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Invasion mechanisms among emerging food-borne protozoan parasites

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Food-borne parasitic diseases, many known to be more prevalent in poor countries with deficient sanitary conditions, are becoming common worldwide. Among the emerging protozoan parasites, the most prominent is *Trypanosoma cruzi*, rarely reported in the past to be transmitted by the oral route but currently responsible for frequent outbreaks of acute cases of Chagas disease contracted orally and characterized by high mortality. Several other food-borne protozoans considered emerging include the apicomplexans *Toxoplasma gondii* and *Cryptosporidium*, as well as *Giardia* and *Entamoeba histolytica*. Here, the interactions of these protozoans with the mucosal epithelia of the host are discussed.

Emerging foodborne parasites

Foodborne parasitic infections are a significant burden on public health services worldwide. Many such infections are termed as 'emergent', despite their probable persistence in human populations for tens of thousands of years [1]. Thus, globalization of travel and food supply, long range introductions of parasite species, changing agricultural practices, human behavior, climate change, health, and lifestyle all contribute to emergent characteristics observed in these parasites, including the exploitation of new hosts and transmission routes [2].

Protozoan enteric infections contracted from contaminated food and/or water include toxoplasmosis (*Toxoplasma gondii*), cryptosporidiosis (*Cryptosporidium* sp.), cyclosporiasis (*Cyclospora cayetanensis*), cystoisosporiasis (*Cystoisospora belli*) sarcocystosis (*Sarcocystis* sp.), which are all apicomplexans, as well as giardiasis (*Giardia intestinalis*) and amoebiasis (*Entamoeba histolytica).* More recently, an 'unusual parasite' was included in this list: *Trypanosoma cruzi*, the agent of Chagas disease. With the exception of *E. histolytica* and *C. belli*, all have a domestic or wild zoonotic reservoir [3]. The variety and severity of symptoms that arise in the human host are reflected by the taxonomic diversity of these pathogenic agents, although in all cases host immune status also plays a significant role. These range from self-resolving diarrheal disease, such as cryptosporidiosis [4] to fatal cardiomyopathy in Chagas disease [5], liver abscesses in *E. histolytica* infection among immunocompetent hosts [6] and *T. gondii*-associated encephalitis among the immunocompromised. In spite of their divergent origins, all foodborne protozoan parasites face similar challenges in invading and colonizing their target tissues in the alimentary tract of the human host. Proteolytic conditions in the stomach must be withstood before penetration of the mucus layer and access to the underlying gut epithelium for attachment or cell invasion. With the notable exception of *T. cruzi* [7] all species form hardy cysts that resist degradation in the stomach. Low pH and host derived proteases trigger excystation of infective lifecycle stages [8,9]. Downstream in the infection process, divergent and diverse strategies are present. Each is highly relevant for understanding disease pathogenesis, the complexity of the host–parasite interaction and thus the design of potential drug and vaccine targets. In this review we examine mucosal and cell invasion across foodborne parasitic protozoa in light of recent advances in the field.

Trypanosoma cruzi

Chagas disease, initially a neglected infection of poor, rural and forgotten populations in Latin America, has since spread to non-endemic countries because of population migration, where transfusion associated transmission is a risk [10,11]. The etiologic agent *T. cruzi* is usually transmitted by haematophagous triatomines although sporadic oral infection has been reported since 1965. The frequency of recent outbreaks of acute Chagas disease (ACD) by this route in diverse areas, including localities where vector borne transmission has been effectively eradicated, demonstrates that oral transmission might be much more common than previously thought. In Brazil, oral infection now constitutes the most prominent mechanism of *T. cruzi* transmission [12]. ACD occurs in microepidemics of orally acquired infection and is found at higher frequency in the Brazilian Amazon, mainly associated with the consumption of açaí palm fruit (Figure 1), crushed to produce paste or juice [13]. Açaí contamination is thought to occur via the crushing of triatomines during the beverage preparation. Vector species implicated in oral *T. cruzi* infection include *Triatoma sordida*, which was found inside or near houses in the 2006 outbreak in Bahia, [14], and *Triatoma tibiamaculata* in the 2005 outbreak in Santa Catarina [15]. In 2007 an ACD outbreak in Caracas, Venezuela, resulted in 1,000 exposed individuals. *T. cruzi* infection was confirmed in 103, and an epidemiological investigation incriminated contaminated fresh guava juice as the sole source of infection [16]. *T. cruzi* is constituted of

