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Giardia duodenalis: NEW INSIGHTS ON AN ANCIENT PARASITE

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Abstract- *Giardia duodenalis* (*G. lamblia, G. intestinalis*) is an enteric protozoan that infects human beings and a wide range of domestic and wild mammals. Giardiasis is considered a parasitic zoonosis by the World Health Organization, and they highlight the importance of animals as *Giardia* transmitters. This parasite is prevalent in areas with a temperate, humid climate, with heterogeneous distribution and the highest infection prevalences found in developing countries. The molecular characterization of *Giardia duodenalis* has permitted the identification of eight morphologically indistinguishable genotypes (A, B, C, D, E, F, G, H) with different host specificity. Several molecular studies have established the presence of genotypes A and B in both humans and animals throughout the world. The clinical presentation of giardiasis in humans is highly varying, ranging from asymptomatic manifestations to chronic diarrhea with malabsorption and childhood stunting. The significant aspects of *Giardia* infection are discussed in this review, and new progresses in topics such as taxonomy, biology, physio-pathogenesis, molecular epidemiology, zoonotic potential, clinical presentation of the infection and treatment of this parasite are emphasized.

Keywords- *Giardia*, genotypes, zoonoses, dogs, cattle, Argentina

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Introduction

Giardia duodenalis (*G. lamblia, G. intestinalis*) is an enteric protozoan infecting human beings and a wide range of both household and wild mammals. The clinical presentation of the infection in humans highly varies, ranging from asymptomatic manifestations to chronic diarrhea with malabsorption and childhood stunting [1]. This parasite has a significant impact on public health and in 2004 was included in the World Health Organization (WHO) Neglected Diseases Initiative.

Giardia is transmitted orally, via ingestion of cysts present in contaminated food or water, or through direct contact with infected people or animals [2]. Human infection shows higher incidence in the summertime, coinciding with the increase of recreational water activities [3]. The activities with a higher risk of sporadic giardiasis include traveling to endemic areas, consumption of untreated waters and raw vegetables, immersion in contaminated (lake or swimming-pool) waters, usage of latrines, close contact with infected people or animals, and inappropriate personal hygiene habits [3-6].

Giardia is a cosmopolitan parasite prevalent in areas with a temperate, humid climate, with an annual distribution estimated in 280 million cases [1,7]. The parasite shows a heterogeneous distribution, with the highest infection prevalences found in developing countries [7,8]. United States, Australia, New Zealand and Western Europe have reported under 8% rates of *Giardia* infection, and the most affected populations were children attending day-care facilities and travelers to endemic areas [2,9]. In developing countries, 200 million people have symptomatic giardiasis and 500,000 new cases are detected per annum [10]. The highest infection rates were found in vulnerable populations, such as inhabitants of irregular settlements, refugees and natural disaster victims [7,11-14]. In Asia and Africa, the prevalence of *Giardia* infection ranged between 2% and 73%. The highest values were those found in Nepal, Uganda, Thailand, Egypt and Kenya [2,15,16]. In Latin America, *Giardia* infection rates were 4-69%. This parasite was found in 15% of the rural population in the region, with a high prevalence in schoolchildren [7,17-19].

In Argentina, several studies have registered the prevalence of *Giardia* in humans. Infection rates in rural areas have reached 6-8%, being higher in urban areas. High infection rates have been registered in vulnerable communities in the country (precarious settlements, native communities) (2-70%) [7,20-31].

Imported *Giardia*: Impact of International Travel and Immigration

Traveler's diarrhea is a frequent gastrointestinal disorder (25-50%) in people traveling to developing countries in tropical and subtropical areas. When identifying the causal agent, bacteria were found to cause 80-85%, parasites cause 10%, and viruses cause 5% of infections. The risk of contracting an intestinal parasite in a trip is related to the length of the stay, hygiene, and socio-economical level of the destination country [32].

The etiology reports of Traveler's diarrhea showed *Escherichia coli* as the most frequent pathogen, present in approximately 30% of the cases. A recent review showed *Giardia* was present in 1.3 and 1.6%, respectively, of travelers with diarrhea heading to Latin America and Africa, as compared to 5.7 and 6.2% in travelers heading to South Asia and Southeast Asia [33].

A wide Swedish study in 25,000 tourists identified *Giardia* as a protozoan involved in Traveler's diarrhea, reaching rates higher than 30‰. This intestinal infection showed a varying incidence, and the most frequent destinations included India, Middle East, Southeast Asia and South America [34].

Diarrhea-related diseases are the greatest cause of morbidity and mortality among refugee populations (from wars or natural disasters) in several countries, included Sudan, Somalia, Ethiopia, Kenya, Iraq, Turkey, Honduras, and more. An African study found prevalences of *Giardia* of 20-40% in children in a refugee camp in Nigeria. Similar results were found in refugee populations in Serbia, Sierra Leona and Palestine [35]. An extensive study on migrant populations revealed the global risk of giardiasis was 1,180 per 100,000 immigrants and refugees, with particularly high values in immigrants from Afghanistan, Africa, Bulgaria, India, Pakistan and Russia [32].

