sciendo

J Biomed Clin Res Volume 15 Number 1, 2022

DOI:10.2478/jbcr-2022-0003

Review

CRYPTOSPORIDIOSIS: HISTORY, ETIOLOGY, BIOLOGY, PATHOGENESIS AND PATHOANATOMY - A REVIEW

Petya V. Dragomirova

Department of Anatomy, Histology, Cytology and Biology, Section of Biology, Medical University – Pleven

Summary

Cryptosporidiosis is a protozoan disease, usually asymptomatic. However, in some cases, the infection can progress to gastroenteritis and, more rarely, to colitis. Intensive studies on the biology of *Cryptosporidium* started in the 20th century after the outbreak of a major waterborne epidemic. The interest in this parasitosis increased after the WHO identified it as an opportunistic parasitosis - an indicator of AIDS. In this regard, the purpose of this article is to provide a scientific overview of this parasitosis, analyzing the state and development of modern research in a global and national aspect.

Keywords: *Cryptosporidium parvum*, cryptosporidiosis, parasitosis, infection, pathogenesis

Introduction

The species of *Cryptosporidium* were first isolated in 1907 by E. E. Tyzzer from the gastrointestinal tract (gastric glands) of laboratory mice. E. Tyzzer described this causative agent as *Cryptosporidium muris* [1]. He also reported a second species - *Cryptosporidium parvum*, again isolated from lab mice. The two species differed in their site of infection and morphological development [2, 3]. Slavin reported cases of infection caused by *C. meleagridis* - a new pathogenic species isolated from the intestines of turkeys. This newly isolated species was associated with an acute clinical disease, manifested with severe diarrhoea and a low death rate [4]. *Cryptosporidium* was isolated from domesticated birds suffering from gastroenteritis with a lethal outcome [4]. Since the 1970s, *Cryptosporidium* has been detected in/or the gastrointestinal or respiratory tract of many mammals, birds, fishes, and reptiles

Cryptosporidiosis in a calf was reported

Corresponding Author:
Petya V. Dragomirova
Department of Anatomy, Histology, Cytology and Biology,
Section of Biology,
Medical University – Pleven
1, St. Kliment Ohridski Str.
Pleven, 5800,
Bulgaria
e-mail: pepaalex@gmail.com

Received: October 05, 2021
Revision received: December 02, 2021
Accepted: February 10, 2022

in 1971 [5] and a pig in 1977 [6, 7]. The first disease cases in humans were reported in 1976: a 3-year-old girl from the USA living in a rural area [8] and an immunosuppressed patient [9].

Since the 1980s, representatives of the genus *Cryptosporidium* have been found to cause infections in humans quite often. The study of these infections is related to improving the diagnostic process and increasing the number of immunocompromised subjects, especially those with acquired immune deficiency syndrome (AIDS). After 1982, the medical community's interest in *Cryptosporidium* significantly increased when the Center for Disease Control and Prevention in Atlanta-USA (CDC) [10] published information about cases of protracted acute diarrhoea caused by *Cryptosporidium* in 21 male patients diagnosed with AIDS. In 14 of them, the outcome was lethal [11, 12]. Later, in 1993, an outbreak of waterborne cryptosporidiosis in Milwaukee-Wisconsin affected 403 000 people. The epidemic prompted the commencement of intensive research of the biological characteristics of the parasite [13, 14]. Cox and McDonald were the first to describe the course of human cryptosporidiosis [15, 16]. Dubey J.P. et al. had earlier published a short and helpful review [17].

The condition was first diagnosed in Bulgaria by M. Halacheva and Belchev in calves with chronic diarrhoea [18]. R. Kurdova diagnosed the first cases in humans [3, 19]. Among the population in the country, cryptosporidiosis is also known as diarrhoea of newborn animals (calves, lambs, pigs). The importance of this parasitosis has increased after WHO defined it as an AIDS indicator of parasitoses [20-24].

In 2002, *Cryptosporidium parvum* was included in agents for bioterrorism Category B, water safety threats [25].

Etiology and pathophysiology

Cryptosporidiosis is caused by intestinal coccidian parasites (apicomplexan protozoa). These parasites belong to class Sporozoasida, subclass Coccidiasina, order Eucoccidiorida, suborder Eimeriarina, family Cryptosporiidae, genus *Cryptosporidium* (from Greek –occult spore).

