as- sociated with giardiasis include conditions that impair host anti- body responses, notably ‘common variable’ immunodeficiency and X-linked immunoglobulin deficiency (XID). Impairment of intes- tinal IgA production is a feature of these particular immunodefi- ciencies that may contribute to increased

susceptibility to giardiasis. Furthermore, HIV-induced acquired immunodeficiency syndrome (AIDS) is associated with an increased prevalence of giardiasis (and other enteric protozoan infections), particularly in patients with low CD4+ T cell numbers and untreated for HIV. An association between selective IgA deficiency and increased prevalence of giardiasis has been mentioned in some reports, although other studies have not seen such an association, suggesting that effective host defence against Giardia can occur in humans in the absence of normal intes- tinal IgA production. Pathogenesis and pathology The mechanisms responsible for diarrhoea and malabsorption in giardiasis are incompletely understood. Giardia is not known to pro- duce classical enterotoxins, making it likely that the host response to infection is mostly responsible for the diarrhoea. A large endoscopic and histological study of 567 patients with confirmed giardiasis in Austria revealed that trophozoites colonized predominantly the duo- denum (83% of cases), but were also found in jejunum (2%), ileum (12%), colon (0.4%), and stomach antrum (9%), suggesting that the relevant host-parasite interactions that lead to diarrhoea most likely occur in the proximal small intestine. The study also demonstrated that the mucosal response to infection was morphologically unremarkable, as 96% of cases displayed normal light-microscopic ap- pearance of the duodenal mucosa. In only a small percentage (4%) of acutely infected patients, moderate villous shortening and mild in- flammation of the lamina propria were observed in the duodenum. These findings indicate that acute giardiasis is not a classical inflam- matory infection as seen, for example, in amoebiasis or shigellosis. Consequently, increased production of inflammatory mediators, such as prostaglandins or interleukin 1, which can mediate diar- rhoeal responses in other enteric infections, is not likely to explain the diarrhoea in giardiasis. Prolonged Giardia infection can be asso- ciated with more pronounced villus shortening and significant in- creases in intraepithelial lymphocytes, as well mixed inflammatory cell infiltrates in the lamina propria of the small intestine, which may contribute to diarrhoeal symptoms, although these tend to become less prominent in protracted infections. Shortening of microvilli on the luminal surface of intestinal epithelial cells has been observed in small intestinal biopsies from patients and animals with giardiasis. In parallel, reduced activity of intestinal disaccharidases occurs in Giardia-infected human subjects and rodents. This functional en- zyme deficiency, which depends on intact immune responses to the parasite, might lead to osmotic diarrhoea (via the presence of undigested disaccharides in the intestinal lumen). Furthermore, intestinal hypermotility occurs in infected patients and animals, which may further contribute to an osmotic form of diarrhoea by reducing the contact time available for complete absorption of nu- trients from the lumen. Other potential mechanisms of diarrhoea may be operational in giardiasis. Electrophysiologic investigations of duodenal biopsy specimens from patients with chronic infection revealed epithelial barrier dysfunction, possibly related to down regulation of tight junction proteins and increased epithelial cell death. Concomitantly, sodium-dependent glucose absorption was impaired and active electrogenic anion secretion was found to be ac- tivated, suggesting that diarrhoea in human chronic giardiasis may be, in part, caused by a combination of leak flux, and malabsorptive and secretory processes. Giardia-induced barrier disruption has also been observed in several in vitro and animal models, giving further support to the notion that paracellular mechanisms may contribute to the infection-associated diarrhoea. Giardiasis is self-limiting in more than 85% of cases in non- endemic areas, strongly suggesting that effective immune defences exist. Consistent with this, experimentally infected rodents eradi- cate the infection over weeks to months. Furthermore, symptoms of giardiasis are much less severe in endemic than nonendemic Fig. 8.8.9.2 Scanning electron micrograph of three Giardia intestinalis trophozoites on a jejunal biopsy specimen from a patient with giardiasis. The dorsal surfaces of two trophozoites are visible (D), and the ventral adhesive

disc of the other trophozoite is shown (V). Courtesy of Dr Robert L. Owen; modified from Carlson JR, Heyworth MF, Owen RL (1984). Giardiasis: Immunology, diagnosis and treatment. Survey of Digestive Diseases, 2, 210–23, with permission.

