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Review article

Enteric protozoal infections in camels: Etiology, epidemiology, and future perspectives

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Abstract

Camels have great potential as a safety valve for current and future food security for pastoralists, agropastoralists, and urban populations. Enteric protozoal diseases are important causes of economic losses in camels; however, they are poorly concerned globally. The most common members of enteric protozoa are *Balantidium*, *Eimeria*, *Giardia*, and *Cryptosporidium*. Some of them threaten human health as humans can be infected by consuming food or water contaminated with camel feces, particularly in poor communities with inadequate sanitation and low-quality healthcare facilities. For these reasons, a comprehensive and careful investigation was conducted on some enteric protozoal diseases of camels to present an updated insight into the etiology, epidemiology, and future trends in diagnosing and controlling camel enteric protozoa. Future studies on the camel enteric protozoa should be carried out to develop advanced diagnostic approaches in diverse farm animal species. Moreover, the protozoan zoonotic potential should be considered to secure human health.

Keywords: Camel, Enteric protozoa, Epidemiology, Diagnosis, Treatment

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Introduction

Camels are multipurpose animals used for meat, milk, wool, hide production, transportation, races, agricultural work, and tourism. They are considered the last species domesticated by man 30-40 centuries ago. Camels have unique physiological and anatomical properties that enable them to live, reproduce, produce milk and meat, and work under severe conditions of heat and aridness, even during periods of drought when other animals barely survive (Abdalla et al., 2018; Abd El Hamid, 2021).

Officially, the total number of the world camel population is 35,525,270 heads. Of these, about 80% of them live in Africa, with 60% in the Horn of Africa (FAOSTAT, 2020). Camels in Egypt are members of the Camelus dromedaries family, known as the Arabian camel (Al-Swailem et al., 2010), and constitute 95% of the world's camel population (FAOSTAT, 2019). Camels were introduced to Egypt after domestication in other Arab countries between 2500 and 1400 B.C (Saber, 1998). Camels are reared in different regions throughout Egypt, including desert regions, the Nile delta, oases, and farming villages (FAOSTAT, 2017). Egypt has 159.000 camels accounting for 1.1%, 0.9%, and 0.7% of camels raised in Arabian countries, Africa, and all over the world, respectively. The world's camel populations have a remarkable yearling growth rate considered moderate in Egypt (Faye, 2015). The breeds of camels that are raised in Egypt are Sudani (common for riding, racing, and meat), Falahi or Baladi (used for transportation and agricultural operations), Maghrabi (dual-purpose, for meat and milk), and Mowallad (hybrid between Maghrabi and Falahi) (Wardeh, 1991).

Lack of animal protein (especially meat) especially in some parts of Africa and Asia has made the demand for camel meat an increasingly important safety valve for future food security. Camel's meat is of high qual-

ity as camel's meat has high glycogen content, less fat, less cholesterol, relatively high polyunsaturated fatty acids like linoleic acid, and essential amino acids than other meat animals (Badawi, 2018).

Imported camels in Egypt come from different African localities, especially Somalia and Sudan, the most camel-populated countries. Camel importation from these countries leads to the occurrence of exotic diseases due to the free movement of camels throughout the borders, which leads to the transmission and spreading of diseases (Abdalla et al., 2018). Camel diseases negatively affect production, especially without adequate veterinary services (Megersa, 2010). High mortality rates among newly born camel calves have been occurring early due to neonatal diarrhea and pneumonia, leading to a decrease in birth and population growth rates (Abdalla et al., 2018). Camel calf diarrhea is a multi-factorial and complex syndrome involving the physiological factors of the calf, the environment, management, and nutrition factors, and infectious agents, and considered the most common cause of mortality in camels all over the world (Salih et al., 1998; Nasr and Meghawery, 2007; Megersa, 2014). Traditional practices such as colostrum overfeeding or colostrum withdrawal are considered possible predisposing factors for diarrhea in dromedary calves (Khanna, 1992).

Several camel outbreaks occur due to contamination of water or food with some intestinal protozoa that have public health importance with several economic losses (Mak, 2004). Meanwhile, little is known about the zoonotic importance of intestinal protozoa and their genetic structure in dromedary camels (El-Alfy et al., 2019). Various outbreaks due to contamination of water or food with camel enteric protozoa have further explained their importance in public health (Mak, 2004). In addition, they cause serious economic losses due to enteritis, diarrhea, poor weight gain, impaired milk and meat production caused by malnutrition, due to intestinal malabsorption, impaired fertility, and low calving rates (Huetink et al., 2001; Al-Megrin, 2015; Innes et al., 2020; Santin, 2020).

Diseases caused by enteric protozoa in camels

Balantidium coli (*B. coli*) of camel

Balantadiasis is caused by *Balantidium coli* (*B. coli*), a zoonotic ciliated protozoan that affects a wide range of hosts, including pigs, camels, ruminants, equines, and even humans. In addition, they have reservoir hosts, including wild and domestic pigs (Schuster and Visvesvara, 2004; Ahmed et al., 2020). In Islamic countries, pig consumption is prohibited, so human infection seems rare. However, human balantidiasis has been reported, and camel has been proposed as one of the reservoir hosts for *B. coli* in Islamic countries (Cox, 2005). Clinical signs of balantadiasis vary from asymptomatic to severe dysenteric forms, such as loose feces to persistent fetid watery diarrhea, loss of appetite, poor body condition, dehydration, retarded growth, and reduced production of the animals (Yazar et al., 2004). So, balantadiasis has severe economic losses for the production and reproduction sectors (Palanivel et al., 2005; Roy et al., 2011).

The life cycle of *B. coli* is simple (Figure 1). Two life stages: cyst and trophozoite. Trophozoites are active, motile feeding and replicating stages found in the large intestine. The ovoid-shaped body of this type has two nuclei (micro and macronucleus) and cilia. The cyst is a non-replicating, resistant form that develops in the colon and is expelled in the feces, and it is responsible for balantidiasis transmission (Schuster and Ramirez-Avila, 2008; Roy et al., 2011). Following ingestion, the cysts were excystated in the small intestine, resulting in the release of trophozoites. These trophozoites migrate to the large intestine, colonizing the lumen and feeding on intestinal bacteria. Balantidia reproduces by binary fission (asexual reproduction) or conjugation (sexual reproduction) (Ahmad and Rasad, 2011). Cysts can survive in the environment for ten days at room temperature outside the host body and persist for weeks in feces under ideal conditions. Whereas trophozoites die within hours of leaving the host body, or they may be infective, according to a study in captive great apes (Schuster and Visvesvara, 2004; Pomajbíková et al., 2010).

Eimeria species of camel

Coccidiosis is a disease caused by a host-specific gastrointestinal protozoan reported in camels (Radfar and Aminzadeh, 2013); however, little information is available known about the prevalence of coccidian parasites in Egyptian camels (Abbas et al., 2019). Five *Eimeria* species (*E. cameli*, *E. dromedarii*, *E. bactriani*, *E. rajasthani*, and *E. pellerdyi*) are believed to have the capability of infecting camels (Dubey et al., 2018). However, *E. cameli* and *E. dromedarii* are mostly associated with disease conditions (Djerbouh et al., 2018). Previous studies also mentioned that *E. rajasthani* is a pathogenic species for young camel calves (Yakhchalim and Cheraghi, 2007).