Molecular Epidemiology of *Giardia*

The genus *Giardia* includes 6 species that can infect a wide range of hosts *G. duodenalis* (humans and mammals), *G. agilis* (amphibians), *G. ardeae* y *G. psittaci* (birds), *G. muris* y *G. microti* (rodents) [36].

Giardia duodenalis is a species complex including eight morphologically indistinguishable genotypes (A, B, C, D, E, F, G, H) with different host specificity [Table-1] [1,2,37-39]. Recently, some investigators have proposed species-specific names for the different genotypes of *G. duodenalis* [Table-1] [40,41]. Genotypes A and B of *G. duodenalis* have been the only genotypes involved in human infection [2,8,10,42].

Table 1- The currently recognized genotypes of Giardia duodenalis and their host distribution [2,9,74].

Genotypes (proposed species name)	Host distribution
A (*G. duodenalis*)	Humans and other primates, livestock, dogs, cats and some species of wild mammals
B (*G. enterica*)	Humans and other primates, livestock, dogs, cats and some species of wild mammals
C/D (*G. canis*)	Dogs and other canids
E (*G. bovis*)	Hoofed livestock
F (*G. cati*)	Cats
G (*G. simondi*)	Rats
H (?)	Marine mammals

Several molecular studies have established the presence of genotypes A and B in humans throughout the world [Table-2]. Genotype B was prevalent in several countries in the Americas, Asia, Europe and Oceania, while genotype A was only prevalent in Africa. Predominance of one genotype in a study area has been attributed to numerous biological and geographical factors. Various researchers suggest that human migrations, the presence of zoonotic transmission cycles and the presence of endemic focal points would act as factors prevailing in the distribution of *Giardia* genotypes [10,43,44].

Our research group has detected a high endemicity of genotype B *Giardia* in humans in Argentina and a smaller proportion of genotype A [Table-2] [45-48]. Our results are in accordance with those found in numerous countries such as Nicaragua, Belgium, Holland, France, Norway, United Kingdom, Bangladesh and Australia, and in contrast with those found in India, Turkey and the United States, where a predominance of genotype A is observed [2,43,49].

Table 2- Giardia duodenalis infection rates and genotypes detected in human

Location (s)	N° of samples with genotype A	N° of samples with genotype B	References
Albania	10	12	[51]
Argentina	14	77	[46,47]
Australia	40	126	[42,106,122]
Bangladesh	20	231	[104]
Belgium	18	54	[118]
Brazil	81	8	[62]
Canada	9	9	[123]
China	16	10	[122]
South Korea	5		[122]
Spain	43	61	[107]
United States	14	2	[38]
Ethiopia	31	13	[56]
France	9	16	[124]
Holland	43	75	[72]
India	11	17	[38,57]
Italy	80	59	[43,64,70]
Mexico	40		[70]
Nicaragua	25	94	[125]
Norway	3	81	[55]
New Zealand	24	11	[59]
Peru	82	106	[18,107]
Portugal	32		[126]
United Kingdom	57	166	[37]
Thailand	35	39	[127]
Turkey	19	25	[49]

Direct transmission of *Giardia* through drinking water has been well documented [50-52]. The parasite has also been found in untreated waters (12-94%), superficial waters (30-98%) and swimming pools (6-96%) in various European countries [3]. In Argentina, this parasite was found in water for human consumption and recreational water in several regions in the country [50,53,54].

Our group found an absolute prevalence of genotype B *Giardia* in a rural area of the province of Buenos Aires, significantly associated with consumption of water from wells and indicating a possible hydrological transmission of genotype B [47]. Besides, prior studies had detected the presence of *Giardia* cysts in animal samples, soil samples and samples of water for consumption in the same town [6,20]. These results match the detection of the same genotype in drinking water that caused the greatest hydrologic outburst of giardiasis in Norway [55]. In rural areas, rains cause agricultural run-off, which can contaminate superficial waters with animal feces. Concentration of *Giardia*-infected cattle around water sources and extended survival of cysts in the environment might contribute to the hydrologic transmission of the parasite [3].

Occurrence of mixed infections has been reported in several countries such as Australia, England, India, Italy and Ethiopia, with percentages ranging between 2% and 21%, being higher in less economically developed countries [2,43,56,57]. In Argentina, prevalence of mixed infection reached 3% in children from an urban area [47]. Infections produced by more than one genotype reflect the complex circulation of the parasite in the environment and indicate the exposure of hosts to multiple infection sources [56].

Zoonotic Potential of *Giardia*

Giardia in Dogs and Cats

The WHO has considered giardiasis a parasitic zoonosis, highlighting the importance of pets as *Giardia* transmitters. Prior studies have determined that parasitic transmission between different hosts increases in locations with high population density or by close contact between people and animals [43]. Consequently, detection of zoonotic genotypes in household and breeding animals turns out to be of great significance for public health [10,44,58-60].

Giardia is a frequent parasite in dogs and its prevalence can exceed 80% in young animals [Table-3]. Dogs may be infected with species-specific parasitic genotypes (C/D) or zoonotic genotypes (A/B). Canine infection prevalence with A and B genotypes varies greatly, with rates between 4% and 87% [61]. Genotype A was reported in dogs in Germany, Australia, Brazil, Canada, Italy and Mexico, while genotype B was observed in dogs in Belgium, India and Thailand [2,61-65].