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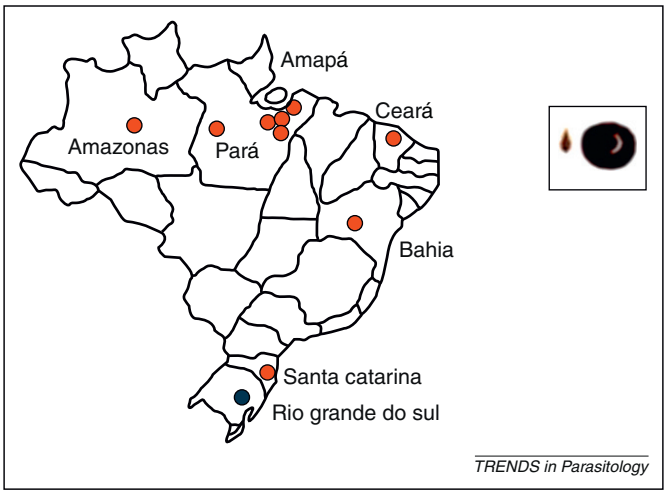

Figure 1. Outbreaks of acute Chagas disease (ACD), associated with oral *T. cruzi* transmission, reported in recent years. **(a)** Contaminated sugar cane juice was the source of infection in Navegantes, state of Santa Catarina, in 2005 [15]. In Macaiba, state of Bahia, where the lethality was 28.6%, the contamination most probably occurred through inadequately stored soft drinks and/or water [14,74]. The possible source of infection in the localities of Ceará state was a soup to which scallion from the vegetable garden was added [75], In the states of Pará and Amazon, the most frequent *T. cruzi* infection has been attributed to consumption of açaí fruit [13,76]. Depicted in dark blue is the locality where the outbreak of ACD by oral *T. cruzi* infection was first reported [77]. **(b)** Shown in the inset is an açaí fruit alongside a triatomine insect, with a similar dark color. The triatomine instars, in particular the first and the second, are so small that they can easily remain unnoticed during handling of the fruit, thus being crushed in the preparation of paste or juice.

genetically diverse populations, now classified into distinct lineages, TcI-VI ([17]. Whether the distantly related isolates use different mechanisms to invade host cells upon oral infection remains to be demonstrated. *In vitro* cell invasion studies with genetically divergent isolates have shown that they rely on distinct mechanisms to enter target cells [18].

The mechanisms of *T. cruzi* infection by the oral route, and the cell types that the insect stage metacyclic trypomastigotes (MT) invade preferentially, have been elucidated by studies in the murine model. Upon oral administration, MT have been shown to invade the gastric mucosal epithelium [19]. When MT reach the mouse stomach, they resist the harsh conditions of the gastric milieu because they express mucin-like surface glycoproteins that are highly resistant to proteolysis. Then, they bind to gastric mucin, the main constituent of the mucus layer that represents the first line of host defense the parasites have to overcome in order to reach the underlying target cells. Apparently, MT bind to gastric mucin in a manner mediated by gp82, the MT-specific surface molecule [20]. Once bound to the gastric mucin, MT migrate through the mucus layer, propelled by parasite ATP [21], and invade the gastric mucosal cells via gp82, which promotes MT invasion of human epithelial cells *in vitro*. Following invasion, MT differentiate into amastigotes, and four days after oral infection with MT, nests of intracellularly replicating amastigotes can be seen in histological sections of the stomach (Figure 2); at a later time

