

***Cryptosporidium* and its public health**

Neveen, S. Satour

Parasitology Department, Alexandria Lab.
Animal Health Research Institute (AHRI), Agriculture Research Center (ARC),
Egypt.

Correspondence author:

Neveen Satour

E.mail: neveen.satour@yahoo.com

Received in 26/8/2024

Accepted in 24/9/2024

Abstract

The protozoan parasite of the genus *Cryptosporidium* is the source of the food and water-borne zoonotic disease known as cryptosporidiosis. The two main species responsible for infections in both humans and animals are *C. hominis* and *C. parvum*. Both the respiratory and fecal-oral routes might spread the disease, as it's highly resistance to antimicrobials and anticoccidials. There are some drugs used for treatment as halofuginone lactate and nitazoxanide. As a result, proper hygiene practices are therefore essential to preventing and controlling disease. This review aims to provide an overview of the infective stage, life cycle, pathogenesis, epidemiology, diagnosis, treatment and control of cryptosporidiosis.

Keywords: *Cryptosporidium*, life cycle, zoonotic disease

Introduction

Cryptosporidiosis is a parasitic disease caused by genus *Cryptosporidium*, this parasite was discovered in the mice's stomach glands in 1910 by **Edward Ernst Tyzzer**, who gave it the name "kruptos" (which means hidden), since the parasite does not have sporocysts within its oocysts and sporulates while still attached to the host wall **Tyzzer, (1910)**. *Cryptosporidium* is one of the most prevalent waterborne diseases and the main global source of outbreaks of waterborne diseases **Helmy et al., (2017 and Hopkins et al., (2013)**. Up to 1.6 million people worldwide have died from diarrheal diseases each year. Due to poor hygiene and contaminated drinking water, a third of these deaths have been recorded among children under the age of five **Dadonaite et al., (2021)**. More than 8 million cases of foodborne illness occur worldwide each year as a result of *Cryptosporidium* **Ryan et al., (2018)**. Oocysts are thought to be one of the causes of waterborne diarrheal outbreaks because of their extreme resistance to standard household disin-

fectants and their ability to persist in water for more than 140 days **Dillingham et al., (2002) and Gururajan et al., (2021)**. The approved treatments for *Cryptosporidium* infections in humans and animals are nitazoxanide and halofuginone. Reducing the incidence of infection, obstructing animal-human transmission pathways and maintaining a good hygienic environment for both humans and animals are the main strategies used to control cryptosporidiosis **Yosra, (2014)**. Information about the route, spread of *Cryptosporidium* and the main subspecies of *Cryptosporidium* that predominate in both humans and animals, is essential to attain successful control. The objective of this review paper is to review available information about *Cryptosporidium* and its significance for public health.

3) Life Cycle of *Cryptosporidium*: -

The life cycle of *Cryptosporidium* is complicated, in which has ability to reproduce asexually and sexually within a single host. The infective stage of *Cryptosporidium* is the sporulated oocyst that contains four sporozoites

Hassan *et al.*, (2020). Transmission occurs mainly by ingestion contaminated water and food, then after ingestion the oocysts, each oocyst releases four infectious sporozoites that attach to the apical surface of intestinal epithelial cells, in which they locate in the microvilli in parasitophorous vacuoles between the cell wall and the cytoplasm that intracellular but extracytoplasmic **Current and Reese, (1986)**. Sporozites mature into trophozoites inside the vacuole, which undergo three cycles of asexual

reproduction, followed by a cycle of sexual stages to produce oocysts with either thin or thick walls that each contain four sporozoites **Current and Reese, (1986)** and **English *et al.*, (2022)**. The majority of oocysts that are formed have thick walls and can survive very well in the external environment but some have thin walls. Usually, these thin-walled oocysts release their sporozoites in the host's gut lumen, where they can cause autoinfection. As shown in figure (1).