The major aetiological agents of the disease in humans are *Cryptosporidium parvum* (previously known as *C. parvum* Type 1, invading only humans) and *Cryptosporidium hominis* (previously known as *C. parvum* Type 2 – zoonotic, invading humans and other mammalians). However, reports of infections with *C. felis*, *C. meleagridis*, *C. canis*, *C. muris*, and *C. cuniculus* have also been published. Livestock, especially cattle, are recognized as one of the main sources of zoonotic infections. It was demonstrated that domestic and wild animals could be infected with several species and genotypes of *Cryptosporidium*, which have a narrow spectrum of hosts, and therefore may not play an essential role in public health (Ryan U. et al., 2014) [26].

The oocysts of *Cryptosporidium* are highly infectious: as few as 10^1 - 10^3 oocysts are sufficient to establish an infection in humans (50% infectious dose, 10^2). They can cause infection immediately after being excreted, and the life cycle of the parasite creates forms that are renewed in the intestine. The location of parasites in the gut is intracellular but extracytoplasmic, which can contribute to a pronounced resistance *of Cryptosporidium* species to treatment. The excreted large numbers of oocysts are resistant to disinfectants (such as chlorine) at levels routinely used in water processing. Cryptosporidiosis is usually manifested by watery diarrhoea. The mechanism by which *Cryptosporidium* causes diarrhoea involves a combination of increased intestinal permeability, chloride secretion, and malabsorption. It is considered that the host response to the infection causes these symptoms. In immunocompetent individuals, the infection is usually restricted to the small intestine.

Cryptosporidium infection has also been observed in immunocompromised individuals such as HIV-infected persons [27].

Taxonomy

The genus *Cryptosporidium* belongs to the phylum Apicomplexa, class - Sporozoasida, subclass - Coccidiasina, order Eucoccidiorida, suborder Eimeriorina, family Cryptosporidiidae [28]. *Cryptosporidium* has been classified together with other intestinal coccidian parasites in the order Eucoccidiorida based on their similar morphology and life cycle [29].

Morphology and species diversity

Currently, 29 species and more than 60 genotypes of *Cryptosporidium* are recognized - valid based on morphological, biological, and molecular data, including recently described *C. rubeyi* in ground squirrels from the genus Spermophilus [30]. More than 17 species have been identified in humans [31, 32, 33]. In addition, *Cryptosporidium viatorum* has been recently identified among travellers returning to the UK from the Indian subcontinent [34]. The occurrence of mixed infection of 3 species of *Cryptosporidium* – *C. meleagridis*, the *Cryptosporidium mink* genotype, and an unknown *Cryptosporidium species* in an immunocompetent individual was reported [35].

Recently, ultrastructural and DNA analyses have shown a high degree of morphological similarity of *Cryptosporidium* spp. with gregarine - as a trophozoite [36, 37]. The identification of stages similar to gregarines and the ability of *Cryptosporidium* to complete its life cycle in the absence of host cells has further confirmed its connection to gregarines. This offers new opportunities to study pathogenesis, epidemiology, treatment, and control of Cryptosporidium infection [32].

C. parvum exists in two biological forms: trophozoite and an oocyst. The parasite has the following stages of development: a) sporozoites (merozoites) (Figure 1) - motile elongated coma-shaped (banana) cells, coated with a three-layer membrane.

They contain a single nucleus in one pole; b) trophozoites (wrapped in 5-layer shells, two of them forming a parasitophorous vacuole) have a large nucleus with a vacuole; c) schizonts - two types with 8 or 4 banana-shaped merozoites are distinguished. Merozoites have a large nucleus with a nucleolus in its posterior pole and a microneme and rhoptries - in the anterior pole; d) microgametocytes and macrogametocytes - the mature forms contain 12 to16 wedge-shaped (cuneiform) non-flagellated microgametes located on the periphery, and residual bodies in the centre. Macrogametocytes possess large polysaccharide and phospholipid granules; e) oocysts (*C. parvum* 6-8μm) have a circular or oval shape (Figure 2).

Sporulated oocysts contain four banana-shaped sporozoites aligned in parallel to each other and a residual body. A sporocyst is absent. Oocysts were detected free in the lumen of the infected organ or attached within the parasitophorous vacuole [19, 24, 38, 39].