Table 3- Giardia sp. infection rates in farm animals and dogs

Type of animal	Country	Number of animals	Prevalence (%)	Reference
Bovine cattle	Argentina	70	40	[128]
	Belgium	499	22	[12]
	Canada	386	73	[129]
	Colombia	308	37	[130]
	Denmark	518	43	[131]
	Spain	734	57	[132]
	Spain	734	40	[132]
	United States	2943	20	[133]
	United States	456	52	[134]
	New Zealand	715	41	[135]
Caprine cattle	Belgium	148	53	[12]
	Brazil	105	14	[136]
Ovine cattle	Belgium	137	36	[12]
	Canada	89	38	[129]
	Spain	466	19	[132]
Porcine cattle	Canada	236	9	[129]
	Denmark	504	38	[131]
	Norway	684	2	[137]
Dogs	Argentina	106	15	[29]
	Chile	582	4	[138]
	Colombia	270	81	[128]
	Peru	385	42	[139]

In Latin America, the canine population exceeds 45 million, with an average dog-human ratio of 1 dog per 10 inhabitants. In Argentina, the number of dogs has been estimated in 9 million. In the city of Buenos Aires, more than 400,000 dogs have been reported, with a ratio of 1 per 3-4 inhabitants [66,67].

The countries in the Americas with studies on *Giardia* genotypes in dogs are Argentina, Brazil, United States, Mexico and Nicaragua. Genotype A was found in 60% of *Giardia* parasite-infected dogs, genotype B in 10% and genotypes C/D in 30% [68]. In Argentina, our group has detected dogs infected with genotype B *Giardia* in two towns in the province of Buenos Aires [46,48]. This genotype was also found in dogs in the province of Santa Fe [69].

There are limited reports indicating the presence of the same genotype in humans and dogs living together in close contact, while there are only two reports among them suggesting independent transmission cycles. As a consequence, the limited number of studies prevents final conclusions to be drawn about the role of dogs in the zoonotic transmission of *Giardia* [46,57,60,62,70-72].

Giardia has been detected in cats throughout the world, with prevalences of 2-80%. These animals may be infected with a species-specific genotype (F) or zoonotic genotypes (A/B) [73]. Zoonotic genotypes in cats have been reported in various studies conducted in Germany, Australia, Canada, Spain, United States, Italy, Japan, Poland and Sweden [65,73-75].

Giardia in Farm Animals

Giardiasis in beef cattle has shown infection rates varying throughout the world [Table-3]. Calves show the highest excretion of *Giardia* cysts (10^5-10^6 cysts/gram of feces) and infection patterns are similar in dairy and beef cattle. They are usually asymptomatic infections but may produce diarrhea and weight loss, thus causing losses in production and economy [2,10].

Cattle may be infected with species-specific genotype E or zoonotic genotypes A and B. Genotypes E and A have been prevalent in beef cattle in Australia, Belgium, Canada, Denmark, United States and Portugal [76,77]. A multi-center study conducted in four European countries Germany, France, Italy and Great Britain- showed a high prevalence of genotype A (45%) in beef cattle. Genotype B in cows has been reported in few studies. In China and New Zealand, beef cattle were found to be infected with genotypes A and B alone, thus indicating cows would act as a zoonotic reservoir of *Giardia* [74].

A recent study from our group found humans and calves infected with genotype B in a rural area in the province of Buenos Aires, Argentina [48,78]. The presence of the same genotype in humans and beef cattle is in accordance with similar studies conducted in Italy and New Zealand, and suggests the presence of a rural zoonotic cycle [10,59,70].

The zoonotic transmission between humans and cattle has been proposed in numerous research studies. In India, a recent study identified genotype A in beef cattle and rural workers in the same dairy farms, suggesting the transmission occurs through direct contact with the animals or through contamination of superficial or drinking waters [2,74].

Ovine and caprine cattle show giardiasis rates close to 40% in young animals [79]. Both sheep and swine are mainly infected with genotype E. However, various studies reveal the presence of genotype A in those animals in Australia, China, United States and several European countries. In contrast, genotype B was detected in few studies in sheep and goat in China, Norway, Italy and Spain [74]. A recent report from Malaysia showed the predominance of genotype E in goat, with less presence of zoonotic genotypes A and B [79].

Giardiasis in porcine cattle has been reported worldwide in swine of all ages with rates 1-30%. Porcine cattle is generally infected with genotype E. Only five studies, in Australia, Canada and Europe, revealed the infection of swine with genotypes A and B [43,74].

Giardia in Wild and Captive Animals

Wild animals (beavers, deer, chinchillas, coyotes, cats, ferrets, wolves, Patagonian maras, marsupials, bears, peccaries, primates) and sea mammals (seals, dolphins) are susceptible to infection with zoonotic genotypes of *Giardia.* Various authors suggest this fauna would have a role as reservoir and source of infection for humans and other mammals [10,74].

Animals in captivity can also be infected with zoonotic genotypes of *Giardia*. There have been several reports of the presence of geno-

type B in lemurs and anteaters in zoos from Italy and Poland. The most frequent routes of transmission would be consumption of fresh vegetables or water, or direct contact with animals infected with the parasite [51,74].