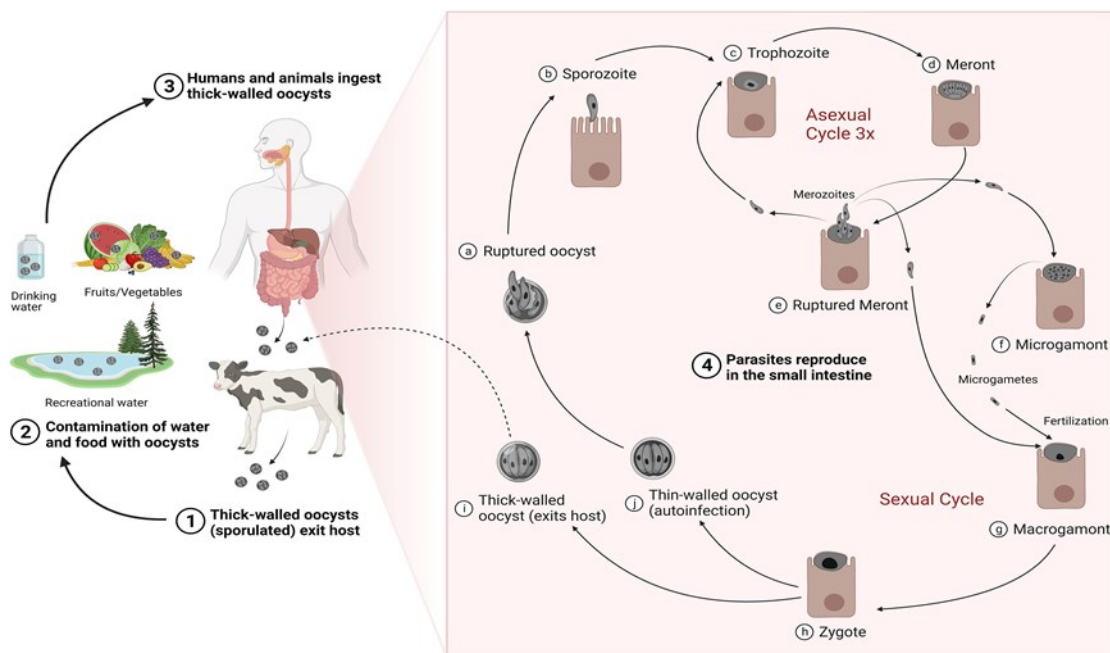


Fig. (1). Life cycle and transmission of *Cryptosporidium* CDC, (2019). Created with BioRender.com.

Pathogenesis of *Cryptosporidium*:

After ingestion of sporulated oocyst with food or water by the host, oocyst rupture and releases sporozoite that attach to the apical surface of epithelial host cells. *Cryptosporidium* infections in calves often occur between one and four weeks of age, although they can also occur in older ages. **Wahba (1994)** when examined histological sections prepared from alimentary tract of experimentally infected mice with *Cryptosporidium* oocysts, found that the most pathological changes were observed to be prevalent in the ileum, stomach, duodenum and caecum. On the other hand, pathological changes in jejunum and large intestine were of a less degree. *Cryptosporidium* oocysts could be noticed on the epithelial surface of intestinal villi and being embedded in the mucosa of

stomach and caecum. The lamina propria of the ileum villi was infiltrated with inflammatory cells. Goblet cells were distended and increased in number. In addition, there was necrosis, hyperplasia and vacuolation of the epithelium. There was diffuse atrophy in the villi which was much more obvious in the ileum. The epithelial cells of atrophied villi have been transformed to low columnar or cuboidal-shaped cells. In sections from the duodenum, there was swelling of some villi, some of which were fused together with the formation of synechia. Vacuolation also appeared in the caecum. Haemorrhages appeared in the lamina propria of ileum and caecum. In the submucosa of stomach and caecum, blood vessels were dilated with phlebitis and formation of thrombi inside. Calves that suffering from diarrhea may

be lethargic, anorexia and dehydrated. The diarrhea is a pale yellow color with mucous. In severe cases, calves die from dehydration and cardiovascular collapse. There are some pathogens such as Rotavirus, *Escherichia coli* and coccidia may also be found in calves during the first month of age, this might cause cryptosporidiosis to appear more severe than it actually is. **Joachim et al., (2003).**