Eimeria is monoxenous (requiring only one host to complete its life cycle). Its life cycle consists of an exogenous phase (sporogony) involving a free-living phase outside the host and a parasitic endogenous phase inside the host. In the host, both asexual and sexual reproduction cycles are observed. Non-sporulated oocysts pass with the feces, and sporulation occurs after 2-7 days, based on species and the environmental conditions, e.g., temperature, oxygen, and moisture are the most important factors influencing sporulation (Chartier and Paraud, 2012) (Figure 2).

The intracellular pathogens are mostly characterized by their resistance to different antimicrobials, so further studying their interaction with host cells is increasingly essential (Zhu et al., 2021a,b; Zhao et al., 2022). *Eimeria* spp. is a gut-dwelling obligatory intracellular intestinal coccidian parasite in which infected animals shed oocysts in feces, and infection is transmitted by fecal-oral route between animals (Al-Megrin, 2015; Al-Afaleq et al., 2018; Abbas et al., 2019). It is considered pathogenic to camel calves due to the destruction of the intestinal mucous membranes by their

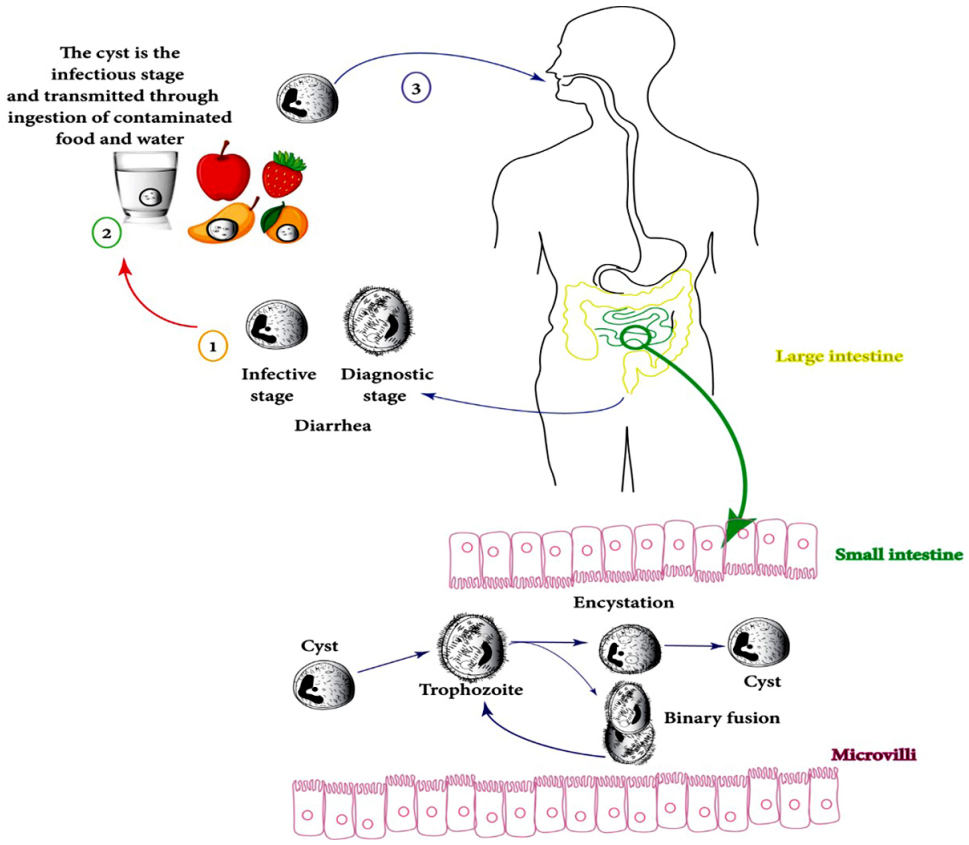

Figure 1: Life cycle of *B. coli*.

giant schizonts. Adult camels were found to be asymptomatic oocyst-shedding carriers or chronic shedders of oocysts without manifesting clinical signs (Kasim et al., 1985; Mahmoud et al., 1998). *E. cameli*, with the largest oocysts, is considered the most pathogenic protozoa. Its gametogenic stages and oocysts development are described in the lamina propria of the small intestines of naturally infected camels. The existence of sexual stages only has been confirmed in camels (Dubey et al., 2018).

Giardia lamblia (*G. lamblia*) of camel

Giardiasis is caused by *G. lamblia* (Synonyms: *G. duodenalis*, *G. intestinalis*), an eukaryotic, unicellular, flagellated pathogenic intestinal protozoan parasite in the small intestine of the host (Adam, 2001). It affects many species worldwide, including humans (Akkad et al., 2002). The high significance of giardiasis infection is because of both the low infectious dose and the tolerance to disinfection of the environmental cyst stage of the parasite (Smith, 1999).

Giardia species have a simple life cycle with two stages: the cyst (the infected stage) and the trophozoite (Figure 3). When the host consumes cysts in the duodenum, they exist, releasing two trophozoites. Trophozoites are not invasive and repeatedly divide through mitosis on the small intestine's mucosal membrane. Later, trophozoites transform into cysts (the environmentally resistant form) that are expelled in the feces in exposure to bile salt and other conditions present in the gut. Factors that lead to the successful spread of giardiasis included releasing a large number of cysts into the environment from infected hosts, cysts becoming instantly infectious after excretion and can survive for a long time under suitable conditions (cold temperatures and moisture), and finally, the low infectious dosage (Erickson and Ortega, 2006; Ryan et al., 2019).

Cryptosporidium spp. of camel

Cryptosporidiosis is one of the most important zoonotic diseases caused by *Cryptosporidium* spp., an obligate intracellular protozoan parasite belongs to the Apicomplexa phylum of parasites (Fayer, 2004; Ryan et al., 2014; Khan et al., 2018; Yang et al., 2021). Infected humans with cryptosporidiosis had a history of close contact with domestic animals like camels. This pathogenic protozoan causes chronic diarrhea in those who have immunosuppression but may induce only acute self-limiting enteritis in those with an intact immune system (Garber et al., 1994; Causapé et al., 2002; Graczyk et al., 2003; Joachim, 2004; Mirzaei, 2007; Razavi et al., 2009; Nichols et al., 2014; Yakhchali, 2016).

