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GIARDIASIS IN HUMAN AND ANIMALS

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INTRODUCTION

Giardia has been known since the times of Antonie van Leeuwenhoek's during 1681 but its public health significance was acknowledged during late 20th century. *Giardia* is known as the main cause of diarrhea now-a-days and is considered as major concern for the health authorities. There are estimated 280 million infections of giardiasis diagnosed every year (Einarsson et al. 2016). It affects the socio-economic condition of people residing in developing countries. Therefore, it is included in "Neglected Disease Initiative" of World Health Organization (Savioli et al. 2006).

Giardia is a unicellular flagellate, belonging to the phylum Protozoa, order Sarcomastigophora and family Mastigophora. Giardiasis is a chronic issue that infects the intestine of the hosts, including mammals, having a specific target on duodenum and ultimately causing bowel diarrhea (Ballweber et al. 2010). The recognized species of the genus include *G. agilis* infecting amphibians, *G. pasittaci* and *G. ardeae* infecting birds, *G. muris* infecting rodents and *G. duodenalis* (syn. *G. intestinalis, G. lamblia*) infecting human and other mammals (Thompson et al. 2012).

Giasrdiasis is considered as a common parasitic infection of companion animals. It also causes substantial pathological changes in laboratory animals like mice. In both human and animals, the disease results in weight loss, diarrhea (either acute or chronic), hypersensitivity and nausea like condition. Asymptomatic infection is also reported in many instances (Geurden and Olson 2011).

The research done so far on giardiasis has been mainly focused on its molecular characterization in various animals in order to identify the reservoir host and calculate the zoonotic risk of disease. At present, identification of specific genotype in animals and humans is possible due to the development of molecular markers (Durigan et al. 2018).

Giardiasis is an important disease, affecting both human and animals, and having millions of cases every year. As per significance of the protozoa, this chapter briefly summarizes the parasite prevalence across the world, pathogenesis in the host and control strategies with a view to reduce the infection rate.

History and evolutionary biology

Giardia is considered as a primitive eukaryote due to lack of several typical eukaryotic organelles like mitochondria and peroxisome. Phylogenetic analysis of gene and protein sequence also confirms its position in the evolutionary tree. Early classification of *Giardia* was based on characteristics of ribosomal RNAs (Van Keulen et al. 1993), but later it was classified on the basis of conserved proteins, like elongation factor (Hashimoto et al. 1994). *Giardia* was typically considered as the most derived member of its order, based on its life history and morphology. Furthermore, phylogenetic analysis of order Diplomonadida further confirmed *Giardia* as the most derived genus of its order (Siddall et al. 1992).

Furthermore, due to large differences in G+C composition of *Giardia* and other eukaryotes and large branch attraction effects, its position in other eukaryotes has been questioned (Dacks et al. 2002). Evolutionary history of *Giardia* is also difficult to trace due to its lateral gene transfer, as in anaerobic metabolism of organism many genes having been acquired from prokaryotes are involved (Andersson et al. 2003). According to the most recent molecular evidences, *Giardia* is classified as a highly derived organism. It is then suggested that it is derived by introns acquisition in genome during eukaryotic evolution, followed by spliceosomal peptides and intron detection in *Giardia* (Nixon et al. 2002). Further sequencing also supported the previous findings and identified the trans-spliced and cis-spliced intron in the genome, having exon dispersal across the genome, with a single transcript being produced by trans-splicing (Kamikawa et al. 2011; Roy et al. 2012).

There are various eukaryotic similarities found in *Giardia* upon genomic data analysis like RNA regulation pathways, such as micro RNAs and RNA slicing (Zhang et al. 2009), processing machinery for sequences encoded for eukaryotic RNA (Chen et al. 2011), meiosis-specific genes (Ramesh et al. 2005) and presence of nucleoli (Jiménez-Garcia et al. 2008). Presence of mitosomes (mitochondrial remnant) has also been demonstrated in *Giardia* and various other amitochondriate protists (Tovar et al. 2003). Upon phylogenetic analysis of genes coded for type-II DNA topoisomerase, it was found that *Giardia* is derived from mitochondrial kinetoplastids and amitochondriate is regarded as polyphyletic. Furthermore, studies also demonstrated that *Giardia* acquired mitochondria from eukaryotes and multiple evolution of organelles has also occurred (He et al. 2005).

The detailed understanding regarding evolution of *Giardia* could be gained by analyzing its genomic data. Due to loss or reduction of metabolic pathways, *Giardia* genome is thought to be in compact form. According to a study, 40%

genes in its genome were found duplicated. Upon phylogenetic analysis, these duplicated genes were found to be encoded for surface protein similar to the placental mammals (Sun et al. 2010).