section 8 Infectious diseases 1444 regions, suggesting gradual build-up of immunity. IgA produced in the intestinal mucosa and secreted into the intestinal lumen has long been considered a primary mechanism of adaptive host defence against the parasite. In human volunteers who were deliberately in- fected with G. intestinalis, an intestinal IgA response to the parasite occurred, as it does in experimentally infected animals. IgA directed against trophozoites can inhibit their attachment to the intestinal epithelium (mostly by randomly cross-linking trophozoites with each other, which presumably interferes with their proper attach- ment orientation and dynamics; specific parasite ligands or recep- tors for attachment that could be blocked by antibodies have not been identified), such that they become susceptible to peristaltic expulsion from the host. Trophozoite antigens recognized by IgA include structural proteins that show little or no variability in amino acid sequence between different Giardia isolates, and surface pro- teins whose amino acid sequences show considerable variability. Giardia-infected human subjects and mice also generate a serum IgG response against trophozoites, although it is not clear whether this contributes to parasite clearance. Despite the compelling nature of secretory IgA as a key antigiardial defence, several clinical and experimental studies have cast doubts on the general importance of IgA, and have explored alternative defence mechanisms against the parasite. Infection in humans and in mice leads to T lymphocyte- dependent increases in small intestinal motility, which can promote clearance of the parasite in the luminal bulk flow. Furthermore, anti- microbial peptides and nitric oxide can directly kill or inhibit the parasite, although their relative contributions to clearance of Giardia remain to be firmly established. Beyond direct effectors, several immune cells and regulators are known to be involved in antigiardial immune defence in animal models. Mast cells and CD4+ T cells, but not CD8+ T cells, are re- quired for clearing Giardia infection in mice. CD4+ T cells might act in part by controlling antigiardial IgA responses. Their functions are probably not related to classical T helper 1 cells or T helper 2 cell subsets, because their signature cytokines, IFN-γ or IL-4, play no role in immune defence. In contrast, IL-6 and IL-17 are important in Giardia clearance. IL-6 appears to act by promoting dendritic cell functions during infection, although it has many other activities, including activation of monocytes, enhancement of follicular helper T cell responses, and stimulation of B cell proliferation and antibody production. Clinical features Giardia infection can be asymptomatic (as shown by cyst excretion in the absence of symptoms) or cause clinical symptoms, ranging from abdominal pain, discomfort and distension, and watery diar- rhoea, to nausea and vomiting, and anorexia and weight loss. Other clinical features are a sensation of fullness, heartburn, flatulence, and steatorrhoea. In one study of adult patients in Austria, the fol- lowing frequencies of symptoms were observed: pain on abdominal palpation (52% of cases), abdominal discomfort (43%), sensation of fullness (41%), abdominal distension (38%), epigastric pain (38%), nausea and/or vomiting (36%), heartburn (27%), and diarrhoea (26%). In contrast, foul-smelling stools, anecdotally considered typ- ical for giardiasis, were observed in fewer than 6% of cases in that study. Micronutrient deficiencies can occur in giardiasis. Impaired absorption of iron and zinc has been observed in Giardia-infected patients, which can be associated with mild to modest anaemia. Occasional case reports have also found defects in vitamin B12 ab- sorption and megaloblastic anaemia, although several larger surveys reported normal serum vitamin B12 levels in Giardia-infected indi- viduals. Together, these findings suggest that no particular symp- toms allow reliable clinical recognition of giardiasis, demanding a high index of