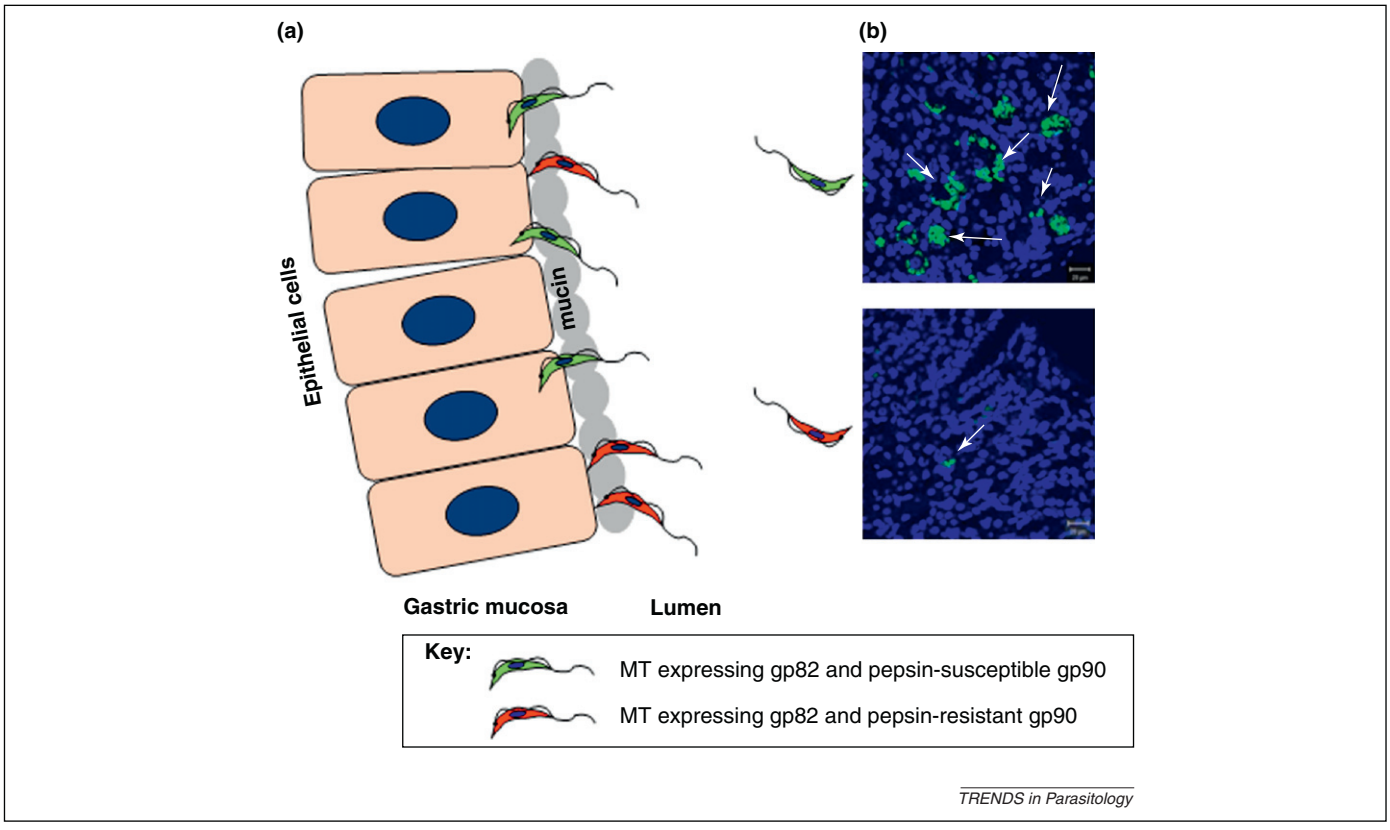

Figure 2. Differential course of oral *T. cruzi* infection in mice. **(a)** Schematic view of interaction of MT of different parasite strains with target cells. In the mouse stomach, MT expressing gp82 and pepsin-susceptible gp90 (green parasites) bind to the gastric mucin, traverse the mucus layer and reach the underlying gastric epithelial cells, which they efficiently invade in a gp82-dependent manner. The journey of MT expressing gp82 and pepsin-resistant gp90 (red parasites) toward target cells is similar, but their entry is hampered by gp90 molecules that remain intact thus preserving their inhibitory effect. **(b)** Nests of amastigotes replicating in the gastric epithelium (arrows), visualized by immunofluorescence in a histological section of the stomach four days after oral infection with MT of different *T. cruzi* strains. Abbreviation: MT, metacyclic trypomastigotes.

point trypomastigotes are detectable in the blood [19]. The crucial role played by gp82 in oral infection is supported by the findings that *T. cruzi* strains that do not express gp82 on the surface are poorly infective in mice by the oral route [7]. The outcome of oral MT infection is also influenced by gp90, a MT surface molecule that negatively regulates host cell invasion [7]. MT of *T. cruzi* strains expressing both gp82 and gp90 at high levels can exhibit distinct infective capacity depending on the susceptibility of g90 molecules to peptic digestion. Parasite strains expressing a pepsin-resistant gp90 isoform invade gastric mucosal epithelium poorly, resulting in subpatent or low parasitemias [7]. The opposite is the outcome of infection by parasite strains expressing pepsin-susceptible gp90 (Figure 2). For instance, MT of *T. cruzi* isolated in 2005 from an orally infected ACD patient, when given to mice by the oral route, produced high parasitemias and high mortality, in sharp contrast with the reduced infectivity *in vitro*, because of high gp90 levels [22]. These *in vivo* and *in vitro* results, although apparently contradictory, were compatible with the finding that MT recovered from the mouse stomach 1 hour after oral administration lacked gp90 molecules, which were completely digested by the gastric juice [22]. Therefore, the interactions of MT with host factors can contribute to further increase their invasive capacity, and if such an exacerbation of infectivity also occurs in humans, it might be responsible for the severity of the disease reported in outbreaks of oral infection, which is characterized by an unusually high mortality rate.