Taxonomy of *Giardia*

The members of the genus *Giardia* are flagellated protozoa, characterized by the presence of diploid nucleus, unique adhesive disc on ventral surface, absence of peroxisomes and mitochondria (Morrison et al. 2007). The unique feature of *Giardia* which differentiates it from other member of family is the ventral disc, which also helps in its attachment with brush border of villi. Ventral disc is supported by cytoskeleton of microfilaments, microtubules and associated fibrous structures and mainly composed of protein tubulin (subunit a and b) and gairdins (Ankarklev et al. 2010).

According to old classification (1980), there are seven phyla placed under subkingdom Protozoa based on morphology. The zoonotic parasites mainly belong to five phyla i.e., Myxozoa, Ciliophora, Apicomplexa, Sarcomastigophora (containing both Sarcodina and Mastigophora) and Microspora. *Giardia* is a member of Phylum Sarcomastigophora, Subphylum Mastigophora, Class Zoomastigophorea, Order Diplomonadida and Family Hexamitidae. According to the most recent classification, Protozoa is recognized as a kingdom containing 13 phyla based on molecular sequence evidence. Former phylum Mastigophora is further divided into Percolozoa, Metamonada, Euglenozoa and Parabasalia. *Giardia* belongs to the Phylum Metamonada, Subphylum Trichozoa, Class Trepomonadea, Order Giardiida and Family Giardiidae (Morrison et al. 2007; Plutzer et al. 2010).

Giardia Species

There are six species of the genus *Giardia* accepted so far, including *G. psittaci, G. ardeae, G. agilis, G. microti, G. muris* and *G. duodenalis*. *Giardia duodenalis* is the only species which is able to infect humans and animals both. Another species has also been reported in reptiles, which resembles *G. duodenalis* in appearance, but it lacks median bodies and contains binucleated cyst. Therefore, it is characterized as *G. varani* (Cacciò et al. 2005). According to a previous study conducted on cultured and wild marine and freshwater fish in Australia, assemblage A and B (zoonotic nature) and assemblage E (artiodactyl-specific) of *G. duodenalis* and *G. microti* have been identified. It was not clear from the study that whether fish acted as mechanical host or actually got infection (Yang et al. 2010). The host range of *Giardia* species is very wide and *G. duodenalis* shows a great public health significance as well (Monis et al. 2009).

Giardia duodenalis, G. intestinalis and *G. lamblia* are the multiple names refer to the same organism in literature. *G. duodenalis* and *G. intestinalis* mostly infect livestock, companion animals and humans. In medical field, *G. lamblia* is used to discuss the giardiasis impact on humans (Xiao et al. 2008). *G. duodenalis* is considered as the only species which can infect humans, pets and livestock and now it is also considered as multiple species complex (Thomas et al. 2008).

There are multiple assemblages found within *G. duodenalis* morphological group which include assemblage A, B, C, D, E, F and G. These all appear to be associated with single species of mammalian host. Historically, allozyme analysis showed that two genetic assemblages, including A and B, are referred as human isolates (Thompson et al. 2004). Multigenic sequence analysis also confirms assemblage A and B to be referred as human isolates, assemblage E to be isolated from artiodactyls, assemblage G from rodents, assemblage C and D from dogs and assemblage F from cats (Cacciò et al. 2005).

Prevalence

Giardiasis affects the humans, as well as animals, across the globe. According to an estimate, around 280 million people across the globe are diagnosed with giardiasis every year, with higher infection in developing countries (Squire et al. 2017). The correct incidence of disease is unknown in many parts of the world due to unreported/undetected cases. In China, almost 28.5 million human cases have been reported every year (Feng et al. 2011). According to Ballweber et al. (2010), the prevalence of giardiasis varies according to different studies, regions, diagnostic methods, symptomatic or asymptomatic, age of the animal and housing conditions. *G. duodenalis* assemblages which mainly cause infection in humans include assemblage A and B (Ballweber et al. 2010). The prevalence of *Giardia duodenalis* in various animal species and humans reported in different studies has been listed in Table 1.

In cats, infection of giardia is often asymptomatic and self-limiting. Therefore, few studies have done on cats regarding presence of *Giardia*. According to these studies, the infection rate in cats in Europe is around 20.3% (Epe et al. 2010), 5.3% in UK (Gow et al. 2009), 10.8 to 44.4% in USA (Fayer et al. 2006; Garrett et al. 2006; Vasilopulos et al. 2007), 5.9% in Brazil (Coelho et al. 2009), 13.6% in Netherlands (Overgaauw et al. 2009), 19% in Chile (Lopez et al. 2006), 4.1% in Canada (Santin et al. 2006), 15.8 to 37% in Italy (Papini et al. 2007) and 2.0% in Australia (Palmer et al. 2008). The variation in infection rate of giardiasis is attributed to differences in age, diagnostic techniques and symptomatic stage of the animal. In many cases, the prevalence rate differs when diagnosed with multiple diagnostic methods i.e., microscopy, Enzyme-Linked Immunosorbent Assay (ELISA), Polymerase Chain Reaction (PCR) or Immunofluorescence Assay (IFA).