Parasite Morphology

Cyst

The cyst of *Giardia* is elliptical *(*8-12µm by 5-9µm), and may contain 2 or 4 nuclei, axonemes, ribosomes, vacuoles, and fragments of ventral disk. The cyst wall is 400nm thick and is made of protein (40%) and carbohydrates (60%). This wall contains fibrils of a homopolymer of N-acetylgalactosamine (N-AcGal) and at least three cyst wall proteins (CWP1, 2 and 3) [36].

The three CWPs are proteins showing high contents of cysteine, several leucine-rich repetition regions (LRR), a preserved and cysteine-rich C-terminal region (CRR) and several glycosylation and phosphorylation sites [80,81]. The genes in the CWPs are transcribed only during encystation, an increase of 140 times the transcribed levels being detected as compared to the non-encysting trophozoites [80,82,83]. A fourth cyst wall protein (HCNCp) has just been discovered [80,81]. Molecular studies have shown this HCNCp protein is also positively regulated during encystation and is part of the cystic wall [84].

CWPs and HCNCp are concentrated in the encystation-specific vesicles (ESV), released in the encysting trophozoite membrane through exocytosis, and exposed to high concentrations of calcium, and they make up the filamentous structure of the cystic wall [82,83].

N-AcGal sugar is produced by a metabolic pathway induced during encystation. Glucosamine-6-P-isomerase (Gln6PI) catalyzes the fructose-6-phosphate to glucosamine-6-phosphate conversion. This enzyme has two forms, one with constitutive expression (Gln6PI-A) in low levels, and the other inducible during encystation (Gln6PI-B). Another enzyme in the same metabolic pathways (UDP-acetylglucosamine-pyrophosphorylase) is allosterically regulated by a product of the path, Glucosamin-6-phosphate, showing an increase of six times its catalytic activity [36].

The mechanism by which carbohydrates join the wall is not quite clear. A recent study showed that the LRR region of CWP1 shows lectine activity joining N-AcGal fibrils to the cyst wall. The authors suggest the formation of the wall involves a coordinated process of synthesis, transport and polymerization of CWP1 and N-AcGal [85].

Trophozoite

The trophozoite is piriform (12-15µm by 5-9µm) and has two transcriptionally active nuclei, an endoplasmic reticulum, lysosomal vesicles and encystation-specific vesicles (ESV) [86]. The parasite has 4 pairs of flagella (anterior, posterior, caudal and ventral) carrying out motility functions and a ventral disk involved in its adhering to the intestinal epithelium [36]. *Giardia* does not have a defined endosomal system but has peripheral vesicles involved in the phenomena of endocytosis, degradation, recycling and secretion of proteins during parasite growth and differentiation [87].

The *Giardia* genome is estimated in $1.34x10^8$ base pairs (bp), organized in 5 lineal chromosomes with sizes between 1.6 and 3.8Mb. Chromosome 1 shows 4 size variants due to the varying number of copies of the DNAr gene. Nuclear and cellular ploidy of the *Giardia* genome varies over the diverse stages of the cellular cycle. During vegetative growth, the trophozoite genome varies from diploid (2n) to tetraploid (4n), the haploid genome being n. When the cyst starts to form, the trophozoite takes two consecutive rounds of chromosome replication with no cellular division, giving rise to 4 nuclei with a ploidy of 4n each. During excystation, the trophozoite splits twice and produces 4 cells with two diploid nuclei [36,88,89].

The ventral disk is a unique structure containing proteins such as actinin, myosin and tropomyosin. These contractile proteins are the biochemical basis of the disk contraction for its adhesion to the intestinal epithelium. This adhesion depends on the active metabolism of the parasite and is inhibited by temperatures under 37ºC, oxygen increase or reduced cysteine concentration. Ultrastructurally, the ventral disk contains groups of microtubules attached to the membrane. These tubules form the dorsal borders extending perpendicular to the membrane and are made up of proteins called giardins [36].

Giardins are structural proteins exclusive of *Giardia*, associated to the microtubules of the ventral disk and the axostyle. These proteins show an alpha helix structure with molecular weight between 29 and 38 kDa. Subsequent studies of giardins show the presence of several proteins such as α1-giardin, α2-giardin, β-giardin y γ-giardin. All giardins are constantly expressed during the parasite life cycle. These antigens are believed to be the first detected by the local host immune system. However, the role they play in the acquisition of immunity has not been reported [90,91].

Tubulins are proteins present in the microtubules in the ventral disk and flagella. Five isoelectrical variants of these proteins have been identified -two α-tubulins and three β-tubulins, of 54 and 58 kDa, respectively. Besides, the γ-tubulin has been recently identified, using monoclonal antibodies towards the C-terminal end of the human γ-tubulin [36,90,91].

The trophozoite contains around 200 genes codifying for a heterogeneous family of proteins called variant surface proteins (VSPs). These VSPs are comprehensive membrane proteins, rich in cysteine (11-12%), whose molecular masses range between 30 and 200 kDa [80]. The VSP genes are distributed among the 5 chromosomes and correspond to approximately 2% of the genome [9,36].