There are 20 species of *Cryptosporidium* that have been reported to infect mammals (Fayer, 2010), and over 40 genotypes were described without species names (Xiao et al., 2004). The most common species infecting camelids are *C. parvum*, *C. muris*, and *C. andersoni*, with public health importance (Wang et al., 2008). *C. parvum* is the most significant in medical and veterinary aspects (Morgan-Ryan et al., 2002). The

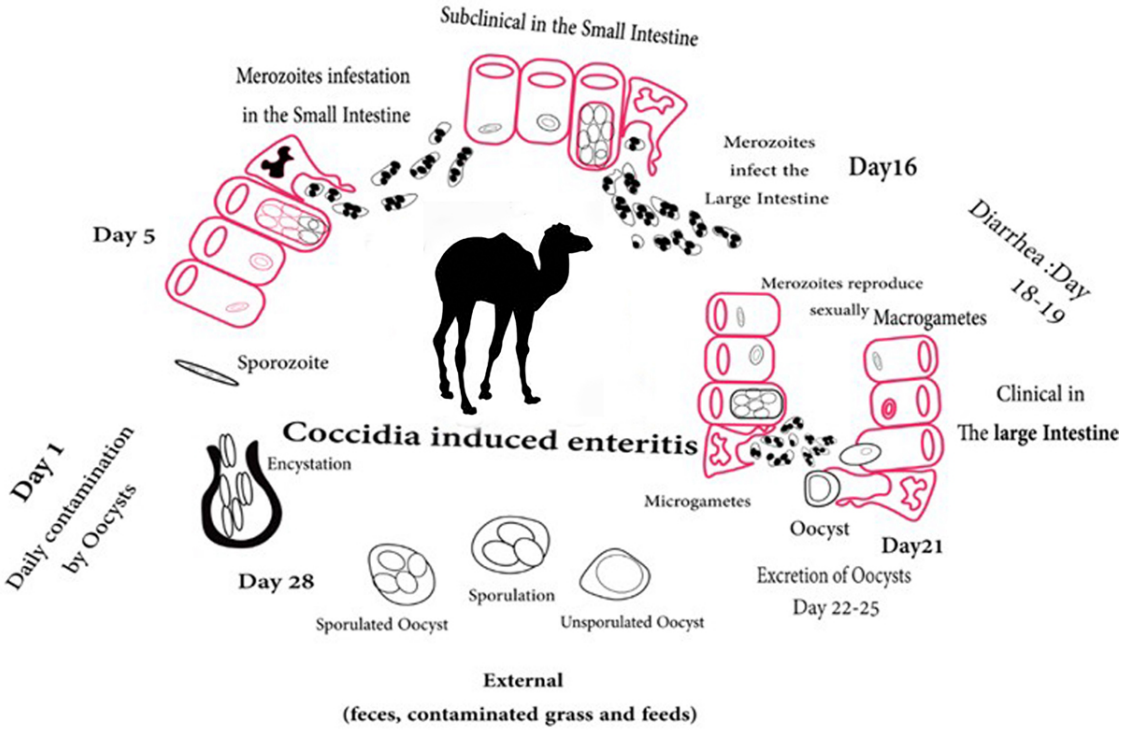

Figure 2: Life cycle of *Eimeria* spp.

pathogenicity of *Cryptosporidium* is different according to species, age, and the immune status of the host (Fayer, 2004; Šlapeta, 2009). Infected hosts with cryptosporidiosis shed up to $10^{5\text{-}7}$ oocysts per gram of feces at the peak of the infection. The oocysts are immediately infective, and because of their solid protective wall, they can survive more than 120 days in soil and for many months in moist conditions and water without losing their infectivity (Fayer, 2004; Medema et al., 2006).

The life cycle of *Cryptosporidium* spp. is mainly similar to the coccidian life cycle (Figure 4), affecting mammals (Bamaiyi and Redhuan, 2017). The life cycle begins with exocystation, which involves the release of infective sporozoites. Next, asexual multiplication (Merogony) occurs within the host's cells, followed by the formation of microgametes and macrogametes (gametogony). Then fertilization, the fusion of the microgametes and macrogametes, occurs, followed by oocyst wall formation, which is needed to form an environmentally resistant stage responsible for the transmission of infection from one host to another. By the end of the life cycle, infective sporozoites within the oocyst wall (sporogony) are formed.

Epidemiology of camel enteric disease

Agent, host, and environment are three important factors influencing the epidemiology of protozoal infection. The agent-associated factors are protozoal virulence, pathogenicity, immunogenicity, and viability. The host factor includes the animal's species, age, sex, breed, resistance, immune system, and nutrition status, while the environmental factors include humidity, temperature, food, and maintenance systems (Budiharta and Suardana, 2007).

Host range

B. coli is a protozoan parasite in the large intestines of pigs, mice, humans, and nonhuman primates. This parasite has been shown to infect a wide variety of domestic and wild mammal species. However, camels, cattle, buffaloes, sheep, goats, ostriches, chimpanzees, horses, donkeys, and dogs are not common hosts (Ahmed et al., 2020). In healthy animals and humans, *B. coli* is a commensal organism. However, it is believed that under certain conditions, *B. coli* might behave as an opportunistic pathogen by invading damaged intestinal epithelial cells caused by other infectious agents (Headley et al., 2008).

In all vertebrate classes, *Eimeria* is an obligate intracellular parasite (Carruthers and Tomley, 2008), with absolute host and tissue specificity (Hofmannová et al., 2018). Still, they are easily transmissible between congeneric hosts (Ogedengbe et al., 2011). Five *Eimeria* spp. (*E. cameli*, *E. dromedarii*, *E. bactriani*, *E. rajasthani*, and *E. pellerdyi*) are believed to have the capability of infecting camels (Djerbouh et al., 2018; Dubey et al., 2018). However, *E. cameli* and *E. dromedarii* are primarily associated with disease conditions (Djerbouh et al., 2018). Previous studies also referred that *E. rajasthani* is a pathogenic species for young camel calves (Yakhchalim and Cheraghi, 2007).

Intestinal flagellate *G. intestinalis* infection affects not only a wide range of domestic and wild animals; but also humans (Hunter and Thompson, 2005). *G. intestinalis* attach and colonize the host small intestine,