Life Cycle

The life cycle of Giardia is simple and direct in nature. It involves two stages; i) the trophozoite stage that is replicative in nature and get attached to brush border of villi epithelium, causing damage to duodenum that

Table 1: Prevalence of *Giardia duodenalis* in multiple animals and humans across the world

Host species	Location	Detection Method	Prevalence (%)	Reference
Ruminants (sheep, goat, cattle, buffalo)	Islamabad, Pakistan	Microscopy	16.0	(Imran et al. 2013)
Dogs	Romania	ELISA	34.6	(Mircean et al. 2012)
Human	UK	Microscopy	30.0	(Waldram et al. 2017)
Human children	Portugal	Microscopy	1.9	(Júlio et al. 2012)
		ELISA	6.8	
Cattle	Ningxia, China	PCR	2.12	(Huang et al. 2014)
Cattle	Beijing, China	PCR	1.09	(Li et al. 2016)
Sheep	Belgium	IFA	36.0	(Geurden et al. 2008)
Sheep	Italy	Microscopy	1.5	(Giangaspero et al. 2005)
Goat	Spain	ELISA	42.0	(Ruiz et al. 2008)
Goat	Brazil	Microscopy	14.0	(Bomfim et al. 2005)
Sheep	Australia	Microscopy	9.0	(Ryan et al. 2005)
Cattle	Denmark	IFA	24.0	(Maddox-Hyttel et al. 2006)
Cattle	Norway	IFA	49.0	(Hamnes et al. 2006)
Cattle	USA	PCR	40.0	(Trout et al. 2004)
Dog	Guangzhou, China	Microscopy	8.61	(Li et al. 2012)
		PCR	11.0	
Human	Ethiopia	Microscopy	13.8	(Wegayehu et al. 2013)
Cattle			2.3	
Human	Lebanon	PCR	28.5	(Osman et al. 2016)
Cattle	Northeast China	PCR	7.9	(Liu et al. 2015)
Human	Cuba	PCR	22.8	(Puebla et al. 2014)
Beef Calves	USA	PCR	33.5	(Santin et al. 2011)
Human	Pakistan	Microscopy	2.75	(Naz et al. 2018)
		ELISA	9.5	
Human	Pakistan	Microscopy	59.54	(Haq et al. 2015)
Human	Pakistan	Microscopy	15.0	(Khan et al. 2018)

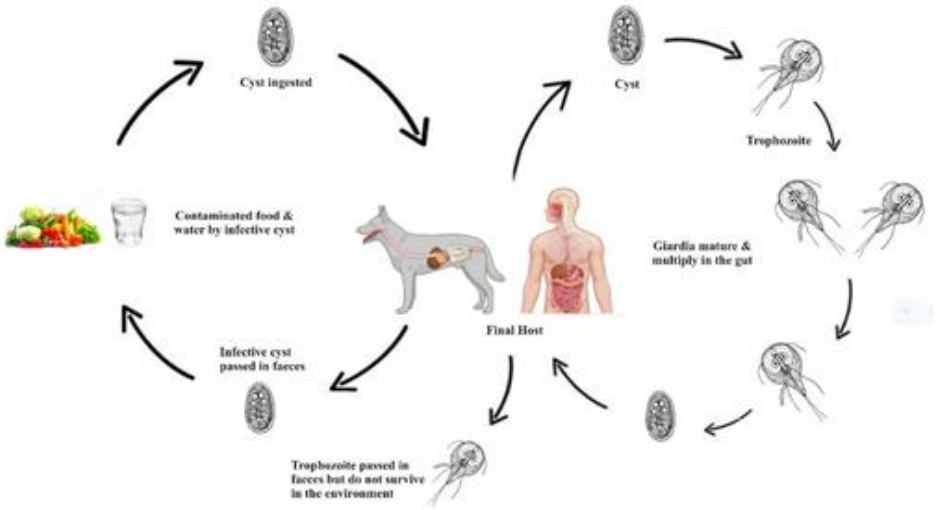

Fig. 1: General stages of life cycle of *Giardia*.