Epidemiology of *Cryptosporidium*: -

Cryptosporidium is a parasitic infection that infects a wide range of vertebrates, including humans. Millions of people have died from gastroenteritis due to cryptosporidiosis throughout the last quarter of the 20th century and at the beginning of the 21st century, with high death rates among children under the age of five. Risk factors for contracting *Cryptosporidium* infection include those with weakened immune systems, children, those who interact with animals, inadequate social infrastructure, sewage-contaminated water supplies, animal dung contaminating fruits and vegetables and using animal dung as fertilizer in agriculture. **Ramadan et al., (2020).** Cryptosporidiosis is the main cause of chronic diarrhea and is associated with a high mortality rate in immunocompromised people, especially those with an AIDS-defining disease. Members of the genus *Cryptosporidium* are thought to be the second leading cause of diarrhea and mortality in the USA, after retroviruses **Hassan et al., (2020).** There are different species and genotypes of *Cryptosporidium* have been identified. Among these are *C. hominis* and *C. parvum* which represent the 95% of cryptosporidiosis cases in humans. A few cases of *C. meleagridis* infections have also been recorded in

humans, as well as *C. canis*, *C. felis*, *C. suis*, *C. muris*, and *C. andersoni* in immunocompromised individuals. A variety of hosts, including fish, birds, reptiles and mammals can become infected with *C. parvum* **CDC, (2022).**

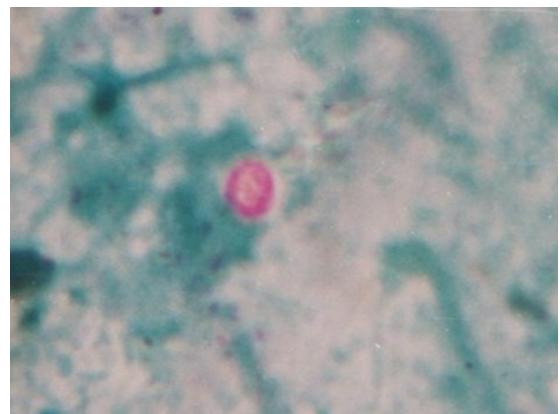
Diagnosis of *Cryptosporidium*: -

The traditional method of diagnosing cryptosporidiosis is based on presence of oocysts in feces. Fecal flotation, molecular, serological and smear analysis using certain stains are some of the approaches that can be used to identify oocysts.

Parasitological method: -

Numerous techniques are available for identifying *Cryptosporidium* in fecal samples. Microscopy is the most common technique among them to detect oocysts. Fecal samples can be examined directly using the concentration flotation method. **Fayer and Xiao, (2007).** Detection of *Cryptosporidium* oocysts is usually done by microscopy without any staining and/or by the modified Ziehl-Neelsen stain technique **Henriksen and Pohlenz, (1981)** under light microscopy with oil immersion lens, whereby the oocysts stain purple with green background figure (2). Also, safranin methylene blue **Baxby et al., (1984)**, methylene blue eosin **Cross and Moorhead, (1984)** and Giemsa stain **Pohlenz et al., (1978).** Another useful technique is immunofluorescence staining techniques. Despite the higher sensitivity of immunofluorescent antibody-based (IFA) procedures, traditional staining techniques like Ziehl-Neelsen stain are still widely used since they are simpler and less expensive **Caccio and Widmer, (2014).**

Fig. (2). Faecal smear from diarrheic lamb stained with modified Ziehl-Neelsen stain technique showed *Cryptosporidium parvum* oocyst (x1000) **Ebied and Satour, (2019).**



Serological method:

Different serological techniques such as enzyme linked immunosorbent assays (ELISA) and immune chromatographic test provide good sensitivity and specificity for detecting of *Cryptosporidium* antigens **Agnamey et al., (2011) and Hawash, (2014).**

Molecular method:

Polymerase Chain Reaction (PCR) technique offers very sensitive, species-specific diagnos-

tics. Compared to traditional microscopical and serological approaches, PCR-based methods for detecting oocysts in faeces are more sensitive in the detection of *Cryptosporidium*. *Cryptosporidium* species oocysts were analyzed by PCR. The 18s rRNA gene sequences used in the PCR process showed positive bands at 1056 bp. However, *Cryptosporidium parvum* subtyping at actin gene sequences reveals positive bands at 400 bps. **Ebied and Satour, (2019)** as shown in figure (3).