Biological Cycle

Infection in the host starts with the entry of *Giardia* cysts per os (infectious dose, 10-100 cysts). Excystation is produced in the duodenal-jejunal segment, after contact with the acid pH in the stomach. Recent studies have suggested this process occurs in at least two stages. First, the cyst is exposed to intestinal proteases degrading the CWPs and weakening the cyst wall, and after that several parasite enzymes such as cysteine protease and glycohydrolases act [81].

The released trophozoites cross the mucus barrier, adhere to the intestinal epithelium through their ventral disk, absorb nutrients via endocytosis and multiply through binary fission. The formation of cysts is induced in the presence of certain stimuli such as absence of cholesterol, a lipid essential for the synthesis of the trophozoite membrane. Encystation starts with the biogenesis of the cystic wall, a coordinated process with 3 stages, i) encystation stimulus and regulation of specific gene expression; ii) CWP synthesis, intracellular transport and secretion; and iii) extracellular assembly of the cyst wall [36,84].

The genes regulating encystation have not been fully studied. However, several encystation-specific genes have been characterized and they have been shown to be positively regulated with identical

kinetic properties, indicating thus a regulation at the transcription level [84]. Finally, the cysts are eliminated with the feces, completing thus the biological cycle by infecting the new host.

Physiopathogenesis of Giardiasis

The physiopathogenesis of giardiasis is a multi-factor process involving several molecular mechanisms not yet totally clear. The initial event in the infection is the adherence of trophozoites to the intestinal epithelium. This process increases the cellular apoptosis, the rupture of tight junctions in enterocytes and transepithelial permeability [9,92,93].

Apoptosis is a physiological mechanism that allows the renewal of enterocytes with no loss of intestinal epithelium integrity. *In vitro* studies have shown *Giardia* can induce enterocyte apoptosis in cell cultures through significant activation of pro-apoptotic genes and formation of intracellular reactive oxygen species (IROS) [92-94].

This apoptotic process favors the loss of intestinal barrier function. *In vitro* observations have shown that *Giardia* increases transepithelial permeability during intestinal colonization. There are various molecular mechanisms involved in this phenomenon, including rupture of proteins from the zonula occludens (ZO-1), F-Actin filaments and alpha-actinin [95].

The enterocyte brush border provides an extensive surface for nutrient absorption. The reduced absorptive area alters the levels of disaccharidases such as sucrase, maltase and lactase, essential enzymes for the appropriate digestion and absorption of sugars. Several agents, both infectious (rotavirus, HIV) and non-infectious (celiac disease, iron deficiency or vitamin A deficiency), are associated to this phenomenon. Studies in infection models (in humans, mice and gerbils) have determined that *Giardia* can reduce the brush border and the activity of disaccharidases [96].

The diffuse shortening of intestinal microvilli is mediated by the host immune response, CD8+ T lymphocytes in particular. This process leads to malabsorption of glucose, sodium and water; chloride hypersecretion; and reduced disaccharidase activity [9,93,95].

This disaccharidase deficiency contributes to diarrhea and nutrient absorption. A recent study revealed that mice infected with genotype B *Giardia* (GS) lacking CD4+ T lymphocytes showed no reduced disaccharidases, clearly indicating that the cellular immune response contributes to the pathology of giardiasis. On the other hand, the authors did not found such reduction when the mice were infected with genotype A *Giardia* (WB). These data suggest the disaccharidase deficiency would be a genotype-dependent phenomenon [96].

The protective role of intestinal flora on *Giardia* has been proposed by several researchers. Experimental *in vivo* and *in vitro* studies have revealed that *Lactobacillus* has antagonistic effects on *Giardia* [97,98]. The differences in microbial communities could be a determining factor in intestinal parasitic colonization. A comprehensive study of intestinal microbiome will permit to know the composition of species and microbial diversity patterns that could be involved in clinical variability of the infection [99].

Host-Parasite Interaction

The *Giardia* trophozoite inhabits the host's intestine and must adapt to diverse abiotic factors such as pH, redox potential, nutrient availability, oxygen tension, as well as biotic factors such as gut microbiota, mucus layer, defensins produced by Paneth cells, intestinal proteases and other intestinal parasites. Also, the host provides a variety of active mechanisms against *Giardia*, such as T lymphocytes, mastocytes, dendritic cells, antibodies and cytokines [9,36].

Giardia has several factors inducing immunity in the host that favor immune evasion and are able to contribute to the pathogenesis of the infection. The parasite produces enzymes such as thiol-proteases and Arginine deiminase (ADI). Thiol-protease affects the immunity of mucous membranes because it cleaves immunoglobulin A in the host. Arginine deiminase inhibits the production of nitric oxide (NO) because it catalyzes the hydrolysis of arginine to citrulline. This amino acid is the substrate necessary for the synthesis of NO, a highly reactive free radical, with antimicrobial activity against bacteria and parasites. *In vitro* studies have shown that NO inhibits growth, encystation and excystation of *Giardia*, not affecting parasite viability [9,84,87,97].

Giardia has two important adaptive mechanisms to survive both inside the host antigenic variation- and outside the host-encystation [84,100].