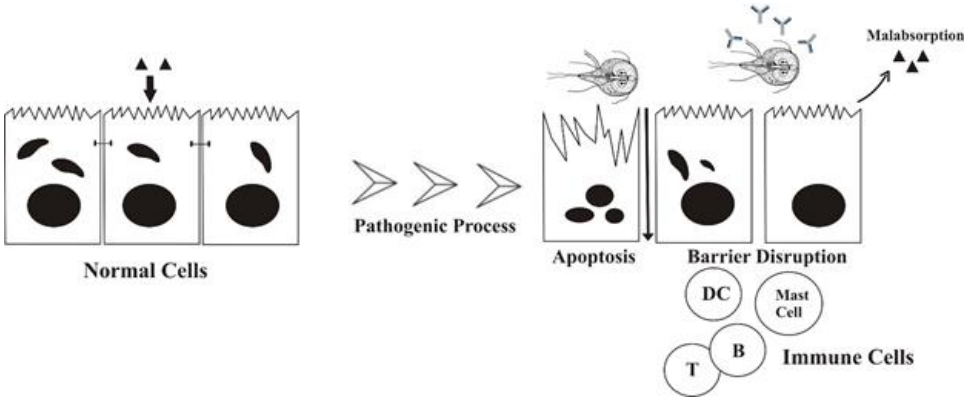

Fig. 2: Pathogenic process during *Giardia* infection.

ultimately leads to mucousal layer sloughing, atrophy of villi and finally increases crypt cell proliferation, and ii) the cyst stage which is infective in nature and passes out in faeces (Fig. 1). Trophozoite ultimately increases mucus secretion due to hyper-secretion of goblet cells (Sazalli et al. 2016).

The transmission of disease is possible directly through feaco-oral route, which may involve direct human to human contact, human to animal contact and animal to animal contact, and indirectly through consumption of contaminated food and water. Acidic environment in the stomach provides suitable stimuli for excystation (trophozoite to cyst conversion) in duodenum (Capári et al. 2013).

Trophozoite undergoes multiple mitotic divisions and then the condition of bile in small intestine converts it into environment resistant cyst. These cysts are shed in feces and able to survive in environment for months. Cysts are infectious in nature and contaminate the environment, leading to the contamination of food and water (Feng et al. 2011). Around 10-100 cysts are considered as the infecting dose in humans. Drinking of contaminated water is a main factor behind major outbreaks of giardiasis. According to a survey, a total of 199 protozoal outbreaks were recorded during the period of 2004-2010 and *Giardia* was responsible for causing 70 outbreaks (35%) (Baldursson et al. 2011).

Clinical Signs and Pathogenesis

The infection ranges from asymptomatic giardiasis to acute or chronic infection. It causes a wide range of infection in domestic, as well as companion, animals and is transmissible to humans. Giardiasis causes potential pathogenic changes in the host. The symptomatic infection appears after 1 to 2 weeks of cyst ingestion and persists for 3 to 4 days (Farthing et al. 1997). The pathognomonic sign of giardiasis is diarrhea with foul smelling, but it may also accompany with nausea, flatulence, weight loss, urticaria, itching and epigastric cramps. Symptoms of disease appear due to the dysfuntioning of small intestine because of the villus atrophy, malabsorption of fat, lactose, electrolytes, vitamin A, D-xylose and vitamin B12, immaturity of enterocytes, luminal enzyme deficiency and decrease in surface area of brush border. Due to malabsorption of electrolytes and nutrients, osmotic gradient is created in intestine, resulting in water accumulation, rapid peristalsis and ultimately diarrhea (Einarsson et al. 2016).

Severe form of giardiasis is usually observed in infants and children in developing countries, where it is associated with poor nutrition status, failure to thrive syndrome, poor cognitive function and retarded growth (Berkman et al. 2002). Sometime giardiasis sequelae may result into a chronic infection. According to a study, in Norway 10% of individuals had persistent infection of giardiasis with mean duration of 7 month after waterborne outbreak of the disease and among them 5% developed fatigue syndrome afterward (Naess et al. 2012). Post-infectious reactive arthritis, irritable bowel syndrome and allergic reactions are also demonstrated from several studies in literature (Wensaas et al. 2012). The pathogenic process of disease is contributable to both ends i.e., parasite and host. The trophozoite of *Giardia* is highly motile and gets attached with the enterocytes in upper portion of small intestine, resulting in establishment of infection (Cotton et al. 2011). The attachment with enterocytes is accomplished by ventral adhesive disc and movement by flagella present on *Giardia* surface. The adhesive disc also protects parasite from elimination by peristaltic movements. In host intestine, there is an active release of products of immune response, bile salts, proteases and lipases which attack on trophozoite to remove it from the intestine. The parasite protects itself by process of antigenic variation (Carranza et al. 2010).