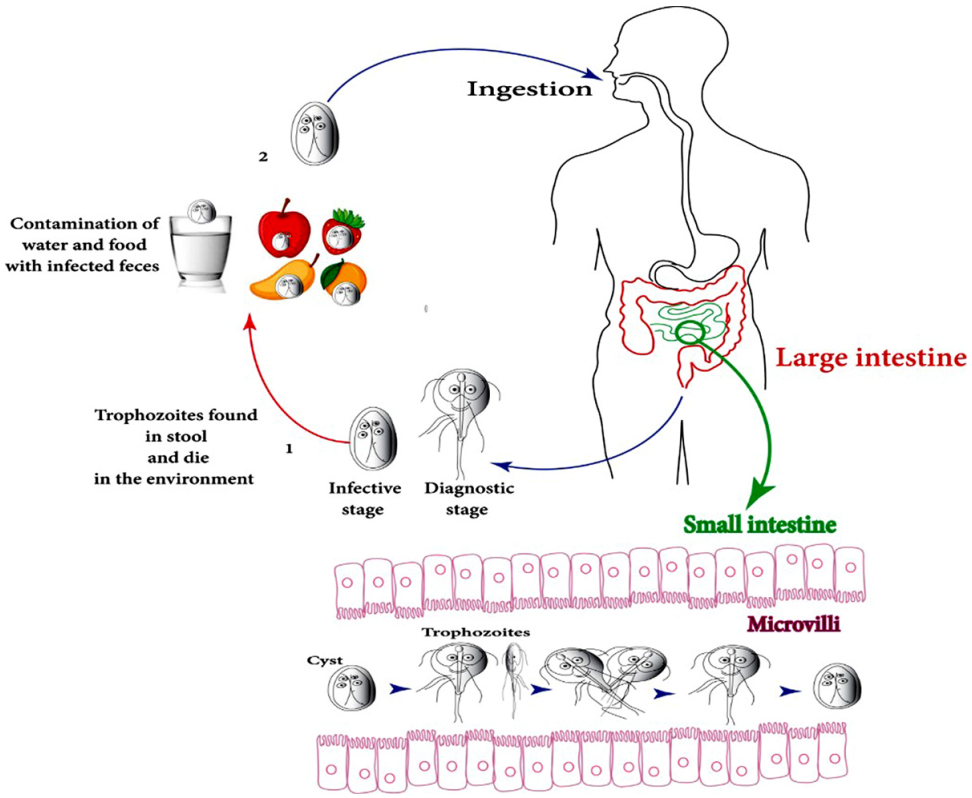

Figure 3: Life cycle of *Giardia* spp.

and its life is divided into two stages: resistance, dormant cyst stage and multiplying, and motile trophozoite stage (Daly et al., 2010). No literature about *Giardia* spp. infection in camels is available (Al-Ani, 2004); however, *Giardia* spp. in the feces of a young llama with diarrhea was found (Kiorpes et al., 1987), which may indicate that camelids, to a lesser extent, are susceptible to Giardiasis (Al-Ani, 2004). Birds, horses, cattle, sheep, camels, rats, and dogs have all been documented as hosts for *Cryptosporidium* spp. (Ranjbar et al., 2018).

Transmission

Balantidiasis is a disease spread through food and water. The fecal-oral route and contact with diseased humans and animals are the main transmission mechanisms for this protozoan parasite. There is no vector or intermediary host (Schuster and Visvesvara, 2004; Schuster and Ramirez-Avila, 2008). The host animal is infected after ingesting cyst-contaminated food or water. Trophozoites are regarded to be unable to survive in stomach conditions (Schuster and Visvesvara, 2004). However, a cyst is considered an infective stage because of an outside protective wall (Wisesa et al., 2015). Poor sanitation and contamination of water resources with contaminated feces are the main causes of transmission in developing countries (Thompson and Smith, 2011; Plutzer and Karanis, 2016). In developed countries, transmission is primarily caused by occasional failures in the sanitation process (Bellanger et al., 2013).

Coccidiosis is a disease transmitted by the fecal-oral route (Chartier and Paraud, 2012). The oocyst of *Eimeria* spp. is characterized by high resistance to environmental conditions (Utebaeva et al., 2021). *G. intestinalis* transmission occurs through direct animal-to-animal contact, fecal soiling (mostly), water contamination, and indirectly by ingestion of water or food contaminated with cysts (water and foodborne transmission) (Taylor and Webster, 1998; Ryan et al., 2019). It is endemic in humans, animal populations, and environmental sources, so complete eradication is likely impossible (Vesy and Peterson, 1999).

Cryptosporidiosis was transmitted by the fecal-oral route to various vertebrates, including humans, and induced pathologic changes in them (Krumkamp et al., 2021). The sample is considered positive when at least one oocyst with the correct morphological characters (4-6 μm, spherical containing a residuum, sporozoites, and usually within a clear halo, against a blue background) is detected (Baxby et al., 1984). Oocysts might not be seen in clinical samples from all cryptosporidiosis cases, and the absence of oocysts from symptomatic hosts does not necessarily indicate the absence of infection. In these cases, and particularly when clinical signs are high, oocyst-negative feces samples should be subjected to another antigen detection, such as sufficient *Cryptosporidium* antigen from asexual life cycle forms should be present (Smith, 2008).

Risk Factors

Inadequate sanitation, hot and humid temperature conditions in subtropical and tropical areas, the presence of *B. coli* infected animals, debilitating diseases, and malnutrition in the host population are all thought to be major factors that promote parasite spread (Giacometti et al., 1997; Solaymani-Mohammadi and Petri,

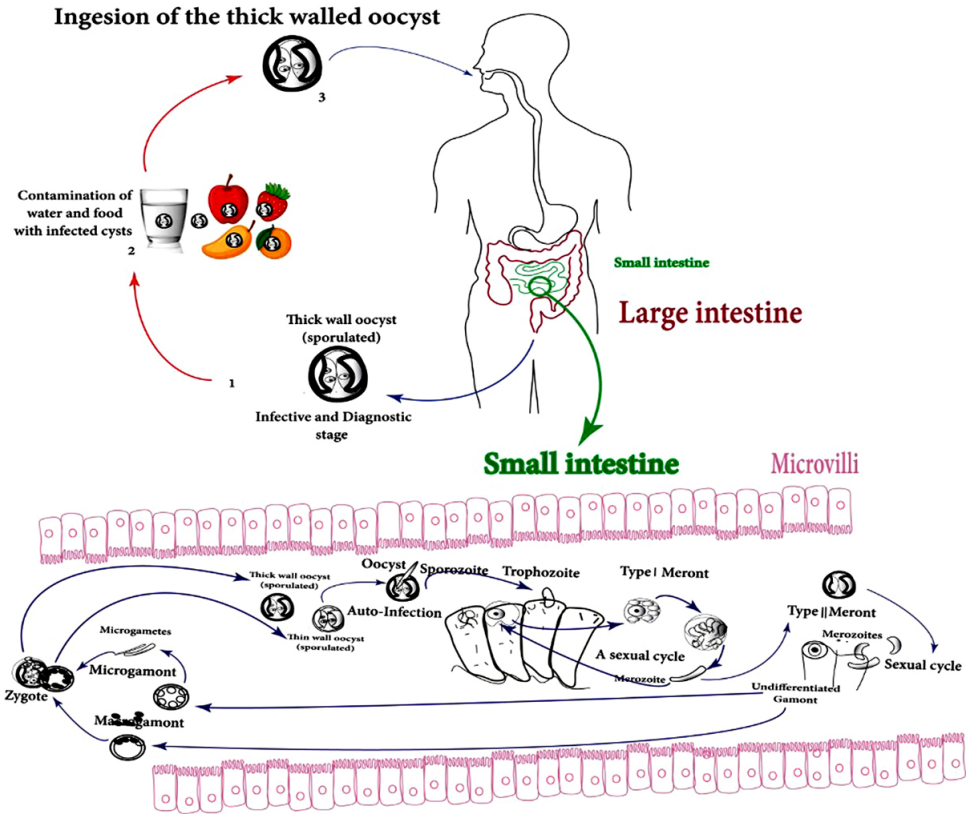

Figure 4: Life cycle of *Cryptosporidium* spp.

2006). This ciliate parasite may survive and thrive at temperatures ranging from 25 to 40°C (Clark and Diamond, 2002) due to ideal geo-climatic circumstances (higher temperature and humidity) for the parasite's development and survival in these regions. This illness has a global distribution with high incidence rates in tropical and subtropical areas (Ahmed et al., 2020).