Antigenic variation, or switching, consists of the continual switch of certain surface proteins in the trophozoite (VSP). Such spontaneous variation occurs both *in vitro* and *in vivo* and it is produced every 6-16 parasite generations [80]. The molecular mechanism involved in the antigenic variation is not fully clear. Unlike other parasites like *Trypanosoma*, the antigenic variation is not directly associated to genomic rearrangements of VSPs. Recent evidence suggests that the mechanism of post-transcriptional regulation of RNA interference is involved in the control of VSP expression. Recent studies have shown that genotypes A and B differ in antigenic-shift rate in VSPs, gene repertoire of VSPs, and occurrence of this phenomenon during encystation and excystation. This phenomenon favors the parasite survival in the intestinal tract, contributes to evading the immune system and increases the diversity of infection to a higher host range [36,80].

Encystation is the second adaptive mechanism involving the formation of a cyst wall resistant to environmental conditions and ensures transmission to susceptible hosts. *Giardia* cysts stay infective in the environment for prolonged periods. At low temperatures (4ºC), cysts remain viable for 49 days in the soil, 56 days in lake water, 84 days in river water, and 65 days in sea water. However, at higher temperatures (20-28ºC) cysts become less infectious faster - 7 days at 25ºC in soil and 14-28 days in water [2].

Clinical Presentation

Giardia produces infections of varying clinical spectrum from asymptomatic manifestations to chronic diarrhea with malabsorption. The more frequent set of symptoms include diarrhea, abdominal pain, flatulence, anorexia, vomiting, weight loss and asthenia [10]. The signs and symptoms of giardiasis usually appear within 7 to 14 days of being exposed to the parasite, although the prepatent period may range from 3 to 25 days. Occasionally, the infection may be associated with extra-intestinal manifestations such as pruritus, uveitis, synovitis, food allergies, fatigue, stunting and impaired nutritional state [101].

Chronic infection usually appears together with diarrhea and intestinal malabsorption, leading to deficiencies in lactase, vitamin A, vitamin B12 and folate [98]. Analysis of duodenal biopsies obtained from patients with chronic giardiasis has revealed a reduction of the epithelial resistance and increase of cellular apoptosis. Reduced expression of a tight junction (claudine 1), increase of anion secretion and glucose absorption alteration would be the mechanisms

responsible for intestinal dysfunction [93,101].

The two components of the host-parasite relationship may have a role in the clinical presentation of the infection. The host factors include variables such as age, immunologic status, prior history of exposure, diet and concurrent gut microbiota, and the parasite factors -probably associated with genotype (A or B)- include multiplication rate, variant surface proteins (VSPs), drug resistance and parasite immune evasion strategies [9,101].

Host defenses against *Giardia* involve innate immunity (toll-like receptors and complement) and acquired immunity (humoral and cellular) mechanisms. Essays in murine models have shown the crucial role of immunoglobulin A in giardiasis control. Also, hosts with hypogammaglobulinemia (both infantile and common variable) have shown a higher predisposition to symptomatic giardiasis and chronic diarrhea [98,101].

A study conducted in India found that children with persistent *Giardia* infection show low concentrations of immunoglobulins A and G. Similar results were observed in Sweden, where 40 people were found with persistent giardiasis and undetectable IgA levels [36,101]. Other authors have indicated that *Giardia* infections have a higher incidence in children under five years of age probably due to their immune system not being completely developed [9,36].

Several studies have suggested that the variability of symptoms of giardiasis would be mediated by the host immune response. A longitudinal study in endemic areas of Brazil indicated that individuals previously infected with *Giardia* were less prone to symptomatic reinfection than individuals who were never exposed to the parasite [102]. Our research group has observed that the frequency of symptomatic giardiasis in schoolchildren in the province of Buenos Aires diminished as age increased [47,48]. The results from these studies suggest that immunity to parasites would build gradually, with the highest complications being detected in children of younger ages [103].

Genotype-Clinical Presentation Relationship

Symptoms of *Giardia* infection vary greatly and some individuals may eliminate cysts with their feces and show no symptoms, indicating that host factors and parasite factors might be related.

Several researchers in Europe, Asia and South America have found a correlation between genotype and symptomatology, while other authors have observed no association [8,37,49,56,92,104-108]. Both *Giardia* genotypes (A and B) are capable of causing symptomatic infection in humans, and the same genotype has been reported in different sets of clinical signs [101,109]. Recently, substantial genomic variations between *Giardia* genotypes A and B have been found that might explain some differences in the clinical presentation [2,3,10,110].

In Nepal, a strong correlation between genotype B *Giardia* and symptomatic infection was found, while asymptomatic individuals were infected with genotype A [111]. In Argentina, genotype B *Giardia* was significantly associated to the presence of abdominal pain, while the presence of diarrhea was independent from the infecting genotype [47]. The significant association between genotype B and presence of symptoms was also reported by authors in Turkey, Ethiopia, Cuba, Malaysia and Saudi Arabia [56,112-115].