suspicion if one or several of the possible symptoms are encountered in patients with gastrointestinal complaints. In immunologically competent persons, untreated giardiasis typ- ically lasts for several weeks to months, with symptoms that fluc- tuate in severity. Most patients clear infection spontaneously over extended periods even without treatment, although asymptomatic carriers exist, and re-infections are probably common in endemic regions. An outbreak of G. intestinalis infection in Norway in 2004 predisposed individuals who had been infected with the parasite to irritable bowel syndrome and chronic fatigue for years after the out- break, despite successful eradication of the parasite by treatment. G. intestinalis infection can be associated with other important long-term sequelae in children, including stunting, low weight, and impairment of cognitive functions, particularly in less developed countries and regions. Laboratory diagnosis The traditional approach to diagnosing giardiasis is detection of G. intestinalis cysts in stool samples by light microscopy, which is often referred to as the ova, cysts, and parasites (OCP) test. Samples can be examined directly as fresh smears, or preserved with a suit- able fixative (e.g. formalin-ethyl acetate) and stained with various dyes, such as trichrome stain. Cysts are recognizable by their oval shape and size of approximately 8 × 12 µm. Internal structures including four nuclei, axonemes, and median bodies might be vis- ible to varying degrees in the cysts. Trophozoites are not commonly observed in faecal samples, but are found in duodenal specimens collected during endoscopy or by a string test. Negative results in the faecal OCP test can be related to variable faecal parasite shed- ding, so repeated stool tests on 2–3 different days might increase the diagnostic yield, although it is generally not necessary to confirm a positive result. The standard OCP test can be performed under conditions of limited resources and is widely used, but the test has a relatively low sensitivity, is labour-intensive and time-consuming, and is highly dependent on operator skill and experience, so many clinical microbiology laboratories no longer offer the test routinely. Assay sensitivity can be increased by staining faecal samples with direct fluorescent antibodies (DFA) and immunofluorescence microscopy. Highly specific antibodies against abundant cyst wall antigens allow ready recognition of brightly stained cysts against a dark background of unstained faecal materials. DFA tests are moderately labour-intensive and have some degree of subjectivity, although less than standard OCP tests, and depend on access to a fluorescence microscope. Nonetheless, compared to the standard OCP test, the method has markedly improved sensitivity and spe- cificity, approaching 95–100% on both measures, and can be con- sidered the current standard in Giardia diagnostics. Enzyme-linked immunosorbent assay (ELISA) tests for detection of faecal G. intestinalis antigen(s) are available, and can be useful as an objective diagnostic technique that can obviate the need for faecal microscopy if there is a high initial suspicion of giardiasis. However, microscopy might be preferable over testing exclu- sively for G. intestinalis antigen(s) in cases of unspecified intestinal

 parasitic infections, particularly because combination DFA tests can be used to detect more than one parasitic species in the same test. While the specificity of ELISA tests is similar to DFA tests (>95%), reported sensitivities of 85–95% are slightly inferior to DFA tests, so stool antigen ELISA tests are less common in routine diagnos- tics. Serum antibodies are elicited by Giardia infection, but cannot distinguish between past and current infection, and are not used in clinical diagnostics. Detection of parasite-specific DNA or RNA in stool by nucleic acid amplification techniques is a relatively new diagnostic strategy with excellent sensitivity and specificity. Numerous protocols and technical approaches have been reported, but they all involve initial sample lysis and nucleic acid extraction, followed by a combination of hybridization-based gene enrichment, reverse transcription (for RNA targets), and one or two rounds of polymerase