Rainfall is one of the most important environmental factors because it provides a favorable environment for parasite proliferation (Azhar et al., 2002). Wetland animals are more susceptible to *B. coli* infection than dry land animals because of excessive rain, high humidity, and a greater probability of feed contamination by parasite cysts (Roy et al., 2011; Wisesa et al., 2015). The differences between infection rates due to gender were not significant in camels, whereas the differences in the infection rate were significant due to age. The highest prevalence was found in camels in the age group 3 to <6 years, while the lowest prevalence was recorded for the group age ≥9 years (Hussein et al., 2016).

There is a negative correlation in *Eimeria* between age and severity of infection, in which the highest proportions of severe cases were recorded in younger calves (Radfar and Aminzadeh, 2013; Gebru et al., 2018; Utebaeva et al., 2021). In addition, camels below 5 years old were more likely to be infected with the *Eimeria* spp. than older ones, but older camels that pass oocysts in their feces have a normal fecal consistency and don't have any sign of coccidiosis (Radfar and Aminzadeh, 2013; Hasan et al., 2021). Sazmand et al. (2012a) found a higher rate in camels aged from 5 to 10 years old. A high prevalence rate was recorded in the winter (Sazmand et al., 2012a; Khedr et al., 2015). There was no significant difference between male and female camel calves (Gebru et al., 2018; Hasan et al., 2021). Health status was found to be a significant factor in convalescent and diarrheic calves (Gebru et al., 2018). The prevalence of coccidiosis in young male camels, adult males, and adult females were 40.46, 26.78, and 31.82%, respectively. Three *Eimeria* spp. were identified; *E. cameli*, *E. rajasthani*, and *E. pellerdyi*; of them, *E. pellerdyi* was recorded in Saudi Arabia for the first time (Metwally et al., 2020). In addition, the prevalence of *Eimeria* in camels aged up to 1, 2, 2-3 years, and in adults were 65.0, 47.5, 35.0, and 22.5%, respectively. Another risk factor investigated was the season of the year, where in summer, autumn, spring, and winter, their prevalence rates were 60.0, 47.5, 41.25, and 21.25%, respectively (Utebaeva et al., 2021).

The incidence of Giardiasis is higher in young camels (under 6 years old) than in adult animals, but this age difference was not significant (Hussin, 2015; Hasan et al., 2021). The highest prevalence was found in spring and summer (Khedr et al., 2015). Though non-significant, females had a higher infection rate than males (Hussin, 2015; Khedr et al., 2015; Hasan et al., 2021).

The highest prevalence of cryptosporidiosis was recorded in camels with symptoms of diarrhea (Yakhchali and Moradi, 2012). No significant differences in prevalence or infection intensity were seen in different age groups (Razavi et al., 2009; Sazmand et al., 2012a). Another risk factor was sex which

showed no significant difference between males and females (Razavi et al., 2009; Sazmand et al., 2012a; Yakhchali and Moradi, 2012; Hussin, 2015; Gebru et al., 2018; Hasan et al., 2021). In addition, the effect of seasons on the prevalence of cryptosporidiosis was not significant (Razavi et al., 2009; Sazmand et al., 2012a). Despite all previous results, the prevalence was significantly higher in camel calves than in other age groups (Gebru et al., 2018; Yakhchali and Moradi, 2012), and the highest infection rate of *Cryptosporidium* spp. in camels recorded at age (up to 6 years) (Hussin, 2015; Hasan et al., 2021). In addition, the infection rate was the highest in female camels (Yakhchali and Moradi, 2012).

Prevalence of enteric protozoa in camel

Different enteric protozoa showed disease conditions in camels with variable prevalence (Table 1).

Diagnosis of enteric protozoa

Laboratory diagnosis for enteric protozoa

Laboratory diagnosis for enteric protozoa was performed using fecal samples by direct smear, simple flotation method (Cebra et al., 2007), and sedimentation techniques (Zajac and Conboy, 2012) . There is no standardized method for detecting *B. coli*. This ciliated protozoan is diagnosed using the same methods as other intestinal helminths and protozoa. For example, identifying trophozoites in ulcers at autopsy or discovering cysts in a biopsy sample collected using a sigmoidoscopy (Tarrar et al., 2008). Autofluorescence is used and is superior to bright field microscopy (Daugschies et al., 2001).

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) have been employed in a few investigations (Nilles-Bije and Rivera, 2010). Recently, diagnosis by PCR targeting the SSU rRNA gene was applied. *B. coli* was confirmed in Tibetan sheep with a history of diarrhea using molecular analysis based on the ITS gene and 18S rRNA sequences (Jian et al., 2018). Fecal samples are tested for the presence of *Eimeria* using a direct smear and zinc sulfate flotation (33 g of dry zinc sulfate crystals plus 67 mL of distilled water) (Dubey and Pande, 1964; Truant et al., 1981). To demonstrate the presence of oocysts, fresh fecal samples are combined with tap water; the combination is then centrifuged (800 ×g for 2 min), and the supernatant is discarded. The sediment is mixed with zinc sulfate (Metwally et al., 2020). The morphology, i.e., form, color, presence or absence of the micropyle and its cap, presence or absence of residual, polar, stiedae bodies, and morphometry (length, width, and shape index) of oocysts are used to distinguish the species as previously described (Kaufmann, 1996; Monnig, 1982).

Giardia diagnostic testing still mainly depends on clinical symptoms and is confirmed by microscopic examination of fecal samples. The cysts shed sporadically during infection, and examination of many fecal samples is necessary to find cysts (Santín et al., 2007). It is recommended to use concentration techniques to increase sensitivity (Soares and Tasca, 2016). In fecal samples, trophozoites and cysts can be seen; however, trophozoites are only seen in cases of watery diarrhea. Under the microscope, trophozoites (10–20 μm long) have a pyriform shape with a ventral sucking disc, two nuclei, two median bodies, and eight flagella, whereas cysts (8–15 μm long) have an oval shape with 2-4 nuclei, fibrils, and median bodies (4 lateral, 2 ventral, and 2 posteriors) (Cama and Mathison, 2015). The cysts can be stained and seen using immunofluorescent antibodies using immunofluorescent microscopy, which aids in the detection process.

Additionally, immunological and molecular techniques are widely used and are reported to be extremely sensitive and specific. Immunoassays for antigen detection have the benefit of being simple to be standardized, quick to produce findings, and having higher sensitivity and specificity than microscopy (Cama and Mathison, 2015; Soares and Tasca, 2016). PCR assays are developing and are thought to be more sensitive than microscopy and immunoassays. SSU rRNA, triose phosphate isomerase, and glutamate dehydrogenase coding genes are the most frequently employed loci in PCR. The ability to carry out further sequence analysis from PCR-positive samples to enable assemblage identification is an obvious benefit of molecular tests. Although they are not frequently employed for diagnosis, PCR and sequencing are used for research (Feng and Xiao, 2011).