On the other hand, a study conducted in Australia revealed that children infected with genotype A had 26 times more probabilities to have diarrhea than children with genotype B [106]. Similar results were observed in Bangladesh, Spain and Portugal, with a significant association between genotype A and symptomatic infection being reported [104,107]. Unlike the above mentioned studies, some reports have not found a correlation between genotype and clinical presentation of giardiasis [102].

Diagnosis of *Giardia* Infection

Microscope examination (morphology and morphometry) of fecal samples is the most commonly used lab procedure for diagnosis of intestinal parasite infections [9]. In recent years, new methodologies have been developed to diagnose *Giardia*. Immunofluorescence (IF) tests with monoclonal antibodies recognize epitopes in the cyst surface and are specially used for environmental samples [9,36]. Enzymatic immunoassays (ELISA) use antibodies that recognize *Giardia* antigens in fecal samples without preservative. These methods detect one or several trophozoite antigens and parasite cysts. The ELISA capturing the GSA65 antigen has shown greater sensitivity and specificity than microscopic study of fecal samples [9,90].

Immunochromatography methods (IC, dipsticks) provide a faster and more convenient diagnostic alternative in feces with no preservative. Several studies have reported similar sensitivity of *Giardia* detection in feces through IF and ELISA, and noticeably greater than the sensitivity shown by IC, particularly in feces with low *Giardia* cyst count [9,116-118]. Molecular methods have shown exquisite sensitivity and specificity. Several authors have shown that polymerase chain reaction (PCR) increases 10^4 times detection sensitivity as compared to IF [117]. The absence of differential morphological features of the parasite makes its identification at species level difficult; therefore, only molecular techniques allow the identification of zoonotic genotypes of *Giardia* [2,9].

Most molecular studies of *Giardia* have used a single locus to assign parasite genotype. The sensitivity of the loci used to identify *Giardia* genotypes shows variations. Some loci represent more preserved genes (*ssu-rRNA*), while others present more variations (*beta-giardin, triose phosphate isomerase, glutamate dehydrogenase*). The main reason for differential sensitivity is the elevated number of copies of the rDNA gene in the *Giardia* genome [36,43].

More recent studies have started analyzing several loci of *Giardia* for genotype determination. The multilocus sequence typing (MLST) method is a molecular biology technique permitting the typification of multiple loci in a microbial genome. The procedure characterizes each isolation through magnification and sequentiation of DNA internal fragments (450-500 pb) in multiple genes (housekeeping genes). The different sequences obtained in each gene are assigned as distinct alleles and the allele set will define the allelic profile or typical sequence of each *Giardia* isolate [9,119].

Parasite Metabolism

Giardia is an amitochondriate eukaryote, with no Krebs cycle or electron transport chain. Final products of the anaerobic metabolism are acetate, ethanol, alanine, hydrogen and CO_2. In eukaryotes, the enzyme catalyzing the conversion of pyruvate to acetyl coenzyme A is pyruvate dehydrogenase. However, in *Giardia*, such function is catalyzed by the pyruvate ferredoxin oxidoreductase (PFOR) enzyme using ferredoxin instead of NAD as electron acceptor [9,36].

Giardia has a glycolytic and fermentative metabolism and lacks the metabolic pathways for the *de novo* production of lipids, purines and pyrimidines. The metabolism of trophozoites is noticeably af-

fected by small changes in oxygen concentration. In strictly anaerobic conditions, alanine is the main product of carbohydrate metabolism. Even adding minimal quantities of O_2 (0.25mM), the production of ethanol is stimulated, while the production of alanine is inhibited. At O_2 concentrations of 0.46mM, the production of alanine is completely inhibited, and acetate and CO_2 are the metabolism predominant products. These oxygen concentrations are probably relevant for the intestinal environment, where trophozoites replicate since oxygen concentration in this environment ranges from 0 to 60 mM [9,36].

Treatment

Giardiasis has historically been treated with mercurials, arsenic products and bismuth. Quinacrine (QUI, a drug derived from acridine) was the first effective drug against the parasite. The drug is interspersed in the DNA of *Giardia* and inhibits nucleic acid synthesis. Laboratory studies have revealed QUI to show viability and excystation rate reduction [101].

Resistance to this drug has been induced *in vitro*, and was correlated to the reduced parasite absorption of the drug. QUI is rapidly absorbed in the host's intestinal tract and is widely distributed among tissues in the body. Clinically, QUI is very efficient; however, its diverse side effects have reduced its usage, particularly in children [120].

The current therapeutic strategy for giardiasis includes numerous antiparasitic agents such as 5-nitroimidazole (metronidazole/MTZ, tinidazole/TIZ, and analogs), nitrofurans (furazolidone/FUR), 5-nitrothiazole (nitazoxanide/NOX), benzimidazole (albendazole/ALB, mebendazole/MEB) and aminoglycosides (paromomycin/PAR) [Table-4].