The dense coat of variable surface proteins (VSP) is present on the surface of trophozoite. Single VSP is dominated during the course of infection. Due to on-off switching of gene expressing VSP, different VSP are expressed during infection, resulting in parasite escape from host immune response. Till date, there are no identical VSP sequences observed among three strains (Jerlström-Hultqvist et al. 2010). Additionally, the involvement of proteins having toxin like activities are also hypothesized but no such protein is identified so far. The four main parasitic proteins that are involved in pathogenesis include elongation factor 1-α, arginine deiminase (ADI), α-enolase and ornithine carbamoyl transferase (OCT) (Skarin et al. 2011). In specific, ADI and OCT interfere in the synthesis of nitric oxide, which is usually cytotoxic for the parasite, resulting in inhibition of innate response by the host. These proteins cause damage to L-arginine, which is needed by the epithelial cells of the host for synthesizing nitric oxide with the help of nitric oxide synthetase (Rópolo et al. 2010). The epithelial cell damage in giardiasis is the key pathogenic change. Patients suffering from chronic disease exhibit a significant damage to the enterocytes, which has also been observed in *in vitro* experiments as well (Troeger et al. 2007). Proapoptotic caspase-3 and 9 appear to induce the process of apoptosis by increasing the pro-apoptotic Bax which ultimately decreases the anti-apoptotic Bc1-2 expression, resulting in the cleavage of poly-ADP ribose polymerase (PARP). After initiation of apoptosis, *Giardia* trophozoite weakens the junctions between enterocytes by breakdown of proteins. During the process of apoptosis, claudin-1, F-actin, α-actinin and zonula-occludens-1 (ZO-1) are relocated to cytosol (Cotton et al. 2011).

Due to caspase-3 inhibition, the relocation of F-actin and ZO-1 is stopped, which indicates a direct relationship between enterocytes barrier function and apoptosis induced by *Giardia* trophozoite. The breakdown in the cells barrier results in the entrance of electrolytes in the sub-mucosal space after bypassing the normal epithelial cells uptake. Nutrient malabsorption occurs due to paracellular uptake of electrolytes, which leads to the activation of innate immune response (Solaymani-Mohammadi et al. 2010). The response of host to parasite also plays a significant role in pathogenesis of the disease. All kinds of proteins secreted from the parasite, including

VSP and major disc protein, are recognized by host sera which is also demonstrated in experimental infection to mice as well and indicates its importance regarding antibody-mediated Giardia immunity. According to a study, IgA antibodies have been recovered after induction of giardiasis to a murine model, indicating its importance in the development of protective immunity (Singer et al. 2000).

The role of T-cells in protection against giardiasis has also been demonstrated in literature. In a study, T-cell deficient mice and patients suffering from immunodeficient syndromes developed chronic giardiasis, indicating the importance of T-cells. Furthermore, there is CD8 and lymphocytes dependent shortening of microvilli, resulting in decreased water, nutrients and electrolytes absorption, as demonstrated in murine model (Scott et al. 2004).

Similarly, in animals, giardiasis results in the decreased crypt to villus ratio and deficiency of brush border enzymes. The severe infection in animals results in the mal-absorptive diarrhea and low weight gain. Diarrhea is a pathognomonic sign for diagnosis similarly as in humans. The disease in calves ranges from acute diarrhea to chronic and intermittent sings. In a study, after experimental infection with assemblage B of *Giardia*, the malabsorptive diarrhea in lambs and goat kids resulted (Aloisio et al. 2006). In another study, decreased feed efficiency and weight gain was noticed in lambs after experimental infection with *Giardia* (Geurden et al. 2010). Therefore, *Giardia* is considered as a potential cause of diarrhea in production animals, resulting in decreased production and economic losses (Geurden et al. 2006).

Cats and dogs exhibit asymptomatic infection of giardiasis, having more severe infection in immature animals. Sometime, infection may causethe acute diarrhea in very young animals but adults remain asymptomatic during the infection. Clinical signs in cats are particularly uncommon (Ballweber et al. 2010).

Diagnosis of *Giardiasis*

Giardiasis is usually diagnosed on the basis of clinical symptoms, environmental condition and by exclusion of other infectious diseases. Its diagnosis is not straightforward due to vagueness of clinical symptoms. Traditionally, Giardiasis is confirmed by identification of trophozoites and cysts in faecal samples through microscopy either by floatation or sedimentation methods (Cama et al. 2015). In the chronic phase of infection, the cyst stage of parasites is released from the host intermittently. Therefore, multiple sampling is sometimes necessary for consecutive three days. In case of animals, young animals of 2-4 weeks age are recommended to be included in sampling method, as they exhibit peak excretion of cysts without displaying clinical disease. Due to no increase in antibody titer after infection of *Giardia*, serum antibody test is not recommended for diagnosis (O'Handley et al. 2003). Apart from microscopy, there are many other methods which can be used for correct diagnosis of the infection. These are biochemical, serological, immunological and molecular methods. Molecular characterization is preferred due to high specificity and sensitivity (Thompson et al. 2004).