Cryptosporidium oocysts can be detected by direct smear without any staining, the modified Ziehl-Neelsen stain under light microscopy, and immunofluorescent antibody-based (IFA) and enzyme-linked immunosorbent assay (ELISA). Despite the lower sensitivity and specificity of traditional staining techniques, e.g., the Ziehl-Neelsen (ZN) stain, they are still frequently used because they are quicker, easier, and less expensive. Ten thousand oocysts per gram of watery stool have been reported to be the cutoff for identifying *C. parvum* oocysts, but 50,000 or 500,000 oocysts per gram are needed for a positive IFA or modified ZN staining test, respectively, in formed feces (Weber et al., 1991; Al-Megrin, 2015; Van den Bossche et al., 2015). Therefore, more accurate and focused methods, such as molecular PCR assay, are required to identify these oocysts in fecal samples. However, due to their high price and shortage of reagents, they are regrettably not yet used as a standard diagnostic method in low-income nations (Agnamey et al., 2011).

Molecular diagnosis of enteric protozoa

B. coli is massive, so it may be easily identified and validated by morphological examination in feces (Schuster and Ramirez-Avila, 2008). Because of the polymorphic characteristics of cysts and trophozoites, morphology is insufficient for distinguishing species and demonstrating *B. coli* intraspecies genetic variability (Hegner, 1934; Schuster and Ramirez-Avila, 2008).

Table 1: Different enteric protozoa showed disease conditions in camels with variable prevalence rates in different countries.

Enteric protozoa	Prevalence (%)	Locality	Sampling time	Health state	Reference
B. coli	Insignificant	Bahrain	Jan.1989-Dec.1990	Diarrhetic young camels	Abubakr et al. (2000)
	7.14	Nagpur region	Early summer, 2008	Apparently healthy migratory camels	Rewatkar et al. (2009)
	4.76	Slaughterhouse, Cairo, Egypt	2009	Apparently healthy camels	Wahba and Radwan (2009)
	53	North Gujarat	2010	Apparently healthy camels	Ghoke et al. (2010)
	Large no. of *B. coli* trophozoites and cysts	Research farm, Central Iran	2013	3 years old diarrhetic camels with anorexia, depression	Tajik et al. (2013)
	Plenty of *B. coli* trophozoites	Saudi Arabia	2014	Diarrhetic 3 years old male dromedary camel	Altayib (2014)
	0.93	Sokoto, Nigeria	Mar-Sept. 2013	Healthy camels	Mahmuda et al. (2014)
	50	Najaf, Iraq	Nov.2014-May.2015	Healthy camels	Hussein et al. (2016)
	7.2	Southern Algeria	Mar.2011-Jun.2012	Healthy camels	Djerbouh et al. (2018)
	6.7	Imported camels to Egypt from Sudan	Jan.-Jul. 2016	Poor body condition, rough coat, emaciation, scrotal swelling, and diarrhea	El-Khabaz et al. (2019)
	22	Dhaka, Bangladesh	Sept-Oct.2015	Apparently Healthy camels	Islam et al. (2019)
	4	Nineveh, Iraq	2021	Healthy grazing camels	Hasan et al. (2021)
	1	Southern Algeria	Feb-May. 2019	Healthy camels	Saidi et al. (2021)
Eimeria spp.	20	Bahrain	Jan.1989-Dec.1990	Diarrhetic young camels	Abubakr et al. (2000)
	25	Nagpur region	Early summer, 2008	Apparently healthy migratory camels	Rewatkar et al. (2009)
	46.60	Slaughterhouse, Cairo, Egypt	2009	Apparently healthy camels	Wahba and Radwan (2009)
	9.90	Northern Tanzania	Jun-Aug. 2010	Free-grazing apparently healthy camels	Swai et al. (2011)
	70 *E. rajasthani* & 90 *E. cameli*	Farm in Bursa district, Turkey	2011	Un-treated 10 camels	Cirak et al. (2011)
	9.51	Yazd province, central Iran	Winter, 2008- Summer, 2010	Apparently healthy camels	Sazmand et al. (2012a)
	24	Central deserts of Iran	Mid-Summer,2011	Healthy camels	Radfar and Aminzadeh (2013)
	8.33	Sokoto, Nigeria	Mar-Sept. 2013	Healthy camels	Mahmuda et al. (2014)
	1.32	Abello district, Oromia state, Ethiopia	Aug. 2011-Ma. 2012	Free-grazing camels in contact with other livestock	Ararsa et al. (2014)
	13.33	Behera province, Egypt	2014 and 2015	Apparently healthy, freshly slaughtered camels	Khedr et al. (2015)
	2 unsporulated *E. leuckarti* oocysts	Farm in Bikaner, India	2016	Camel calves <2 years of age	Kumar et al. (2016)
	0.65	Mogadishu, Somalia	Dec. 2015- Mar. 2016	Adult lactating apparently healthy females	Ibrahim et al. (2016)
	5.80, 27.10,23.3	Ethiopia	April. 2013-Dec. 2014	Apparently healthy, clinically diseased, and convalescent camel calves, respectively	Gebru et al. (2018)
	11	Karamoja, Northeastern Uganda	March 2016	dromedary camels	Jesca et al. (2017)
	14.4	Southern Algeria	Mar.2011-Jun. 2012	Healthy camels	Djerbouh et al. (2018)
	38	Cairo- Egypt	2019	Cairo slaughterhouse	Abbas et al. (2019)
	47.30	Babile district, Ethiopia	Dec- Jun. 2019	Clinically diarrhetic camel calves	Abraha et al. (2019)
	28.33	Imported camels to Egypt from Sudan	Jan.-Jul. 2016	Camels with poor body condition, rough coat, emaciation, scrotal swelling, and diarrhea	El-Khabaz et al. (2019)
	35.88	Saudi Arabia	Feb- Oct. 2018	West Abattoir (Riyadh) and Onaizah Modern abattoir (Al-Qassim)	Metwally et al. (2020)
	42.50	Turkestan region, Kazakhstan	2019	Apparently healthy camels	Utebaeva et al. (2021)
	29	Nineveh, Iraq	2021	Healthy grazing camels	Hasan et al. (2021)
	20	Southern Algeria	Feb-May. 2019	Healthy camels	Saidi et al. (2021)
Giardia spp.	NA	Saudi Arabia	March 2005	Female camel in sternal Recumbency and severe intermittent diarrhea	Al-Jabr et al. (2005)
	0	Croatia	Jan. and Feb. 2005	Captive camels in zoo	Beck et al. (2011)
	100	Zawraa zoo, Baghdad, Iraq	Nov. 2012- May. 2013	Four non-diarrheic fecal camel samples	Radhy et al. (2013)
	24	Najaf, Iraq	Nov. 2014- May. 2015	Randoml 100 fecal samples from different ages and sex of camels	Hussin (2015)
	5	Behera, Egypt	2014 and 2015	Apparently healthy, freshly slaughtered camels	Khedr et al. (2015)
	20	Al-Diwaniyah and Najaf, Iraq	Oct. 2015- Apr. 2016	Two hundred fecal samples (healthy and diarrhetic camels)	Jawad and Jasim (2016)
	6.70	Nineveh, Iraq	2021	Healthy grazing camels	Hasan et al. (2021)
Cryptosporidium spp.	37.86	Isfahan, Iran	2009	103 camels from Najaf-Abad Slaughterhouse	Razavi et al. (2009)
	3.8	Slaughterhouse, Cairo, Egypt	2009	Apparently healthy camels	Wahba and Radwan (2009)
	19.30	Egypt	2011	Healthy camels	Abdel-Wahab and Abdel-Maogood (2011)
	20.33	Yazd, Iran	Winter, 2008- Summer, 2010	Apparently healthy camels	Sazmand et al. (2012b)
	10	Northwestern Iran	Nov. 2009- Jul. 2010	170 randomly selected camels	Yakhchali and Moradi (2012)
	2.26	Central deserts of Iran	Mid-Summer,2011	Healthy camels	Radfar and Aminzadeh (2013)
	61	Najaf, Iraq	Nov. 2014- May. 2015	Randoml 100 fecal samples from different ages and sex of camels	Hussin (2015)
	100	Ardabil, Northwest Iran	2016	Apparently healthy camels	Asghari et al. (2016)
	15.1	Riyadh, Saudi Arabia	2016	Thirty-three diarrhetic camel calves	El Wathig and Faye (2016)
	55	Al-Diwaniyah and Najaf, Iraq	Oct. 2015- Apr. 2016	Two hundred fecal samples (healthy and diarrhetic camels)	Jawad and Jasim (2016)
	4.20, 37.90, 43.30	Ethiopia	April. 2013-Dec. 2014	Apparently healthy, clinically diseased, and convalescent camel calves, respectively	Gebru et al. (2018)
	8.3	Imported camels to Egypt from Sudan	Jan.-Jul. 2016	Poor body condition, rough coat, emaciation, scrotal swelling, and diarrhea	El-Khabaz et al. (2019)
	20.20	Qena, Egypt	Sept. 2016- Dec. 2017	Healthy camels	Elshahawy and AbouElenien (2019)
	46.10	Babile district, Ethiopia	Dec- Jun. 2019	Clinically diarrhetic camel calves	Abraha et al. (2019)
	17.4	Al-Ahsa, Saudi Arabia	2020	Diarrhetic dromedary camel calves	El Hassan et al. (2020)
	60	Nineveh, Iraq	2021	Healthy grazing camels	Hasan et al. (2021)
	58	Southern Algeria	Feb.- May. 2019	Healthy camels	Saidi et al. (2021)