Table 4- Current antigiardial agents [9,101,121]

Antigiardial agents	Efficacy* (%)	Adverse effects reported
Metronidazole	36-100	Gastrointestinal discomfort, metallic taste, headache, vértigo, insomnia, irritability, neuropathy, seizures, rash, leukopenia.
Tinidazole	74-100	Better tolerated than metronidazole rare: hepatitis and colangitis.
Quinacrine	84-100	Vomiting, bitter taste, nausea, headache, urticaria, exfoliative, dermatitis, exacerbation of psoriasis. Haemolysis in G6PD-deficiency.
Furazolidone	20-92	Nausea, vomiting, diarrhoea. Haemolysis in G6PD-deficiency and Interaction with MAO inhibitors. Haemolytic anaemia in neonates.
Albendazole	35-96	Nausea, vomiting, diarrhoea, epigastric pain.
Mebendazole	42-86	Transient abdominal pain.
Paromomycin	40-91	Gastrointestinal discomfort.
Nitazoxanide	64-94	Abdominal pain, diarrhoea, vomiting, headache.

The mechanism of action of the main drugs against *Giardia* is based in cell damage by two types of reactive species free radicals splitting the DNA threads and oxidizing biological membranes-, or reactive electrophile species forming covalent bonds with the N groups in DNA and proteins in parasites, and producing cell damage. The transference of electrons to the nitro group may be direct, through parasite enzymes (nitazoxanide), NADH oxidase (furazolidone), or indirect, through parasite proteins depending in turn on parasite enzymes to be reduced (metronidazole and nitazoxanide) [101].

The drugs more commonly used to treat giardiasis are 5-nitroimidazoles. MTZ is an antimicrobial agent with a broad spectrum of activity against anaerobic bacteria and protozoa. The drug enters the trophozoite where it is reduced, generating a toxic nitro radical which covalently binds to DNA, causing loss of helicoidal structure and thread rupture. The drug uses the anaerobic metabolic pathways present in *Giardia* and acts as a terminal electron acceptor [9,36,101].

Resistance to MTZ has been induced *in vitro* and correlates to the negative regulation of pyruvate ferredoxin oxidoreductase (PFOR) and ferredoxin (Fd), enzymes activating MTZ to its highly toxic free-radical state. Laboratory studies have shown a negative regulation of PFOR activity in MTZ-resistant *Giardia*. On the other hand, this phenomenon was not observed with either FUR or QUI [121].

Among nitroimidazoles, MTZ and TIZ have consistently proved the highest activity *in vitro*. MTZ is rapidly and completely absorbed after oral administration and penetrates tissues and secretions such as saliva, breast milk, semen and vaginal discharge. Other highly substituted nitroimidazoles such as clotrimazole, itraconazole, miconazole and ketoconazole have developed antifungal activity and are not effective agents against *Giardia* [9,121].

FUR is one of numerous compounds derived from nitrofurans. This drug has been used as an antigiardial agent since 1950, though its mechanism of action is not fully known. The drug enters the trophozoite and is activated by reduction through the NADH oxidase enzyme. Its lethal effect is correlated to the toxicity of reduced products that may damage cellular components, including DNA [120].

FUR resistance may be correlated to a reduced drug entrance or increased enzyme levels degrading toxic free radicals. The drug is easily absorbed through the gastrointestinal tract and is rapidly metabolized in tissues. It has the advantage of minimum adverse effects and availability of a fluid suspension suitable for treatment of children [101,120].

NOX is a derivative of nitrothiazole-salicylamide discovered in 1975. This drug has shown *in vitro* activity in the presence of numerous protozoan and helminth parasites. NOX enters the parasite and becomes an active metabolite (desacetyl nitazoxanide or tizoxanide). The antiparasitic action is produced by direct interference of the PFOR enzyme, though its mechanism is different from that of nitroimidazoles. Several studies have shown that NOX effectively acts on MTZ-resistant giardiasis and in the treatment of intestinal mixed helminth and protozoan infections [9,120].

Two benzimidazoles -albendazole (ALB) and mebendazole (MEB)- have been used to treat giardiasis. These drugs produce their toxic effect by binding to the b-tubulin in the trophozoite cytoskeleton, preventing its polymerization, that is, inhibiting the microtubular function. ALB resistance can be induced *in vitro* and is correlated to changes in the parasite cytoskeleton. Benzimidazoles are badly absorbed from the gastrointestinal tract. The systemic effect of albendazole is owing to its primary metabolite, albendazole sulphoxide, rapidly forming in the liver after absorption. Other drugs such as nocodazole, thiabendazole, oxfendazole and fenbendazole have also shown some efficiency *in vitro* [101,120].

Paromycin (PAR), a member of the aminoglycoside family, was first isolated in 1956. This drug inhibits the protein synthesis in *Giardia* interfering with ribosomal subunits 50S and 30S. PAR reaches a high concentration in the intestinal lumen due to its low absorption and is prescribed as antigiardial in resistant infections as well as during pregnancy [121,122].

Conclusion

Giardia is a zoonotic parasite prevalent in areas with a temperate, humid climate, with the highest infection prevalences found in developing countries. Determination of the infection source is crucial to understand its epidemiology. However, the situation has resulted more complex due to a great variety of infection reservoirs and transmission cycles. The application of molecular techniques has significantly changed the knowledge of the zoonotic potential of *Giardia*. The evidence accumulated in recent decades has determined that giardiasis is a zoonotic infection. However, identification of infection sources and transmission dynamics require further studies integrating molecular analysis and epidemiological aspects of giardiasis in endemic areas.

Conflicts of Interest: None declared.

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