chain reaction (PCR) amplification of pathogen-specific genes. PCR products are then de- tected by DNA-binding dyes, or with fluorescent microspheres coated with matching capture probes for hybridization. Specific methods differ in the lysis conditions, amplification parameters, and target genes, whose sequences must be sufficiently conserved to allow de- tection of different strains and isolates of the respective pathogen, but sufficiently different from other microbes to avoid false-positive results. A major advantage of PCR-based diagnostics is the ability to detect multiple (>20) relevant pathogens in one sample, increasing diagnostic yield and shortening the time to diagnosis. This is particu- larly useful for identifying species of pathogens that might not have been suspected as the cause of a patient's gastroenteritis symptoms. PCR-based technologies have outstanding sensitivity and specificity, although they can exhibit an increased risk of false-positive results due to their exquisite sensitivity (<10 organisms can be routinely de- tected). Even minor contaminations during initial sample collection or subsequent laboratory processing can pose problems, particularly in endemic regions where environmental contamination might be high. Furthermore, the need for highly specialized equipment and technical expertise limits the use to well-equipped clinical laboratories with stringent work practices. Nonetheless, PCR-based diagnostics are increasingly supplanting microscopic methods in the routine la- boratory diagnostics of giardiasis and other enteric infections. As an alternative to PCR-based nucleic acid detection, loop- mediated isothermal amplification protocols have more recently been developed. The initial assay steps, including meticulous sample collection, lysis, and nucleic acid extraction, are similar to PCR methods, and the technical development and validation of new assays is at least as challenging as it is for PCR-based methods. However, unlike PCR, loop-mediated isothermal amplification as- says do not require a thermal cycler for the amplification steps and can be performed with a water-bath or heating block at a constant temperature, a feature that can simplify use in resource-limited con- ditions. This technology has the promise to promote point-of-care diagnostics over a wide range of clinical settings, although it is not currently employed in routine laboratory testing. Treatment Table 8.8.9.1 summarizes the major drug regimens used in the treat- ment of giardiasis. The most commonly utilized drugs worldwide are members of the 5-nitroimidazole class, including metronidazole and tinidazole. Metronidazole, which was developed and approved for clinical use in the 1950s and 1960s, is typically given in three divided daily 250 mg oral doses for 5–10 days, and has a reported efficacy of 80–95%. More recently, tinidazole, first approved in 2004, has become a good alternative for giardiasis because of its efficacy (85–90%), tol- erability, and convenience (a single oral dose is recommended). All 5- nitroimidazole drugs are prodrugs whose microbial specificity is due to the requirement for reduction to toxic free radical intermediates by low redox potential reductive reactions present only in the anaer- obic target microbes including Giardia. The radicals that result from nitro drug reduction form covalently bonded adducts on microbial target molecules, leading to their inactivation. The specific molecular targets of 5-nitroimidazole drugs have not been defined in G. intesti nalis. In spite of the general efficacy of 5-nitroimidazole drugs, treat- ment failures in giardiasis are not uncommon (up to 20%), clinical resistance is proven, and in vitro resistance can be induced so that parasites grow in clinically relevant levels of metronidazole. Nitazoxanide is a nitrothiazole with broad-spectrum activity against intestinal parasites. Like 5-nitroimidazole drugs, it is a pro- drug that must be reduced to form toxic radicals, which inactivate various microbial target molecules. It is usually given in two daily 500 mg doses for three days, which is more convenient dosing than metronidazole, but has slightly lower efficacy (70–80%) than 5- nitroimidazole drugs and can also be impacted by metronidazole resistance. In a clinical trial involving children with diarrheal illness, nitazoxanide reduced symptom duration in those afflicted with giar- diasis as well as in those

without a microbiological diagnosis, further underlining its utility as a broad-spectrum antimicrobial agent. Benzimidazoles, such as albendazole and mebendazole, are gen- erally used to treat intestinal helminth infections. Albendazole is also effective in giardiasis, although its efficacy varies markedly (25–90%) depending on the dosing regimen. The drug binds to β-tubulin in G. intestinalis and blocks tubulin polymerization and hence microtubules assembly and normal parasite motility and at- tachment. Albendazole can be taken once daily for five days, making it more convenient than three-times-a-day metronidazole, and its antihelminth activity makes it an attractive agent for dual use Table 8.8.9.1 Major oral drug regimens for treating giardiasis Drug Dose Treatment duration Metronidazole 250 mg/dose, 3 x doses/day (adult) 5–10 days 5 mg/kg/dose, 3 x doses/day (paediatric) 5 days Tinidazole 2 g/dose (adult) Single dose 50 mg/kg/dose (paediatric) Single dose
(2 g maximum) Nitazoxanide 500 mg/dose, 2 × doses/day (adult) 3 days 100 mg/dose, 2 × doses/day
(age 1–3 years) 3 days 200 mg/dose, 2 × doses/day
(age 4–11 years) 3 days Albendazole 400 mg/dose, 1 × dose/day
(adult and paediatric >2 years) 5 days Paromomycin 500 mg/dose, 3 × doses/day (adult) 7–10 days 10 mg/kg/dose, 3 × doses/day (paediatric) 7–10 days Quinacrine 100 mg/dose, 3 × doses/day (adult) 5–7 days