As inferred from experiments of cell invasion in cultured mammalian cells, *T. cruzi* entry into gastric epithelial cells relies on the host cell actin cytoskeleton rearrangement and lysosome mobilization. Host cell cytoskeletal remodeling induced by *T. cruzi*, a Ca^{2+}-dependent process that is the hallmark of the early stages of invasion and parasite retention [23], has been observed during MT internalization as well as by exposure to the gp82 molecule [24]. Actin cytoskeletal disruption that facilitates lysosome fusion with invading or recently internalized *T. cruzi*, crucial for cellular retention of parasites [25], is an event easily visualized following interaction of MT with human epithelial cells and is apparently associated with mTOR signaling [26]. As a strategy to enter host cells, MT might also rely on lipid rafts, the specialized membrane domains enriched in certain lipids, cholesterol and proteins. Cultured mammalian cells depleted of cholesterol by treatment with methyl-β-cyclodextrin were found to be less susceptible to invasion by MT [27].

Apicomplexan *Toxoplasma gondii* and *Cryptosporidium sp.*

Out of many apicomplexan species, only a few are known to infect humans as a result of ingestion. These include *T. gondii*, *Cystoisospora belli*, *Cyclospora cayetanensis, Sarcocystis hominis*, *Sarcocystis suihominis* and several species of *Cryptosporidium*. Among the latter, the anthroponotic *Cryptosporidium hominis* and zoonotic *C. parvum* are responsible for the vast majority of disease. With the exception of *T. gondii,* most ingested protozoan parasites cause self-resolving cases of acute diarrhoea in immunocompetent humans. Aside from sporadic outbreaks of ocular toxoplasmosis, *T. gondii* rarely causes recognizable symptoms in healthy adults; however, severe encephalitic disease can occur among newborns and the immunocompromised. Transmission of these species occurs via ingestion of resilient oocysts discharged into the environment via defecation, and/or through the ingestion of bradyzoite-containing cysts in the tissues of intermediate hosts, as in *T. gondii*. As such, contaminated water [28], soil [29] and meat [30] are all potential sources of infection. Most human infections by food-borne apicomplexans are accounted for by *T. gondii*; indeed, over a third of the population of the world could harbor this incredibly successful parasite. In the USA, overall age-adjusted seroprevalence has been estimated to be as high as 22.5% [31]. *Cryptosporidium,* the most common water-borne parasite, is less prevalent, with an estimated 0.25% infected in the same population [32]. Infection with other food-borne apicomplexans is sporadic. Excystation, the process by which infective sporozoites emerge from thick walled and resistant oocysts, is an important precursor to invasion of target cells in the ileum, jejunum and duodenum for both *Cryptosporidium* and *Toxoplasma*. Several host derived triggers are important, including temperature and pH [33,34], exposure to trypsins, bile salts and other reducing factors present in the mammalian digestive tract [34,35]. The net effect of these triggers is to reduce the thickness and increase the permeability of the oocyst wall, which facilitates detection of excystation signals by the sporozoite(s) inside. Proteomic analysis of excysting *C. parvum* oocysts supports evidence that several parasite-derived enzymes also have a role, including serine and cysteine proteases [36]. For *Cryptosporidium* species this process of excystation has been the subject of rigorous recent review [8,37].

Once mature and released from the ruptured oocyst, sporozoites must preferentially bind and invade target cells in the gut epithelium. Apicomplexans coordinate membrane secretory processes with gliding motility, easing entry into their target cell, minimizing mechanical damage and directly modulating the composition of the host cell membrane at the parasite synapse [38]. In doing so, they rely on parasite actin-based motility to generate the force required for entry and their own secretory organelles to furbish the membrane of the parasitophorous vacuole (PV) into which they enter. A significant body of recent research now describes several stages in *T. gondii* host cell invasion, important in our understanding of homologous processes among other apicomplexans. After initial adhesion of the parasite to the host cell surface and parasite re-orientation such that its apical end has contact with the host cell, activity of several key organelles, including the microneme, rhoptry and dense granules, results in the coordinated discharge of multiple proteins that facilitate invasion [39]. An important initial step in the invasion process is the formation of the moving junction (MJ), which dynamically anchors the parasite to the host cell surface (Figure 3) while the parasite 'glides' into a host cell vacuole, mediated by the actin-myosin motor of the parasite [40]. Fluorescent antibody labeling in concert with time lapse video and confocal microscopy reveals that complex partitioning occurs at the MJ [41]. In this manner, multiple host membrane proteins are excluded from the