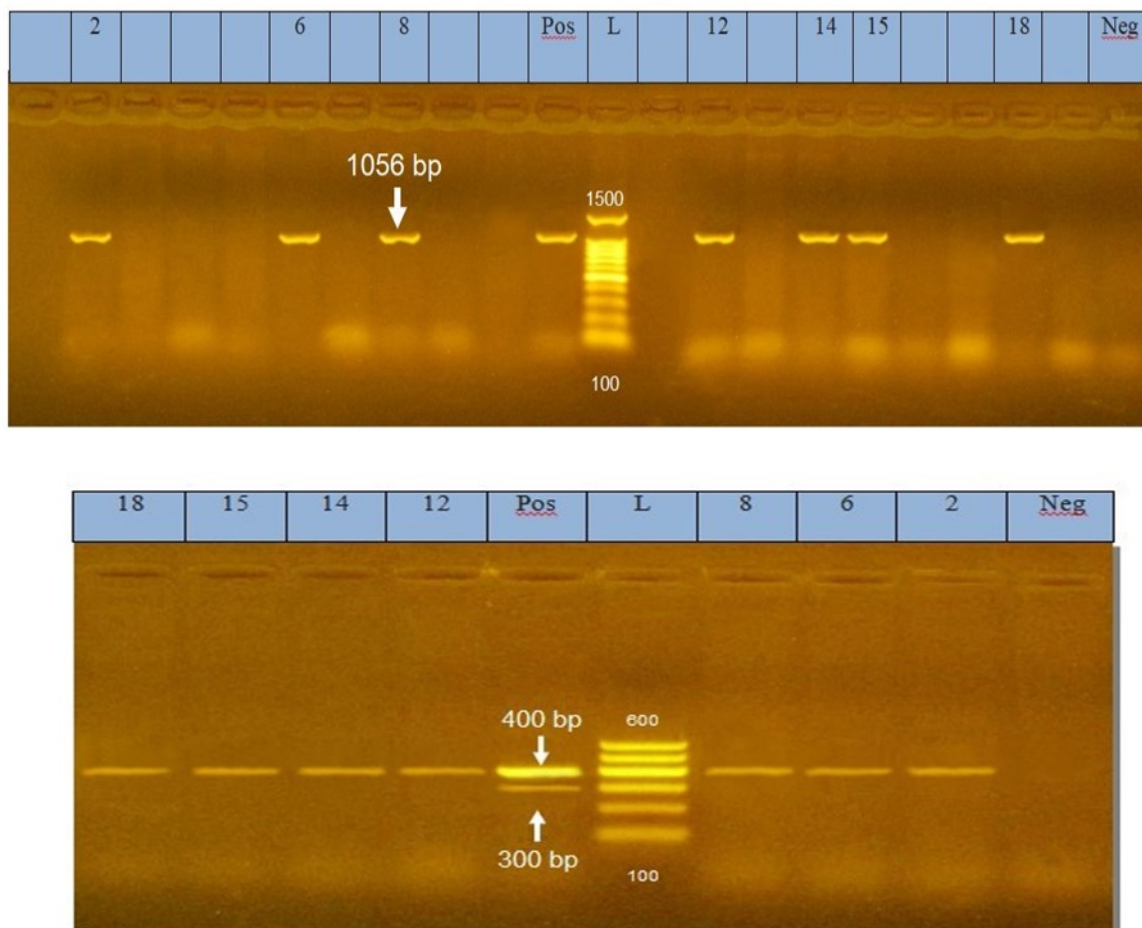


Fig. (3). Sub typing of *Cryptosporidium parvum* at actin gene sequences showing positive bands at 400bp. **Ebied and Satour, (2019).**

Treatment and control of *Cryptosporidium*:

People with healthy immune systems will recover without treatment. Drinking lots of fluids helps in preventing dehydration, which is one way to manage diarrhea. While intravenous therapy may be required and nutritional support may be helpful, replacing fluids and electrolytes through oral rehydration solutions is the most important aspect of treatment **Zintl et al., (2009)**. Pregnant women and small children should drink enough of fluids during illness as they may be more susceptible to dehydration from diarrhea. Diarrhea can cause rapid fluid loss, which can be fatal for infants. Anti-diarrhea medications could be able to reduce diarrhea. Nitazoxanide has been used for treatment of diarrhea caused by *Cryptosporidium* in people with good immune systems **CDC, (2012)**. halofuginone lactate has been used in both prevention and treatment of cryptosporidiosis in calves at a dose of 0.10 mg/kg of body weight per day for seven consecutive days. Halofuginone lactate treatment shortens the duration of diarrhea and oocyst discharge but does not entirely prevent or cure disease **Jarvie et al., (2005)** and **Trotz-Williams et al., (2011)**. In order to prevent and control cryptosporidiosis, continuous efforts must be made to prevent the spread of *Cryptosporidium* through food, water and contact with animals and people who are affected. In any setting, practicing good hygiene is important.