section 8 Infectious diseases 1446 purposes. However, results from a Bolivian cohort study (a region with endemic Giardia and helminth infections) found that treat- ment with mebendazole reduced hookworm infections but para- doxically led to an increase in giardiasis, suggesting an antagonistic relationship between the two parasites and complicating the pro- spect of multitarget therapies. Quinacrine, an old malaria drug, is an acridine derivative that binds to DNA and interferes with transcription and DNA replica- tion in many target microorganisms, although different mechan- isms of action, including membrane breakdown and inhibition of critical enzymes, appear to be operating in G. intestinalis. The drug has excellent efficacy against giardiasis (c.90%). In a randomized trial in children with giardiasis, chloroquine was equally effective as tinidazole and superior to albendazole. Anecdotal reports also sug- gest that quinacrine can be effective when other drug regimen failed. Despite its efficacy, quinacrine has potentially severe adverse effects, including major psychiatric and dermatologic manifestations, and is no longer commercially available in the United States or Canada. Finally, paromomycin, an aminoglycoside antibiotic that inhibits bacterial protein synthesis by targeting ribosomal RNA, is active against G. intestinalis in vitro and in vivo. Although Giardia is an eu- karyote, its ribosomal RNAs display features that resemble those in susceptible bacteria (but not human cells), thus providing a poten- tial explanation for the antigiardial activity of paromomycin. Oral formulations of this antibiotic are not absorbed from the gastro- intestinal tract, which presumably accounts for the rarity of systemic adverse effects (such as nephrotoxicity and ototoxicity) compared to other aminoglycosides. Paromomycin is moderately effective against giardiasis, with reported response rates of 55–90%. It has also been used in cases of metronidazole-refractory giardiasis. Treatment failure and resistance of G. intestinalis to all common antigiardial drugs have been reported. For the most commonly used 5-nitroimidazoles, resistance has been observed in 1–20% of cases. Therapeutic strategies for treatment-refractory giardiasis include longer duration and/or higher doses of the original agent, switching to a different class of drug, or combination therapy. In a case series of ten patients who failed 5-nitroimidazole therapy, all were cured with one of the following combinations: metronidazole or tinidazole

- paromomycin + albendazole in three cases, metronidazole + paromomycin in two cases, tinidazole + paromomycin in two cases, tinidazole + quinacrine in two cases, and metronidazole + quinacrine in one case. All the drugs were administered for 7 or 10 days except for tinidazole, which was given for 1 to 7 days. The combinations were well tolerated and had no serious adverse effects. In another case report, a HIV patient with giardiasis was unsuccessfully treated five times with metronidazole and albendazole, but was cured with high and prolonged doses of nitazoxanide (500 mg twice a day for 10 days and then 1 g twice a day for 15 days). Susceptibility testing showed the strain to be resistant to metronidazole and albendazole, but sus- ceptible to nitazoxanide.
  Combination therapy generally decreases the risk of developing antimicrobial drug resistance, although it is presently not known how this concept applies to giardiasis. Increasing reports of drug resistance and the ever-present po- tential for new forms of drug resistance have prompted a con- tinuing search for alternative therapeutic agents against giardiasis. Methods to develop new drugs for treating giardiasis include: (1) modifying the structure of a historically effective drug, such as metronidazole, by attaching ‘novel’ side chains to the nucleus of the drug molecule, and testing the resulting compounds for improve- ments in antigiardial activity; (2) designing inhibitor molecules for enzymes (and metabolic processes) that are unique features of Giardia species, but not found in human cells (e.g. Giardia en- zymes involved in arginine metabolism and in cyst formation); and (3) empirical testing of chemical compound libraries for activity against Giardia. Efficient testing is achieved with high-throughput automated systems, such as multiwell microtitre plates containing cultured trophozoites, and by screening for the ability of candi- date drugs to kill the organisms in vitro and in animal models in vivo. Subsequent safety testing and clinical trials of drugs that show promise in vitro and in animals may then be warranted in human subjects with giardiasis. As an alternative to the development of spe- cific antimicrobials for individual treatment of a diagnosed infec- tion, dietary supplementation strategies may be helpful in reducing the incidence or clinical severity of giardiasis in cohorts of vulner- able individuals. For example, zinc supplementation was found to reduce the rate of diarrhoea caused by Giardia in children and was found to upregulate humoral immune response in Giardia-infected mice. Experimental studies also suggest that probiotic bacteria can alter susceptibility to G. intestinalis infection, although much re- mains to be learned about any consistent changes in the commensal microbiota during and after giardiasis, and systematic strategies to alter the infection dynamics by microbial interventions that do not directly kill the parasite. Prevention G. intestinalis cysts can be removed from water by adequate filtra- tion, for example using membrane filters with a pore diameter of less than 5 µm. Cysts in water are also killed by boiling or exposure to ultraviolet light. In contrast, cysts are relatively resistant to several common disinfectants, including chlorine- and iodine-based chem- icals. The most reliable water decontamination is achieved by com- binations of treatment methods, such as high-rate sand filtration, ultrafiltration, and ultraviolet disinfection. Complete inactivation or removal is particularly important for preventing the spread of the parasite, because G. intestinalis is highly contagious, as evidenced by experimental human infection after oral ingestion of as few as ten cysts. Water intended for human consumption can be screened for G. intestinalis by capturing and concentrating cysts with magnetic beads coated with antibodies against cyst antigens, followed by DFA assays or PCR tests of the concentrates. Viable and dead cysts re- trieved from water can