Microscopic Examination

Both cysts and trophozoites are considered as diagnostic stages for detection of giardiasis. These can either be detected through direct microscopy of fecal smear or after treating with floatation solutions i.e., sodium nitrate, zinc sulfate or sucrose. The specificity of diagnostic test is improved when performed with standard protocols (Dryden et al. 2006). Fat malabsorption and diarrhea are the characteristic features of giardiasis, which can interfere in the process of floatation after treating with sucrose, but this problem can be addressed by treating the sample with chloroform. Sometimes, trophozoite stage can be detected from diarrheic samples but immediate microscopic observation is required to observe trophozoites due to their characteristic movement. Alternatively, microscopic observation of the cyst is preferred over trophozoite detection which is mostly present in faeces (Elmi et al. 2017). Staining is usually done to observe the cyst under microscope. The most frequently used stains for giardiasis include Iodine, Zeil Nelson stain and modified Acid-Fast stain. Parasitic cyst and fecal debris can easily be separated by using floatation technique. Separation is done on the basis of difference of specific gravity. High specific gravity liquids, such as NaCl, $NaNO_3$, $ZnSO_4$, are used as floatation solutions. In comparison, sedimentation method requires centrifugation to obtain parasitic cyst from the sediment, which makes the process of diagnosis difficult. The cyst stage of parasite is preserved in 10% formalin solution. (Smith et al. 2011). The main advantage of microscopic method is its limited cost, but it requires skilled and experienced person for identification. Moreover, microscopy is less sensitive than immunological techniques (Geurden et al. 2004). The chances of false positive results are more in microscopy due to small size of *Giardia* cyst, which is sometimes confused with pseudoparasites (yeast, paricles) (Dryden et al. 2006).

Antigen Detection

There are many immunological methods commercially available for the detection of parasitic antigen, including rapid solid phase qualitative immunochromatography assay, immunofluorescent assay (IFA) and enzyme linked immunosorbent assay (ELISA) (Garcia et al. 2003). These techniques were originally developed for diagnosis of infection in humans. Monoclonal antibodies are used against protein of cyst wall in ELISA and IFA techniques. EPG score of 1000 could easily be detected by immunofluorescence assay (Vidal et al. 2005). For diagnosis of infection in calves, immunological techniques are considered as more specific and sensitive than microscopy (Geurden et al. 2004). Similarly, in dogs IFA is found more sensitive technique for diagnosing giardiasis (Geurden et al. 2008). The main disadvantage of using any

immunological technique is its elevated cost due to expensive laboratory instruments and trained personnel.
The alternative technique for diagnosis is the immunochromatographic assay, which enables field-based diagnosis of infection within 15 minutes. In this method, monoclonal antibodies are used against parasitic cyst or trophozoite stage (Garrett et al. 2006). In human medicine, various assays including rapid membrane assay and dip-stick etc. have become commercialized for quick detection of infection. Similarly, SNAP® Giardia test is available in veterinary science for detection of infection in animals, particularly in dogs (Geurden et al. 2008). Speed® Giardia is an immunochromatographic assay commercially available for detection of infection in production animals (Geurden et al. 2010). Fecal ELISA kits are also commercially available for cats and dogs.

Polymerase Chain Reaction

Polymerase chain reaction is a molecular technique used for species identification and genotyping of Giardia. This technique has limited diagnostic uses and mostly used in research laboratories for sub-typing purpose, in order to determine assemblage or sub-assemblages of Giardia (Hooshyar et al. 2017). For molecular studies of Giardia species, the target gene sequences, including genes encoding small subunit (SSU) ribosomal RNA, glutamate dehydrogenase (gdh), triosephosphate isomerase (tpi) and β-giardin genes (a protein in the adhesive disk of Giardia) (Hooshyar et al. 2019). The most commonly used gene for genotyping and diagnosis of Giardia is 18S rDNA (Read et al. 2004). PCR is highly sensitive test, as it can detect even 1 cyst in the sample (Amar et al. 2002). DNA inhibition may interfere in correct diagnosis. Furthermore, DNA extraction from faeces also needs to be standardized (Da Silva et al. 1999). Currently, PCR is extensively used in diagnosis of human infection but has very limited use in veterinary science due to the need of expensive instruments. It is yet to be evaluated as a diagnostic assay in production animals (Hooshyar et al. 2017). Moreover, another technique that can be used for diagnosing giardiasis is Fluorescent *in situ* hybridization (FISH), in which targeted sequences within RNA or DNA can be detected specifically with the use of fluorescent-labelled probes or oligonucleotides (Amann et al. 1995).