Some studies done related to *Cryptosporidium* species in Egypt:

Wahba (1994) when examined faecal samples of different animals with modified Ziehl-Neelsen stain found *Cryptosporidium* oocysts in 6.6% buffalo calves, 8.2% in cattle calves, 5.2% in lambs and 2.5% in kids slaughters at Giza and Kalyobia abattoirs.

According to a study by **El-Seify et al. (2012)**, 698 fecal samples were examined with modified Ziehl-Neelsen staining technique for *Cryptosporidium* species oocysts. The prevalence of *Cryptosporidium* spp. was 34.1 %.

Satur (2012), examined 397 domestic birds, the highest infection rate with *Cryptosporidium* species oocysts was that in *Cryptosporidium baileyi* where it reached 23.5% and 13.7% in domestic duck and broiler chicken respectively, followed by *Cryptosporidium meleagridis*

was 8.8% and 2.4% in farmed quail and broiler chicken respectively.

Abdel-Hady et al. (2014), examined 100 diarrheic fecal samples from calves aged 5 to 90 days to evaluate the presence of bovine coronavirus antigen by ELISA method and *Cryptosporidium* oocysts with modified Ziehl-Neelsen staining technique. Ziehl-Neelsen positive specimens for *Cryptosporidium* were confirmed by PCR. Both coronavirus antigen and *Cryptosporidium* were detected in 3 out of 100 tested specimens.

In a study by **Essa et al. (2014)**, A total of 717 fecal samples from Friesian and buffalo calves were collected for microscopical examination from Minufiya governorate. The results indicated that one-week-old calves had the highest percentage of *Cryptosporidium* (56.32%). The safranin staining method is used to examine these samples.

Mahfouz et al. (2014) claimed that a total of 2283 fecal samples were collected from farm animals and examined for detection of *Cryptosporidium* oocysts. The prevalence was 1.29%, 7.07% and 2.50% in buffalo, cattle and sheep respectively.

According to a study by **Ebied and Satour (2019)**, by microscopic examination and PCR analysis, the prevalence of *Cryptosporidium parvum* in lambs and goat kids were 14% and 6.2%, and 27.7% and 8.3% respectively.

In study by **Hamza et al. (2020)** using Ziehl-Neelsen stain and Multiplex PCR for detection of *Cryptosporidium* spp. in diarrheic buffalo calves. The results revealed that prevalence of *C. parvum*, *C. bovis* and *C. andersoni* were 50%, 35% and 30% respectively. No *C. reyne* was detected in the examined samples.

Elmahallawy et al. (2022), examined 608 fecal samples collected from cattle and buffalo in Upper Egypt. The prevalence of *Cryptosporidium* spp. was 38.27% and 28.16% in cattle and buffalo respectively.

Recommendations and conclusion:

- Preventing the transmission of *Cryptosporidium* requires practicing basic good hand hygiene. Hands should be cleansed for at least 20 seconds with soap and water. Alcohol-based hand sanitizers do not completely kill *Cryptosporidium*.

- Avoid drinking untreated water from lakes, rivers, ponds and shallow wells, as well as swallowing the water you swim in.
- Clean any surfaces that have obvious contamination according to manufacturer's instructions. Use normal detergents in a washer/dryer to clean clothes and bedding.
- Avoid swimming and water activities of any kind (lakes, splashpads, hot tubs and pools) if you've been sick.

Conclusion: *Cryptosporidium* is the second most common cause of moderate to severe diarrhea in infants under two years old and it is also a major global cause of mortality. *Cryptosporidium* is a serious global health concern, especially for immunocompromised individuals and children. The incidence of it varies widely and this wide variation in incidence can be attributed to a variety of factors, including the patient's age, immune status and environmental habitats. Cryptosporidiosis is a dangerous protozoal disease as its sporocysts excreted with faeces to outside the infected host already sporulated and not take a time to be sporulated outside the host like some other protozoa.