be distinguished by staining with fluores- cent dyes that selectively stain living or dead cysts. Without knowing the genotype of G. intestinalis cysts found in a water sample, it may be difficult or impossible to comment on the possible infectivity of such cysts to human subjects; this caveat applies, for example, to pre- 1990s literature documenting the presence of Giardia cysts in water, which predates awareness of G. intestinalis genotypes. If domestic pets and farm animals are sources of human giardiasis (a likely, though not rigorously proven, scenario), avoidance of unhygienic interactions with these animals would predictably help to avoid interspecies transmission of G. intestinalis. A human vaccine against giardiasis is not available. A crude vet- erinary vaccine (GiardiaVax), composed of total cell lysates of a mixture of sheep, dog, and human isolates, reduces symptoms and duration of cyst output in cats and dogs, suggesting that a human

8.8.9 Giardiasis and balantidiasis 1447 vaccine may be feasible. Several vaccine antigen candidates have been identified. The veterinary vaccine has also been used as an immunotherapeutic agent in dogs with chronic giardiasis that had failed standard drug treatment, raising the possibility that a future human vaccine may also be effective postexposure. Balantidiasis Aetiology Balantidium was first described by Malmsten in 1857 in the stool of two cases of dysentery in Sweden, and was given its current designation as Balantidium coli in 1863. It has long been con- sidered one of the rarest pathogenic organisms, with only 600 cases reported in the literature over a 100-year period until the 1950s. However, the parasite is now recognized as being more prevalent than earlier appreciated, partly due to more compre- hensive epidemiologic surveys, as well as the realization that many infected individuals are colonized but exhibit no overt dis- ease symptoms. B. coli, the cause of balantidiasis, is the largest protozoan and only ciliated parasite of man. The parasite has a two-stage life cycle comprised of nonmotile cysts and motile, replicating tropho- zoites (Fig. 8.8.9.3). Spread of the infection to new hosts occurs Fig. 8.8.9.3 Life cycle of Balantidium coli in humans. Transmission occurs by ingestion of cysts from contaminated food or water (step 2). Excystation occurs in the small intestine, and trophozoites colonize the large intestine (step 3), where they replicate (step 4) and potentially invade the mucosa. Trophozoites undergo encystation to produce infective cysts (step 5), which are passed with faeces (step 1). Reprinted from the Public Health Image Library of the Centers for Disease Control and Prevention, Atlanta, Georgia, USA; image generated by Dr Alexander J. da Silva and Melanie Moser.

section 8 Infectious diseases 1448 by the faecal-oral route via ingestion of the cyst form, which is presumably resistant to gastric acid during stomach passage, and release of the vegetative trophozoite form in the small intestine or colon. Trophozoites are large (30–150 µm) and ovoid in shape with a funnel-like depression (peristome, mouth) at the tapering anterior end, and a cytopyge (anus) at the rounded posterior end. The entire trophozoite surface is covered by numerous short (4–6 µm) hair-like cilia (Fig. 8.8.9.4), whose beating leads to a straight swimming motion combined with rotation around the longitudinal axis. Cilia in the peristomal region are elongated and have feeding functions. Trophozoites carry two nuclei, a large macronucleus and a small micronucleus, whose relative roles in metabolism, growth, and reproduction are poorly defined in B. coli. In other ciliates, macronuclei are typically polyploid and primarily re- sponsible for the metabolic cell functions in the vegetative stage, while micronuclei are diploid and contribute little during vege- tative metabolism, but play a key role in reproduction. Although the morphology of the parasites has been well characterized, not much is presently known about its biochemical and molecular biologic features. Selected DNA regions have been sequenced for taxonomic