Zoonotic Transmission

Both host specific and zoonotic *Giardia duodenalis* can be harbored by the animals and are morphologically identical. Therefore, molecular typing is needed to trace the transmission of parasites (Feng et al. 2011).
Zoonotic transmission of Giardia from cattle to humans has been documented in numerous studies. The major way of transmission is through direct contact between cattle and humans. In addition, zoonotic transmission may also occur due to contaminated surface and ground water (Budu-Amoako et al. 2012). Calves play a major role in zoonotic transmission as they can shed up to 105-106 cysts per gram of faeces. Mostly the infection in cattle is caused by assemblage E of *Giardia duodenalis* and assemblage A infection occurs rarely and only in young animals (Mark-Carew et al. 2013).
There is very limited data available regarding zoonotic transmission of giardiasis from cattle to humans. In a study conducted in India, genotype A1 was identified from both cattle and workers from the same farm. However, cattle are mostly infected with sub-assemblage AI of Giardia, while humans are mostly infected with sub-assemblage AII of parasite (Xiao et al. 2008), indicating zoonotic transmission of parasite from cattle to farm workers (Khan et al. 2011).
The role of dogs in zoonotic transmission of Giardia is very important and is a subject of intense investigation. The dogs in urban areas of developed countries play a vital role in zoonotic transmission (Ballweber et al. 2010). People living in cities often have a close association with one another and sometimes they may live with pets i.e. dogs and cats. They consider them as their true and loyal friends, but some other benefits are also seen, like emotional and physiological attachment and social development. Canines and felines can also serve as a reservoir for a large number of zoonotic parasites like Giardia. These parasites can enter into the body of host through multiple routes, like mucosa and faeco-oral route. Humans serve as the final host for more than 100 parasites. It has been revealed that 85% of adult people have parasitism in their bodies (Martinez-Moreno et al. 2007). Cats and dogs are also proved to adopt non-zoonotic host-specific assemblages of Giardia after molecular surveys. Although, dogs and cats are infected with assemblage A and B of Giardia, but it is very difficult to access the frequency of zoonotic transmission without obtaining data from the owner (Feng et al. 2011).
There are numerous studies which were performed on a specific focus, involving both pet animals and humans and gave very interesting results. A study performed in tea growing area of India (Assam) indicated identical genotypes circulating between humans and dogs. The molecular evidence of this was not very convincing, however epidemiological data showed a significant relationship between prevalence of Giardia in human and household dogs (Traub et al. 2004). The zoonotic species of Giardia was also reported in both dogs and humans of Temple communities of Bangkok (Inpankaew et al. 2007) and indigenous communities in North Canada (Salb et al. 2008). Alternatively, another study which was performed in endemic regions of Peru showed different assemblages of Giardia in human and dogs, indicating the zoonotic transmission from dogs to humans is very uncommon (Cooper et al. 2010). Therefore, it is a matter of intense research to identify the zoonotic potential of Giardia from dogs to humans.
Cross transmission of Giardia between different hosts is possible. Wild animals living in aquatic environment can also be source of spread of giardiasis to human. In 1980 in Canada giardiasis outbreak occurred in beavers after consuming municipal water. The isolated cysts of Giardia from beavers were found identical to the cysts found in gerbils and later on it was confirmed by growing these

cysts under *in vitro* condition in laboratory (Appelbee et al. 2002). That's why giardiasis is referred as "beaver fever" in Canada. According to a study, cyst from human sources can also cause infection to beavers but needed in large quantity. Although it remained unclear that either beavers were the source of infection or they got infected by cysts from human source (Erlandsen et al. 1988).

The transmission of *Giardia duodenalis* is also possible from monkeys to humans. According to a study performed on rhesus monkey (*Macaca mulatta*) in China, assemblage A and B were found in the monkeys living in public parks. Assemblage AII is the main source of human infection and some strains of assemblage B were also detected from human sources in China, indicating the possibility of zoonotic transmission from monkeys (Ye et al. 2012).

Giardia can also be transmitted to humans from wild primates. A study conducted at Uganda indicated high degree of cross transmission of *G. duodenalis* among wild primates, livestock and humans at locations where humans and livestock interact at high degree with wildlife (Ghariebet al. 2016).

Treatment and Control

There are several compounds with known *in-vitro* and *in-vivo* efficacy against Giardia, and this has also been demonstrated in laboratory animals. There is no published data available regarding good efficacy of any treatment against giardiasis in sheep and goats. Although different studies were conducted on ruminants to evaluate the efficacious treatment against the disease (Gultekin et al. 2016; Karademir et al. 2016; Santin 2020), but no licensed treatment is available in ruminants yet. However, several compounds were evaluated and found to be effective in dogs and cats.

Chemotherapeutic Treatment

In human medicine, nitro-imidazoles (NZs), including furazolidone, metronidazole, are frequently used to treat giardiasis. Although, there are chances of side effects from these compounds, yet these show very good efficacy in humans against giardiasis. Metronidazole is even considered as carcinogenic in nature. Furthermore, there are also chances of resistance development against both metronidazole and furazolidone (Harris et al. 2001).

In veterinary medicine, dimetridazole and metronidazole are used against giardiasis in companion animals and ruminants and exhibit very good efficacy. In cats, metronidazole and dimetridazole is used for a period of 15 days in order to achieve cyst reduction. In some countries, the use of nitro-imidazoles in farm animals is contra-indicated (Scorza et al. 2004).

Furthermore, nitazoxanide has proved to be a promising drug against giardiasis in *in-vitro* trials (Cedillo-Rivera et al. 2002). According to Zygner et al. (2008), azithromycin is also effective against giardiasis in companion animals but only few numbers of animals were included in the trial.