The incidence and genetic variation of *B. coli* in hosts have recently been investigated using molecular methods (Anh et al., 2007; Ponce-Gordo et al., 2008, 2011), small subunit rRNA (SSU rRNA) and ITS1-5.8S rRNA-ITS2 segments of the rRNA genes are mainly targeted. At the genus level, the SSU rDNA was widely used to highlight the taxonomic issues of *Balantioides* spp. (Cameron, 2003). The ITS1-5.8S rRNA-ITS2 gene locus of *B. coli* has been studied for genetic diversity at the species/subspecies level. In humans and animals, such as pigs, ostriches, and nonhuman primates, sequence analysis of the hypervariable region of ITS1-5.8S rRNA-ITS2 detected at least two genetic variations (A and B) (Ponce-Gordo et al., 2008, 2011).

In *Giardia*, assemblages show different levels of host specificity. For example, assemblages A and B occur in humans and many other hosts, C and D in canids, E in hoofed animals, F in cats, G in rodents, and assemblages H in pinnipeds. Only a portion of the factors determining host specificity is recognized, although it is evident that both the host and the parasite are involved. Very few molecular investigations exist on the genotypes and *Giardia* spp. Like other hoofed animals, the most common spp.*G. duodenalis* assemblages in camels will mostly have the zoonotic assemblage E. The presence of other assemblages may reveal the zoonotic potential of camel giardiasis (Al-Jabr et al., 2005; Cacciò et al., 2018; Sazmand et al., 2019). The prevalence of *G. lamblia* from camel feces samples, depending on the PCR technique based on the subunit ribosomal rRNA gene, was 39% (Jawad and Jasim, 2016).

Acid-fast staining is the traditional technique for the laboratory detection of cryptosporidiosis (Adeyemo et al., 2018), which is also easy and inexpensive, but less sensitive, time-consuming, cost-effective, and requires significant experience (Van den Bossche et al., 2015; Camargo et al., 2018), while PCR is a more accurate and highly sensitive diagnostic tool, but high cost and shortage of reagents are the most disadvantages of it in the developed countries (Agnamey et al., 2011; Ahmed et al., 2016; Ryan et al., 2019). In addition, detecting cryptosporidiosis in dromedary camels depends on microscopy, and little molecular data is available (Razavi et al., 2009; Sazmand et al., 2012a; Yakhchali and Moradi, 2012). Molecular characterization of camel *Cryptosporidium* revealed that *C. parvum*, *C. muris*, and *C. andersoni* could infect camels (Wernery and Kaaden, 2002). The molecular identification of *C. parvum* positive isolates (14%) from camels was carried out using target primers for heat shock protein gene, which was designed using the NCBI-Genbank database (Genbank code: GQ259151.1) and primer3 plus program for primer design at 180 bp product size (Ahmed et al., 2016). In addition, *C. parvum* was identified by PCR from a formalin-fixed intestinal tissue specimen from a one-week-old dromedary camel in which extracted DNA.

Treatment, prevention, and control for enteric protozoa

Treatment

The most common illness syndrome that causes morbidity and mortality is diarrhea. Therefore, the usage of potent medications along with sound management practices is advised. The reduction in parasite load in feces and the absence of clinical symptoms are indicators of the efficacy of any treatment. Infection with *B. coli* has been treated with various therapeutic medicines in farm animals worldwide (Hassan et al., 2017). Due to the high prevalence of *B. coli* among camels, it is essential to treat both asymptomatic and clinically diseased camels to reduce and prevent environmental pollution and human infection. Oxytetracycline is also recommended for the treatment of camel balantidiasis (Osman, 2019). Additionally, Infection with *B. coli* has been effectively treated with secnidazole in various animal species (Jamil et al., 2015).

Ionophoric antibiotics (monensin, lasalocid, or salinomycin) used to treat coccidiosis in poultry have serious side effects on camels, such as skeletal muscle degeneration (Al-Nazawi and Homeida, 2009). The treatment of coccidiosis in old-world camels is poorly understood. Dromedary calves were treated with sulfadimidine orally for 10 days as an aqueous suspension at a dose of 30 mg/kg body weight (Hussein et al., 1987). Gerlach made an effort to evaluate Toltrazuril's effectiveness in experimentally infected dromedaries; when administered for 6, 12, or 22 days after inoculation, toltrazuril failed to prevent patent infections, although showing high serum levels that were promising in pharmacokinetic analysis (Gerlach, 2008).