References

- Abdel-Hady, H.A.; El-bakry, K.M. and Satour, N.S. (2014).** Some Studies on Coronavirus and *Cryptosporidium* as causes of diarrhea in young calves in Alexandria Governorate. *Animal Health Research Journal*, 2 (2): 6-18.
- Agnamey, P.; Sarfati, C.; Pinel, C.; Rabodoniriina, M.; Kapel, N. and Dutoit E. (2011).** Evaluation of four commercial rapid immunochromatographic assays for detection of *Cryptosporidium* antigens in stool samples: a blind multicenter trial. *J Clin Microbiol.*, 49(4):1605–7
- Baxby, D.; Blundell, N. and Hart, C.A. (1984).** The development and performance of a simple, sensitive method for the detection of *Cryptosporidium* oocysts in faeces. *J. Hyg. Camb.*, 92: 317-323.
- Caccio, S.M. and Widmer, G. (2014).** *Cryptosporidium: Parasite and Disease.* Springer, Wien, Heidelberg, New York, Dordrecht, London.
- CDC (2019).** DPDx cryptosporidiosis. In: Centers for disease control and prevention. Available at: <https://www.cdc.gov/dpdx/cryptosporidiosis/index.html> (Accessed December 30 2022).
- Centers for Disease Control (CDC) (2012).** Treatment of Cryptosporidiosis. www.cdc.gov/parasites/crypto/daycare/Treatment.
- Centers for Disease Control (CDC) (2022).** Cryptosporidiosis. <https://www.cdc.gov/dpdx/cryptosporidiosis/index.html> accessed on 22 January 2022).
- Cross, R.F. and Moorhead, P.D. (1984).** A rapid staining technique for *Cryptosporidia*. *Mod. Vet. Pract.*, 65 (4): 307.
- Current, W.L. and Reese, N.C. (1986).** A comparison of endogenous development of three isolates of *Cryptosporidium* in suckling mice. *J. Protozool.*, 33 (1): 98–108.
- Dadonaite, B.; Ritchie, H. and Roser, M. (2021).** Diarrheal Diseases. In *Our World in Data*. diarrheal-diseases (accessed on 26 January 2021).
- Dillingham, R.A.; Lima, A.A. and Guerrant, R.L. (2002).** Cryptosporidiosis: epidemiology and impact. *Microbes and Infectious Diseases*, 4:1059-1066.
- Ebied, S.K. and Satour, N.S. (2019).** Diarrhoea syndrome in lambs and goat kids and its relation to some bacteria and Parasites. *Animal Health Research Journal*, 7 (4): 143-159.
- Elmahallawy, E.K.; Sadek, H.A.; Aboelsoued, D.; Aloraini, M.A.; Alkhaldi, A.A.M.; Abdel-Rahman, S.A.; Bakir, H.Y.; Arafa, M.I.; Hassan, E.A.; Elbaz, E.; Hassanen, E.A.A.; El-Gohary, F.A. and Gareh, A. (2022).** Parasitological, Molecular, and Epidemiological Investigation of *Cryptosporidium* Infection Among Cattle and Buffalo Calves From Assiut Governorate, Upper Egypt Current Status and Zoonotic Implications. *Frontiers in Veterinary Science*, Vol 9: 1-11.
- El-Seify, M.A.; Desoukey, M.A.; Khalafalla, R.E. and Sorour, S.S. (2012).** Prevalence of *Eimeria* and *Cryptosporidium* species in cattle in Kafr El-sheikh province. *Kafrelsheikh Vet. Med. J.*, 10 (2): 1-22.
- English, E.D.; Guerin, A.; Tandel, J. and Striepen, B. (2022).** Live imaging of the *Cryptosporidium parvum* life cycle reveals direct development of male and female gam-