Biology and life cycle

The parasite is monoxenic (one-host). The

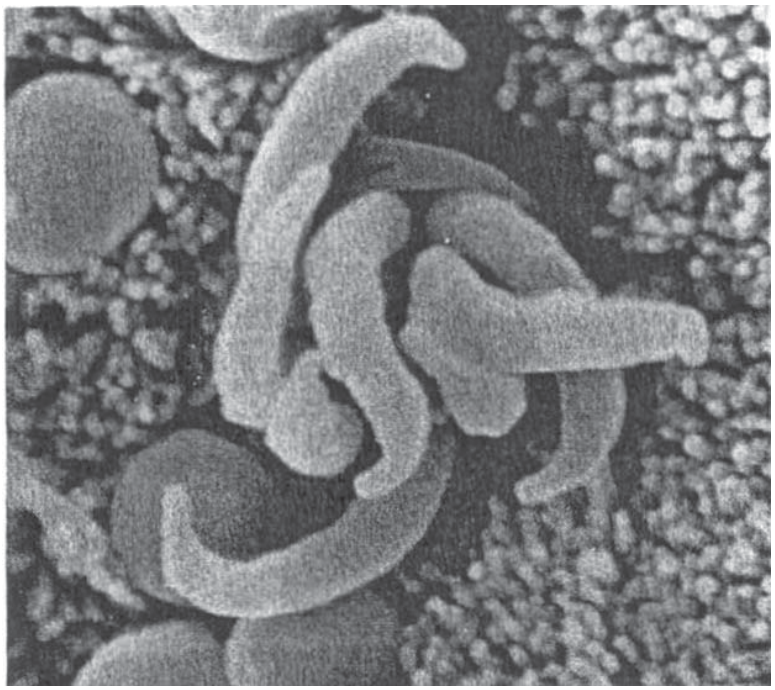

Figure 1. Electron microscopy of *Cryptosporidium sp.* from a lamb. First-generation of a cryptosporidium meront with 8 merozoites (x 1970) [24]

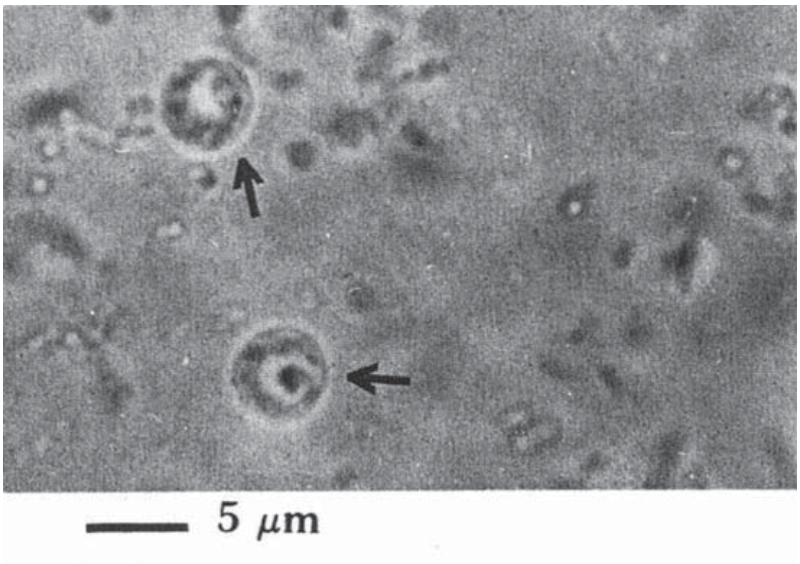

Figure 2. Oocysts of *Cryptosporidium sp.* (on light microscope under immersion, scale bar -5 μm) in a fecal sample from a homosexual male with AIDS and persistent watery diarrhoea [24]

oocyst-to-oocyst development occurs in the host organism without an intermediate host. It passes three stages of development - schizogony, merogony (of asexual reproduction), gametogony (of sexual reproduction), and sporogony (sporulation) (Figures 3, 4) [40, 41].

A sporulated oocyst is excreted through the faeces of an infected person or animal in the external environment (exogenous development). After its ingestion or inhalation, it undergoes excystation within the host and releases the sporozoites, sized up to 5.5 μm. They are motile and attack the cell wall of the epithelium of the gastrointestinal or respiratory tract of various animals and humans. Sporozoites are sucked into the microcrypts of the epithelial cells. The endogenous stage of development of the cryptosporidia occurs in the parasitophorous vacuole, which is composed of two cells of the host and two parasitic membranes. Thus, the location of cryptosporidia in the intestine is intracellular but extracytoplasmic. The duration of excretion of oocysts in the faeces lasts 7-30, possibly more days, depending on the