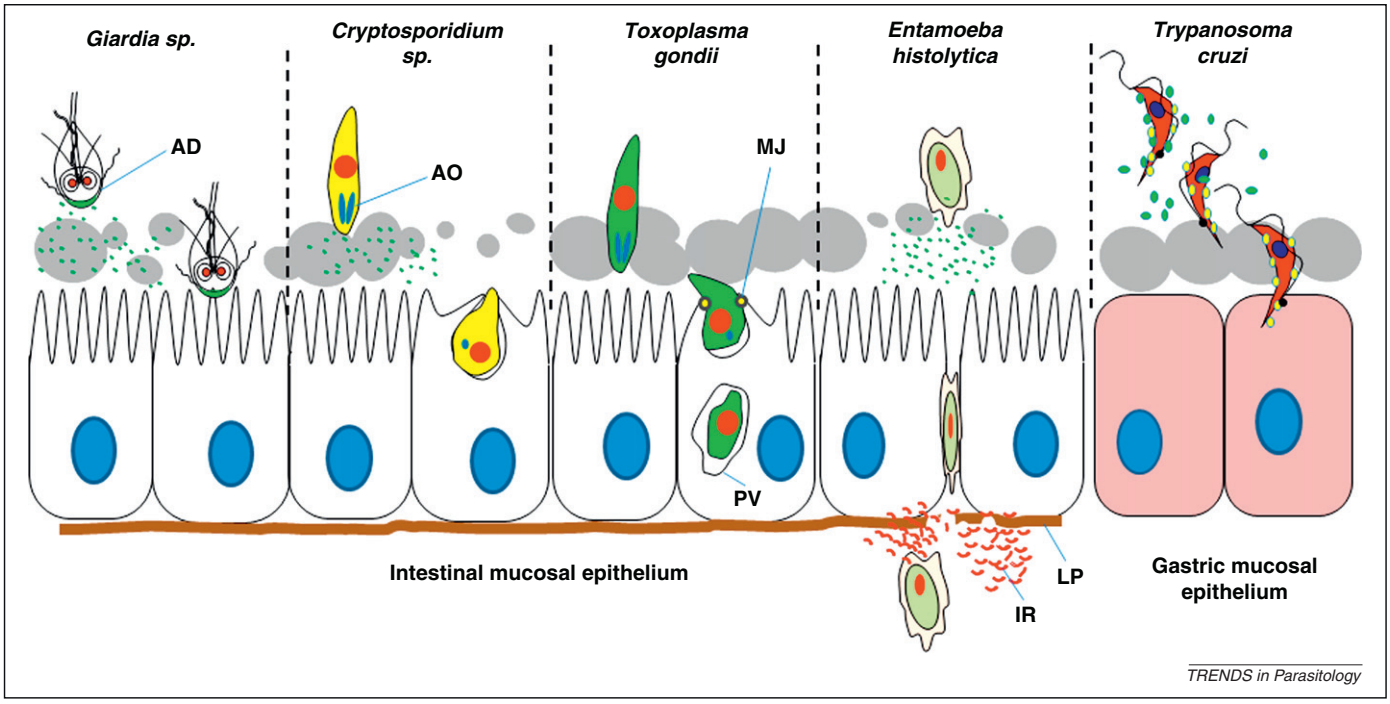

Figure 3. Parasitic protozoa and their interactions with the human intestinal mucosa. From left to right, *Giardia sp.* trophozoites release proteases (green dots) to degrade the mucosal layer (grey) and adhere to epithelial cells via a specialized adhesive disc on their anterior ventral surface. *Cryptosporidium sp.* sporozoites secrete chemicals (green dots) from their apical organelles (AO) including proteases and gp30 lectin to provide traction for their gliding motility across the mucosa before invasion of their extracytoplasmic niche. *T. gondii* tachyzoites traverse the mucosal layer via an as yet unknown mechanism. Host cell invasion occurs via the moving junction (MJ), and the organism remains sequestered in a parasitophorous vacuole (PV). *E. histolytica* trophozoites secrete multiple proteases (green dots) that strip off the mucosa, induce an inflammatory response (IR), and facilitate penetration of the lamina propria (LP) of the gut lumen. *T. cruzi* metacyclic trypomastigotes expressing gp82 (yellow) and pepsin-susceptible gp90 (green, sheared off in stomach) bind to the gastric mucin, traverse the mucus layer and reach the underlying gastric epithelial cells, which they efficiently invade in a gp82-dependent manner. Abbreviations: AD, adhesive disk; AO, apical organelles; MJ, moving junction; PV, parasitophorous vacuole; IR, inflammatory response; LP; lamina propria.

(PV) in which the sporozoite resides (Figure 3), a mechanism thought to preclude fusion of host cell lysosomes to the PV and resultant destruction of the parasite. Targeted disruption of several proteins involved in MJ formation demonstrates its importance in the invasion process. Rhoptry neck protein (RON) 8 has a role in anchoring the MJ to the host cytoskeleton [42]. Knockouts exhibit abortive host cell attachment and invasion. Dynamic connection of this protein to the parasite itself is mediated by several other proteins including AMA1, a transmembrane micronemal protein present in the parasite membrane, and RON2 [43]. RON2 forms the membrane spanning contact with the host cell surface, and the stable association between this and AMA1 is thought to contribute to the wide variety of cells that *T. gondii* can infect [44]. Cleavage of AMA1 by the intermembrane rhomboid protease ROM4 provides the signal by which the parasite is triggered to proliferate once invasion is complete [45].