Benzimidazoles

Benzimidazole compounds (BZs) belong to the class of broad spectrum anthelmintics and can be used against giardiasis due to their broad safety margins and less toxicity. In *in-vitro* trials, BZs have also proved to be more efficacious than metronidazole against Giardia. Tubulin is the structure found in the trophozoite cytoskeleton and BZs interfere in its polymerization, resulting in inhibition of all activities of ventral disc and median body. As a result, trophozoite will not be able to get attached and colonized in intestine. BZs seem to be ineffective against flagellar tubulin due to their different structure. BZs also get attached with giardin which is a Giardia-specific protein present in the ventral disc (Shaharyar et al. 2017).

In calves, use of albendazole and fenbendazole in combination showed promising results regarding decrease in cyst excretion. The total dose of benzimidazole used against giardiasis (5-20 mg/kg/day for three consecutive days) is higher than that used against helminth infection (Leitsch et al. 2015). According to another study, BZs showed very limited cyst suppressing effect against giardiasis in calves under field condition. This might be due to high infection pressure, less efficacious treatment or rapid re-infection. Due to less efficacy, it was concluded that calves need continuous treatment with BZs for long period of time in low dosage (O'handley et al. 2000). However, the continuous treatment with BZs may lead to a resistant development in animal.

In dogs, fenbendazole, oxfendazole and albendazole are used against giardiasis and show very good results regarding cyst elimination and alleviation of clinical symptoms. The recommended treatment is for consecutive three days along with good hygiene practices. Furthermore, there are few reports of albendazole causing bone marrow depletion and carcinogenic effects, specifically in pregnant bitches (Chon et al. 2005). In cats, fenbendazole is not recommended against sole giardiasis infection due to its less efficacy to remove cysts but it shows good efficacy against co-infection of Giardia and Cryptosporidium (Keith et al. 2003).

Pyrantel-febantel-praziquantel combination

Various studies have shown that combined use of pyrantel, febantel and praziquantel in dogs and cats resulted in a significant reduction in cyst excretion (Payne et al. 2002; Scorza et al. 2006; Montoya et al. 2008; Bowman et al. 2009). Another study suggested that combination of pyrantel and febantel showed very promising results compared to febantel alone (Olson et al. 2009).

Paromomycin

Paromomycin is a broad-spectrum antibiotic, belonging to the amino-glycoside group and shows very good efficacy against giardiasis in humans (Wright et al. 2003). It inhibits the synthesis of proteins after binding with small subunit of rRNA, resulting in either destruction of Giardia

or withdrawal of its nutrients (Rossignol et al. 2010). It is well tolerated by calves due to prolonged absorption period from gastro-intestinal tract (Grinberg et al. 2002). The recommended dose in calves is 50-75 mg/kg/day for a period five consecutive days (Geurden et al. 2006).

Control

Although the treatment of giardiasis by chemotherapy showed good results, even then most of treated animals show re-infection after several days (2-3 weeks) due to contaminated environment (Geurden et al. 2006). The cysts of this parasite can survive for 7 weeks in soil and 1 week in feces, while the treatment is mostly done for three to days which is very short to prevent occurrence of re-infection in a contaminated environment. So, there is need of integrated control approach, including both treatment of infected individuals and cleansing of environment in order to minimize the chances of re-infection. Giardia cysts survive well after disinfectant treatment like chlorine. Ultraviolet irradiation, chlorine dioxide and ozone are included in the category of disinfectants which can be used in drinking water treatment for research purpose and strongly contra-indicated to be used in calf facility (Saleh et al. 2016). Alternatively, quaternary ammonia disinfection and heat treatment can be used safely in housing facilities. According to a study, when environment is disinfected with 10% ammonia along with treatment of animals with fenbendazole, the efficacy of treatment is improved, resulting in the reduction of excreted cysts from animals. This indicates that treatment efficacy can be improved effectively by breaking the transmission cycle of the parasite (Geurden et al. 2006).

As the chances of getting re-infection in companion animals from fecal contaminated limbs or fur increased in contaminated environment, so the practice of introducing the treated animals into a clean environment is emphasized. Animals should be washed after treatment and before introducing in to a clean environment (Uehlinger et al. 2007).

Good management is the key practice to achieve good curative results after every treatment regimen. There are several management practices that can prevent the occurrence of infection if applied correctly, including proper cleaning of housing facilities and limiting the number of animals per housing facility (Maddox-Hyttel et al. 2006). Indoor animals are more likely to get giardiasis infection than outdoor animals, indicating the importance of housing facilities management (Itoh et al. 2009). As Giardia cysts can persist for long period of time on moist areas, the management of manure is another alternative approach to reduce the infection load at farm level (Van Herk et al. 2004).

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