purposes, but the entire genome sequence has not been reported to date. Epidemiology Humans are not primary hosts for B. coli (or any other intestinal ciliates), and the pathogen is maintained in populations of mam- malian reservoir hosts, yet the exact relationship between animal and human infections has not been established. Substantial cir- cumstantial evidence suggests that humans can acquire B. coli from animals, making it likely that the infection is zoonotic in origin. The parasite is frequently identified in pigs and several species of nonhuman primates, and more rarely in cows, donkeys, horses, and ostriches (but not cats, dogs, or rodents). Parasites from the faeces of infected humans can infect piglets, and cause severe diar- rhoea and mucosal destruction from terminal ileum to rectum, strongly implying that no species barrier exists between humans and swine in regard to B. coli infectivity and pathogenicity. A high prevalence of the infection has been seen in communities that live in close proximity to B. coli-infected pigs (e.g. in New Guinea and subtropical China). Consequently, it is assumed that pigs are an important reservoir for the spread of B. coli to humans. However, balantidiasis has also occurred in human subjects who had no known contact with pigs or other animals. It is possible that veget- ables and fruits grown on fields fertilized with contaminated animal manure carry the parasite, as suggested by identification of B. coli on field-grown strawberries in Brazil. In India, B. coli cysts have been found in water for drinking and cooking, and the organism has been identified on cockroaches in Nigeria and Ethiopia, and house flies in Egypt. Overall, the prevalence of B. coli infection varies considerably across the world, with the highest levels gener- ally in tropical and subtropical regions. Prevalences as high as 0.4% in the general population in rural Thailand, 1–5% in asymptomatic children in Bolivia, and 2.4% in children with diarrhoea in India have been reported. Infections are rarely seen in most developed nations, although clusters of B. coli infection have been described in institutional facilities such as psychiatric hospitals and prisons in the United States and Italy, and sporadic cases have been reported worldwide. Pathogenesis and pathology Once trophozoites are present in the intestinal lumen, they pri- marily interact with the host in the colon, where they can invade the mucosa and cause ulcerations that are accompanied by inflam- matory cell infiltrates (Fig. 8.8.9.4). The parasite is not known to produce toxins. It can probably break down extracellular matrix, and may be able to lyse host cells during invasion, but the detailed mechanisms responsible for tissue invasion and destruction are not known. It is also not clear why some infections cause marked tissue destruction and dysenteric symptoms, while most are asymptom- atic and presumably not accompanied by significant tissue damage. Differences in virulence genes of the parasite or host susceptibility to the actions of such genes are predictably important but remain to be investigated. Clinical features Human subjects infected with B. coli are commonly asymptomatic, but a subset of individuals can develop diarrhoea with stools that are either watery or consist of blood and mucus, resembling amoebic dysentery. In severe cases, patients can develop colonic perforation, peritonitis, gangrene of the appendix. Spread of the parasite to the liver or lungs can occur, although balantidiasis is only an exceed- ingly rare cause of liver abscess and of pulmonary haemorrhage. Severe colonic balantidiasis may be clinically indistinguishable from amoebiasis, bacillary dysentery, ulcerative colitis, and Crohn’s disease, and can be fatal if undiagnosed and untreated. B. coli in- fection in the lungs has been described in occasional patients with Fig. 8.8.9.4 Light micrograph of Balantidium coli trophozoite (arrow) in colonic tissue (×705). Cilia are visible on the surface of the organism (arrow). Arrowheads indicate tissue plasma cells. Modified from Neafie RC (1976). Balantidiasis. In: Binford CH, Connor DH (eds) Pathology of tropical and extraordinary diseases, vol. 1, pp. 325–7. Armed Forces Institute of Pathology, Washington DC, with permission.

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