Cryptosporidium sporozoites also use gliding motility for cell invasion. To traverse the intestinal mucus and infect the underlying epithelia, the sporozoites secrete lectin-like surface proteins from their apical organelles, notably p30 [46], which are believed to bind host mucins (Figure 3) and provide the traction against which the parasite gliding motility can act to penetrate the mucus layer and attach to the underlying epithelial cells [8]. Sporozoites do not fully enter the cells, but rather form an intramembranous yet extracytoplasmic niche on the apical surface (Figure 3). Using cell culture models of infection it has been determined that interaction between *C. parvum* and the apical surface of an epithelial cell results in the localized activation of signaling cascades, culminating in the polymerization of actin filaments directly subjacent to the region of host–parasite interaction within the host cell cytoplasm. In a cell culture model of biliary cryptosporidiosis, it was demonstrated that PI3k/Cdc42 signaling is initiated at the parasite synapse leading to actin polymerization via N-WASP activation [47,48]. Involvement of cortactin phosphorylated by c-Src was also previously demonstrated [49] and together with the clustering of host cell receptors involved in actin nucleation suggests a role for sphingolipid-enriched microdomains (lipid rafts) in the process, and this occurs for both the *T. gondii* and *T. cruzi*. In *C. parvum*, a role for the acid sphingomyelinase has also been described [50]. In addition, it was found that the Na^+/glucose co-transporter 1 (SGLT1), and aquaporin 1 (Aqp1), accumulate at the parasite synapse and can participate in the overlaying of membrane induced by the parasite [51]. Finally, a role for mucins and/or mucin like glycoproteins, including gp40, gp15, gp900, CSL p30, CpMuc 4, and CpMu 5 has also been identified in epithelial cell adherence and invasion [52], facilitated by the online availability of the *C. parvum* genome.

Entamoeba histolytica

Among the *Entamoeba* species that infect humans, *E. histolytica* stands out as a pathogenic amoeba that can cause invasive intestinal and extra-intestinal disease worldwide. It has recently been recognized as an emerging

pathogen in homosexual men in Asian Pacific countries where amebiasis is not endemic, such as Japan, Taiwan, Republic of Korea, and in Australia, with increased pathogenicity of invasive amoebiasis associated with HIV [53–55].

Unlike *T. cruzi* and *T. gondii*, and to a lesser extent *Cryptosporidium*, *E. histolytica* does not invade individual cells. Rather the parasite penetrates and disrupts the lamina propria via the mucosal epithelium, causing significant inflammation and damage to the wall of the caecum. Upon invasion, *E. histolytica* disrupts the protective mucus layer and the colonic epithelial barrier (Figure 3), a process in which cysteine proteinases play a key role [56,57]. *E. histolytica* cysteine proteases (EhCPs) are known to dissolve mucus gels by targeting the less glycosylated C-terminal domain of MUC2 mucin, the major structural component of colonic mucus [56]. In an *ex vivo* human colonic model of amoebiasis, *E. histolytica* stripped off the protective mucus coat during the first two hours of incubation, detaching enterocytes, and penetrating the lamina propria; after four hours of incubation significant cell lysis and inflammation were detected [58]. In a parallel experiment, the closely related nonpathogenic *Entamoeba dispar*, which also colonizes the human colon, induced none of these affects [58]. Inhibition of EhCP dramatically reduces invasion [59]. When cysteine protease 5 was silenced, *E. histolytica* did not penetrate the colonic lamina propria and failed to induce pro-inflammatory cytokine secretion of the host [60]. A specific inhibitor of CP4, the dominant cysteine protease expressed during invasion and colonization in a mouse cecal model of amoebiasis, significantly reduced parasite burden and inflammation [57]. Evidence suggests that NFκB-triggered inflammatory responses, stimulated by binding of the RGD motif of immature cysteine proteinase (PCP5) to α(V)β(3) integrin on Caco-2 colonic cells, could represent an important invasion mechanism for *E. histolytica* trophozoites [60]. Analysis of CP5 mRNA levels in samples of *E. histolytica*, isolated from patients presenting different clinical profiles that correlated with differential virulence in hamster liver cells, revealed differences among trophozoites freshly isolated from hepatic amoebic lesions [61].