Infection with giardiasis is usually characterized by spontaneous recovery. Metronidazole, secnidazole, and tinidazole are three effective drugs that can be used to treat an acute condition (Huetink et al., 2001). The Cochrane Collaboration's meta-analysis studies show that compared to the gold standard of metronidazole, albendazole has equal efficacy with very few adverse effects, such as neurological and gastrointestinal symptoms (Upcroft and Upcroft, 1993). Additionally, three medications, including quinacrine, nitazoxanide, bacitracin zinc, and furazolidone, provide good results for treatment. Paromomycin is the chosen medication during pregnancy due to its weak intestinal villi absorption and, thus, low fetal involvement (Solaymani-Mohammadi et al., 2010).

For a long time, we have been unable to treat humans and animals with cryptosporidiosis effectively. Numerous medications and drug combinations, including rifaximin, azithromycin, and paromomycin, have been designed and explored to treat many resilient microbes such as *Cryptosporidium*. Still, the outcomes have been unsatisfactory or variable (Algharib et al., 2020). Fortunately, nutritional and supportive therapy helps the majority of healthy hosts recover. However, in immune-compromised hosts, cryptosporidiosis continues to be a serious illness that can be fatal (Bamaiyi and Redhuan, 2017).

Prevention and control

The prevention programs for *B. coli* are mainly based on decreasing the exposure of susceptible animal species to domestic pigs and wild boars by farming domestic pigs away from crop areas, water sources, and other domestic animal populations. In Muslim countries, the prevalence of balantidiasis is less due to restrictions on pig farming (Hussein et al., 2016). *B. coli* is a water-borne parasite; hence animals living in places with insufficient sewage and water infrastructure are more susceptible to the disease (Khan et al., 2013). As a result, animal grazing in such places should be avoided, and animals should be reared in enclosed spaces where their health can be monitored (Bilal et al., 2009).

Separation of carrier animals of *B. coli* from susceptible animals and good sanitary measures (as pest control) should be practiced between enclosures of carrier animals and susceptible animals. Treatment of carrier animals should be occurred to reduce environmental contamination. The most effective technique for controlling *B. coli* infection is to provide clean water to animals for drinking and other purposes (Schuster and Ramirez-Avila, 2008). Despite being a self-limiting disease, anticoccidials drug may be used to treat and control coccidiosis outbreaks in camelids. Young camels are more susceptible to *Eimeria* infection than adult camels. Still, adult camels play an important role in spreading disease as chronic carriers of infectious oocysts. Hence, investigations must include all age groups for economic and successful control of Eimeriosis in camels (Sazmand et al., 2012a). In addition, the distribution of coccidiosis is affected by many factors, such as environmental conditions, animal physiology and health, farming practices, stress, and sickness (Barth, 2003).

The most common method of *Giardia* spp. transmission is through contaminated food and water by infected feces. Consequently, lowering this pollution is the most important prevention method through hygienic sanitation and avoiding water consumption from untreated sources such as rivers, lakes, and streams. Using chlorine and filtration is an effective way to eliminate parasites (Scorza and Tangtrongsup, 2010). However, the widespread distribution of *Cryptosporidium* in nature, and the resistance of the oocysts to most common detergents and disinfectants (WHO, 2003; Chako et al., 2010), make the control of the infection challenging. Cryptosporidiosis can be avoided by routinely disinfecting with ammonia or hydrogen peroxide (Chako et al., 2010). In the absence of a viable vaccination against cryptosporidiosis, maintaining good hygiene is essential for infection control and prevention (Bamaiyi and Redhuan, 2017).

Conclusion and future perspectives

It is evident that *B. coli* is an emerging zoonotic protozoan disease affecting many host species. Balantidiasis requires immediate international attention, and additional epidemiological studies should be conducted to determine the actual prevalence of *B. coli* infection in various domestic and wild animal species in developing and developed countries. More research on the survival time of *B. coli* cysts in the environment should be undertaken to determine the period of infection in susceptible hosts. To develop efficient disease prevention and control strategies, other mechanisms of *B. coli* infection transmission from feed and water contaminated with pig feces should be investigated. Advanced diagnostic approaches for diagnosing balantidiasis in diverse farm animal species and the zoonotic character of this protozoan parasite should be investigated further to protect human health. Because cysts are infrequently detected in human feces while trophozoites are frequently observed in cases of dysentery, more research into the role of trophozoites as an infective stage is needed to understand better human-to-human transmission (Ahmed et al., 2020).

G. lamblia is the most prevalent and severe protozoal infection for animal and human health. Despite having a wide range of effectiveness depending on the viability of the host, *G. lamblia* is neglected as parasitic infections affect many hosts. Further research on the mechanism of resistance to infection and strategy for good control measures should be considered to eradicate the giardiasis infection. Moreover, the challenges and restrictions related to control planning for eliminating the parasite, specifically cleaning the source of drinking water, should be studied comprehensively (Al-Sabbawi, 2007).

Cryptosporidiosis as a global emerging zoonotic disease is still poorly understood and largely neglected. Though literature is often sporadic or nonexistent, there are now enough reliable reports of the disease. The mechanisms by which *Cryptosporidium* can adapt to many hosts and its ability to resist various challenging circumstances are still poorly understood and researched. Despite discovering oocysts in newborn animals, there are no clear studies regarding the transplacental transfer of oocysts in animals and humans. Considering no known effective therapeutics to treat infection, the parasite poses a threat by quickly changing and increasing its host range. Due to its extensive geographic distribution, the number of infected animal species, and its potential for zoonotic transmission, *C. parvum* seems to be the most significant *Cryptosporidium* spp. (Bamaiyi and Redhuan, 2017).

Since the 1970s, the creation and use of typing techniques and next-generation sequencing technologies have enabled a significant advancement in our knowledge of the taxonomy and epidemiology of enteric protozoa. The identification of additional genetic loci that need to be more thoroughly validated has been made possible by whole genome sequencing. This has helped us better understand population structure, the degree of host adaptation, and the possibility of zoonotic transmission from domestic animals and wildlife (Dawood et al., 2022).

Characterizing isolates from field research and outbreak investigations has increasingly used whole genome sequencing. Although extensive research has been done in developed countries, studies in low- and

middle-income developing countries are needed. These regions have various lifestyles, levels of hygiene, and degrees of agriculture, which frequently favor the incidence of anthroponotic disease transmission. Because of this, variable infection origins, transmission dynamics, and pathogen development may occur in industrialized countries depending on how those genotypes are distributed (Ryan et al., 2021).

To avoid infection, various anti-protozoan medications are utilized; however, protozoans can develop resistance considering the extensive usage of antiprotozoal medications. This poses a major public health threat and cross-transfer of resistance caused by animal products' residues entering the food chain. In addition, medicines can enter the environment through animal secretions and excretions, exposing people to even more risks. To solve these problems, vaccination will be a different approach to managing protozoan infections (Stephen et al., 1997; De Ruyck et al., 2000; Geary, 2002). The development of effective commercial vaccines against numerous serious protozoan infections is moving slowly, even though more efficient recombinant vaccines are being introduced (Patra et al., 2017). The utilization of modern molecular techniques, our growing understanding of immunological systems, and methods to maximize immune responses to obtain maximum protection have all contributed to the rapid advancement of vaccine technology.

Article Information

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