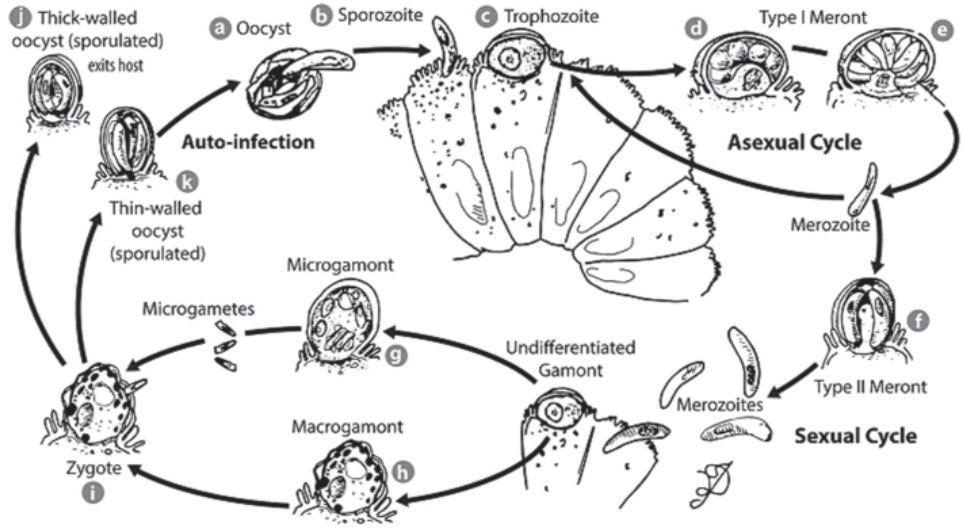

Figure 3. Biological cycle of *C. parvum* and *C. hominis* [40]
*Developmental stages: a - oocyst; b - sporozoite; c - trophozoite; d, e - Type I meront = asexual reproduction; f - Type II meront = sexual reproduction; g - microgamont (male germ cell); h - macrogamont (female germ cell); I - zygote; k - thin-walled oocyst (sporulated) - re-invasion; j - thick-walled oocyst

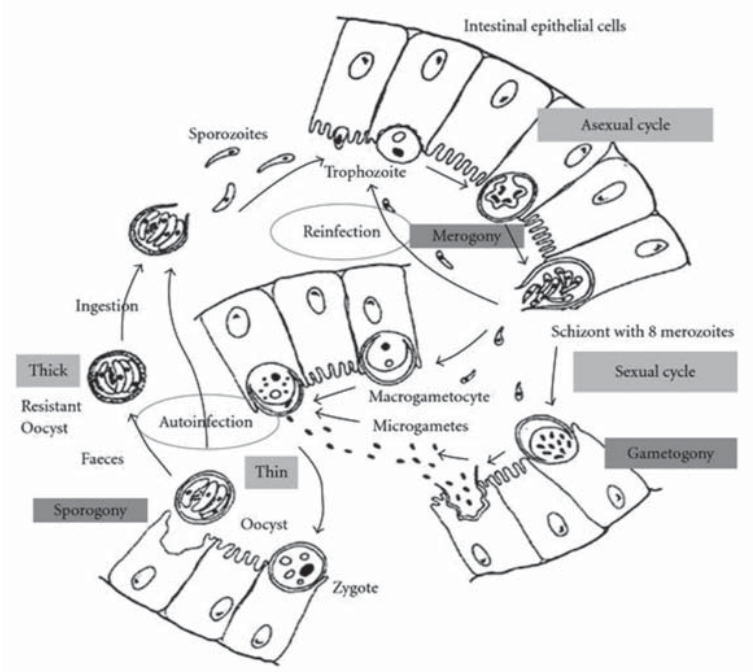

Figure 4. The life cycle of Cryptosporidium in the enterocyte) [41]

immunological status of the host. Thick-walled oocysts shed in faeces represent the parasite's infective stage for humans and animals. Thin-walled oocysts undergo excystation within the small intestine lumen to re-invade the intestinal epithelium, thus facilitating the endogenous reinvasion, which explains the chronic course of cryptosporidiosis [22, 42, 43, 44, 45].

The genome of *Cryptosporidium* consists of eight chromosomes containing nearly 9.2 million base pairs. Comparison of the genomes of *C. parvum* Type1 and *C. parvum* Type 2 showed that both genomes have 3-5% sequence variations without significant removal or rearrangement of genes. The phenotypic differences between the two types are assumed to be due to selective pressures occurring in the interaction between host cells and parasites [46].