Adhesion is an important virulence function for *E. histolytica*, which expresses diverse adhesion molecules. Recently a novel *E. histolytica* fibronectin receptor (EhFNR) was described with 99% homology to the intermediate subunit-2 of Gal/GalNAc-specific lectin [62], one the major factors implicated in the pathogenesis of amoebiasis. A close association of the purified EhFNR complex to adhesion plates and phagocytic invaginations was observed. In *E. histolytica*, lipid rafts participate in interactions with the host cell as well as with the extracellular matrix, and it appears that raft-associated Gal/GalNAc lectin serves as a receptor for collagen [63]. Following the colonization of the mucin layer by *E. histolytica*, a proinflammatory response is induced. *E. histolytica*-derived prostaglandin E(2) was identified as one of the major virulence factors involved, triggering acute inflammation that causes nonspecific tissue damage and can facilitate parasite invasion of the underlying colonic mucosa [64].

Giardia

Giardia intestinalis (syn. *G. duodenalis*, *G. lamblia*) causes diarrheal disease in humans worldwide, is transmitted via ingestion of contaminated drinking or recreational water, consumption of contaminated food or contact with infected persons, or animals. It was included in 2004 in the WHO 'Neglected Diseases Initiative', which consists of heterogeneous groups of diseases that impair development and socio-economic improvements [65]. More common in countries where poor sanitary conditions prevail, giardiasis is sometimes referred to as a re-emerging infectious disease for its role in outbreaks of diarrhoeal disease in day care centers and water-associated outbreaks in industrialized countries [66].

Giardia is an extracellular parasite that resides in the lumen of the small intestine. Adhesion of *Giardia* trophozoites to the intestinal epithelium is via a specialized disc (Figure 3) and is crucial to initiate colonization as well as to maintain the infection. Alpha-giardins, proteins related to annexins that are highly expressed in *Giardia*, are involved in parasite attachment to the intestinal mucosa and in the cytoskeletal disassembly and reassembly that marks the transition from infectious trophozoite to transmissible cyst [67].One surface protein (200 kDa) has been identified as playing a role [68]; when compared to wild type *Giardia* trophozoites, those with reduced expression of the 200 kDa surface protein showed reduced capacity to establish infection in Mongolian gerbils [68]. By attaching to the epithelium and overlying mucus layers, *Giardia* can induce alterations in the intestinal epithelial cells through its secreted products, which include proteases [69]. *Giardia* is believed to cause malabsorbtion and diarrhea primarily through disruption of epithelial tight junctions of the upper small intestine brush border epithelium, which in turn increases intestinal permeability. This loss of epithelial barrier function results from enterocyte apoptosis [70]. *Giardia* was found to induce apoptosis in a human intestinal epithelial cell line [71]. The apoptotic effect induced by *Giardia* is somewhat mitigated by the activity of host cell sodium activated glucose transporters (SGLT-1), particularly in the presence of higher glucose levels [72]. Analysis of duodenal biopsy specimens from patients with chronic giardiasis indicated that *Giardia* infection causes downregulation of the tight junction protein claudin 1, an increase in epithelial apoptosis, impairment of Na^+-dependent D-glucose absorption and activation of electrogenic anion secretion [73].

Concluding remarks

Food-borne parasitic infections are a worldwide problem, and protozoan parasites already place a considerable burden on public health (Box 1). Globalization of travel and food supply as well as HIV co-infections all serve to exacerbate the problem, whereas the warmer and wetter conditions attributable to climate change could encourage increased transmission in the future. *T. cruzi* represents a recent arrival on the scene, exploiting a recently disclosed oral transmission route, and thus joining an already populous group of orally transmitted protozoans. With the exception of *T. cruzi*, these parasites: *T. gondii*, *Cryptosporidum sp.*, *E. histolytica*, and *Giardia,* are ingested as