- etes from type I meronts. *PloS Biol.* 20 (4), e3001604.
- Essa, S.H.; Galila, E.M.; Abdelwahab, M.G.; Moustafa, A.M.; Hamouda, F.K. and El-Akabawy, L. (2014).** The incidence of *Cryptosporidium* infection among Frisian and buffalo calves in Minufiya governorate. *Benha Vet. Med. J.*, 26 (1): 195-204.
- Fayer, R. and Xiao, L. (2007).** *Cryptosporidium* and cryptosporidiosis, 2nd edition. CRC Press/Taylor & Francis Group, Boca Raton, London, New York, pp. 580.
- Gururajan, A.; Rajkumari, N.; Devi, U. and Borah, P. (2021).** *Cryptosporidium* and waterborne outbreaks - A mini review. *Tropical parasitology*, 11 (1): 11-15.
- Hamza, D.A.; Soliman, M.; Soliman, S.M.; Salem, N.Y. and Nader, S.M. (2020).** Bovine Cryptosporidiosis and the Egyptian Diarrheic Buffalo Calves. *Int. J. Vet. Sci.*, 9(3): 448-452.
- Hassan, E.M.; Örmeci, B.; DeRosa, M.C., Dixon, B.R.; Sattar, S.A. and Iqbal, A. (2020).** A review of *Cryptosporidium* spp. and their detection in water. *Water Sci Technol*, 28: 1-25.
- Hawash, Y. (2014).** Evaluation of an immunoassay-based algorithm for screening and identification of giardia and *cryptosporidium* antigens in human faecal specimens from Saudi Arabia. *J Parasitol Res.*, 2014:213745.
- Helmy, Y.A.; El-Adawy, H. and Abdelwhab, E.M. (2017).** A Comprehensive Review of Common Bacterial, Parasitic and Viral Zoonoses at the Human-Animal Interface in Egypt. *Pathogens*, 6, 33.
- Henriksen, S.A. and Pohlenz, J.F.L. (1981).** Staining of *Cryptosporidia* by a modified Zeihl-Neelsen technique. *Acta Vet. Sc.*, 22: 594-596.
- Hopkins, J.; Hague, H.; Hudgin, G.; Ross, L. and Moore, D. (2013).** International Perspectives: An Outbreak of *Cryptosporidium* at a Recreational Water Park in Niagara Region, Canada. *J. Environ. Health*, 75, 28–33.
- Jarvie, B.D.; Trotz-Williams, L.A.; McKnight, D.R.; Leslie, K.E.; Wallace, M.M.; Todd, C.G.; Sharpe, P.H. and Peregrine, A.S. (2005).** Effect of halofuginone lactate on the occurrence of *Cryptosporidium parvum* and growth of neonatal dairy calves. *J Dairy Sci*, 88: 1801-1806.
- Joachim, A.; Krull, T.; Schwarzkopf, J. and Dauschies, A. (2003).** Prevalence and control of bovine cryptosporidiosis in German dairy herds. *Vet. Parasitol*, 112: 277–288.
- Mahfouz, M.E.; Mira, N. and Amer, S. (2014).** Prevalence and Genotyping of *Cryptosporidium* spp. in Farm Animals in Egypt. *J. Vet. Med. Sci.*, 76(12): 1569–1575.
- Pohlenz, J.; Moon, H.W.; Cheville, N.F. and Bemrick, W.J. (1978).** Cryptosporidiosis as a probable factor in neonatal diarrhea of calves. *J. Am. Vet. Med. Assoc.*, 172 (4): 452-457.
- Ramadan, R.M.; Khalifa, M.M.; Kamel, N.O.; Abdel-Wahab, A.M. and El-Bahy, M.M. (2020).** The use of Giardia immunogenic protein fraction to distinguish assemblages in humans and animals. *World's Veterinary Journal*, 10, 3:421-8.
- Ryan, U.; Hijjawi, N. and Xiao, L. (2018).** Foodborne cryptosporidiosis. *Int. J. Parasitol.*, 48, 1–12.
- Satur, N.S. (2012).** Studies on protozoal parasites of some birds. Ph. D. Thesis Fac. Vet. Med. Kafrelsheikh Univ. Egypt.
- Trotz-Williams, L.A.; Jarvie, B.D.; Peregrine, A.S.; Duffield, T.F. and Leslie, K.E. (2011).** Efficacy of halofuginone lactate in the prevention of cryptosporidiosis in dairy calves. *Vet Rec*, 168: 509.
- Tyzzar, E.E. (1910).** An extracellular Coccidium, *Cryptosporidium Muris* (Gen. Et Sp. Nov.) of the gastric glands of the Common Mouse. *J Med Res*, 23: 487- 510.
- Wahba, A.A. (1994).** Morphobiological studies on *Cryptosporidia* and *Sarcocystis* in animals and their impact on their health. Ph. D. Thesis Fac. Vet. Med. Zag. Univ. Egypt.
- Yosra, A. (2014).** Epidemiological investigations on the public health significance of *Cryptosporidium* parasites in livestock and people in the Ismailia Canal Zone of Egypt, pp. 245.
- Zintl, A.; Proctor, A.F. and Read, C. (2009).** The prevalence of *Cryptosporidium* species and subtypes in human faecal samples in Ireland. *Epidemiology and Infection*; 137: 270-277.