Box 1. Current epidemiological trends for food-borne protozoan parasites

- **Chagas disease.** Following a coordinated multi-country program in the Southern Cone countries, the transmission of Chagas disease by vectors and via blood transfusion was interrupted in Uruguay in 1997, in Chile in 1999 and in Brazil in 2006, and the incidence of new infection by *T. cruzi* across the South American continent has decreased by 70%; similar initiatives have been launched in the Andean countries and in Central America [78]. The special characteristics of the disease in the Amazon region require a new model of epidemiological surveillance, taking into account the enormous surface of the territory and serious operational difficulties [78]. In nonendemic countries, the estimated total number of *T. cruzi*-infected individuals is higher than 390,000 [10]: 300,000 in the US, >5,500 in Canada, 80,000 in Europe, >3,000 in Japan, and >1,500 in Australia.
- **Toxoplasmosis.** *T. gondii* is a globally distributed parasite and the prevalence of human infection worldwide could be up to one third. It is transmitted via the ingestion of contaminated water and uncooked meat and only completes its lifecycle within a cat. The vast majority of *T. gondii* infections described in humans and domestic mammals are caused by three recently emerged clonal genotypes. As well as causing significant disease in human populations, *T. gondii* also has an important veterinary impact, and is a major cause of stillbirth among goats and sheep.
- **Cryptosporidiosis.** The disease is often associated with waterborne outbreaks even in the developed world because the oocyst is particularly resistant to chlorinated water. Disease prevalence is greatest in rural communities in the developing world, and young children and the immunosuppressed are particularly susceptible to infection. Although eight species of *Cryptosporidium* are able to infect humans, two are responsible for the vast majority of cases: the predominantly anthroponotic *Cryptosporidium hominis* and the predominantly zoonotic *Cryptosporidium parvum,* which is associated with a broader host range including cattle and sheep.
- **Amebiasis.** *E. histolytica* is responsible for an estimated 35 to 50 million cases of symptomatic disease and approximately 100,000 deaths annually; the majority of morbidity and mortality occurs in Asia, Central and South America and Africa, and children are especially vulnerable [79].
- **Giardiadis.** *Giardia intestinalis* causes one of the most common parasitic infections worldwide. It contributes to an estimated 280 million symptomatic human infections per year. The parasite survives in the environment for prolonged periods and the need to control waterborne transmission results in large economic losses for industry [80].

Table 1. Developmental forms of food-borne protozoan parasites implicated in the establishment of infection

Parasite species	Ingested form	Adhesive/invasive form	Main contaminated source
T. cruzi	Trypomastigote	Trypomastigote	Fruit juice
T. gondii	Oocyst	Sporozoite	Soil/water
	Cyst		Meat
Cryptosporidum sp.	Oocyst	Sporozoite	Water
E. histolytica	Cyst	Trophozoite	Food/water
G. intestinalis	Cyst	Trophozoite	Water

thick-walled oocysts (Table 1) and adapted for persistence in hostile environments, including those they encounter in the gastrointestinal milieu. Although relatively susceptible to the environment, *T. cruzi* metacyclic forms can survive for several hours in sugar cane juice and are uniquely capable of invading the gastric mucosal epithelium. Furthermore, the sophisticated role of gp90 and proteolytic gastric conditions in capacitating *T. cruzi* mucosal invasion suggests that oral transmission might be ancient among insectivorous sylvatic reservoir hosts. Our improved understanding of the early mechanisms and events that occur during infection with food-borne protozoa reveals a rich array of biochemical strategies to overcome and perhaps exploit innate immune responses to facilitate cell invasion. Also varied are the pathophysiologic mechanisms operating in infections by different food-borne protozoan parasites (Box 2). Comparisons between protozoan genera can pave the way for future investigative strategies and ultimately the identification of new drug and vaccine targets.

Box 2. Pathogenesis of food-borne protozoan parasites

- **Chagas disease.** In recent decades, clinical and experimental investigations have shown that a low-grade but persistent parasitism, along with an accompanying immune response, either parasite-driven and/or autoimmune-mediated, plays an important role in producing Chagas cardiomyopathy, which appears in 20–40% of *T. cruzi*-infected individuals between 10 and 30 years after the original acute infection and is responsible for high morbidity and mortality [5].
- **Toxoplasmosis.** The risk of pathology by *T. gondii* infection appears to vary with infective dose, and virulence is associated with parasite genotype; type I parasites are more frequently associated with acute retinal damage and with congenital toxoplasmosis that can result in foetal hydrocephaly, chorioretinitis, and miscarriage. Pathogenesis is also profoundly affected by the host, its genetics, immune response and in particular the cytokines elicited by the infection, with IFN-gamma playing a key role; accordingly, in AIDS patients, reactivation of the disease can lead to fatal encephalitis.
- **Cryptosporidiosis.** Recent studies on infection by *Cryptosporidium*, which triggers secretory diarrhoea upon attachment and penetration of the sporozoites into enterocytes, suggest keys roles for inflammatory mediators including TNF-α and substance P.
- **Amebiasis.** In addition to the apoptosis-inducing activity of *E. histolytica* on target cells, which can underlie disease pathogenesis, recent studies have begun to unravel the host genetic determinants that influence the outcome of infection [79]. Genetic variants in the receptor for leptin, a hormone produced by adipocytes that inhibits food intake, has been associated with susceptibility to infection in children and with amebic liver abscess in adult patients [81].
- **Giardiasis.** The proposed mechanisms of giardiasis include apoptosis of enterocytes, loss of epithelial-barrier function, hypersecretion of chloride ion, malabsorption of glucose, water and Na^+, diffuse microvillus shortening, immune reaction, inhibition of brush-border enzymes, and interference with bile salt metabolism [80].

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