Several metabolic pathways, numerous organelles, and genes common to eukaryotes or limited to Apicomplexa are reduced or absent in *Cryptosporidium. Cryptosporidium* lacks enzymes for synthesizing key biochemical building blocks, such as sugars, amino acids, and nucleotides. Genomic analysis of Cryptosporidium indicates that it encodes more than 80 genes with strong similarities with known transporters and several hundred genes with transporter-like properties.

Therefore, *Cryptosporidium spp.* relies heavily on extracting nutrients from the host rather than biosynthesis on substrate level for energy production [27].

Pathogenesis and pathological anatomy

The pathogenesis of *Cryptosporidium* has not been studied sufficiently. It is similar to the pathogenesis of enteric and rotaviruses, intestinal protozooses like amebiasis, giardiasis, etc. The major damage inflicted in cryptosporidiosis is in the gut, but cryptosporidia can be found in other organs such as the lungs, conjunctiva, and the digestive tract. In rare cases, enlargement of mesenteric lymph nodes is present. In the pathogenesis of the disease, the toxic and sensitising effects of the parasite through its metabolic products and toxins in endogenous development probably play a role. The intestinal damages include atrophy of the villi, epithelium hyperplasia in the crypts of the villi, and degenerative changes in the lamina propria, the latter being rich in macrophages and neutrophils, eosinophils, and lymphocytes. Thus, the small intestine's enzyme activity and absorptive surface decrease. Malabsorption syndrome develops with profuse diarrhoea, lactose, and enzyme deficiency, and the protein metabolism and water and electrolyte balance are severely

disturbed. The urogenital tract can be damaged in the presence of intestinal parasites in an ascending way. Haematogenous spread is also possible [2, 23, 47, 48].

Approximately 10^7 oocysts can cause infection [49]. An infected person excretes the highest number of oocysts during the first week. According to data about *C. parvum* and *C. hominis*, oocysts can be excreted for weeks after the diarrhoea subsides. However, excretion of oocysts has been observed in immunocompetent individuals infected with *C. muris* for as long as seven months. Significant progress in identifying the putative virulence of *Cryptosporidium* is achieved due to the establishment of the genomes of *C. parvum* and *C. hominis* with describing over 25 putative virulence factors identified by various immunological and molecular techniques [31]. The immune system reduces the formation rate of merozoites type 1 and the number of thin-walled oocysts. This helps prevent autoinfection [50]. A previous infection in immunocompetent people leads to a low resistance against future infection but can also reduce the severity of disease and the number of excreted oocysts [51].

Conclusions

The above analysis gives grounds for recommending more precise criteria for the true incidence and prevalence of cryptosporidiosis and for *Cryptosporidium* identification to be included in routine screening of fecal samples of immunocompromised and immunosuppressed individuals.

Developing a monitoring system of early detection of an increased frequency of *Cryptosporidium* oocysts in adult fecal samples may be a potential indicator of water transmission.

Acknowledgments

The author would like to express gratitude to the Medical University - Pleven, project №3/ 2019, for the help and support of this article.

References

1. Tyzzer EE. A sporozoan found in the peptic glands of the common mouse. Proc. Soc. Exp. Biol Med. 1907-1908;5:12-3.
2. Thompson RC, Olson ME, Zhu G, Enomotos S, Abrahamsen MS, Hijjawi NS et al. Cryptosporidium and cryptosporidiosis. Adv. Parasitol. 2005;59:77-158.
3. Kurdova R, Yordanova D. Opportunistic parasitoses and HIV infection in Bulgaria, Infectology. 2000;4(37):16-9.
4. Slavin D. Cryptosporidium meleagridis (sp. nov.). J. Comp. Pathol. Therapeut. 1955;65:262-6.
5. Panciera RJ, Thomassen RW, Garner FM. Cryptosporidial infection in a calf. Vet. Pathol. 1971;8:479-84.
6. Bergeland ME. Necrotic enteritis in nursing piglets. Proc. Am. Assoc. Vet. Lab. Diagn. 1977;20:151-8.
7. Kennedy GA, Kreinter GL, Strafus AC. Cryptosporidsis in three pigs. J. Am. Vet. Med. Assoc. 1977;170:348-50.
8. Nime FA, Burek JD, Page DL, Holsher MA, Yardley JH. Acute enterocoltis in a human being infected with the protozoan Cryptosporidium. Gastroenterology. 1976;70:592-8.
9. Meisel JL, Perea DR, Meligro C et al. Owerhelming watery diarrhea asocieted with a Cryptosporidium in an immunosuppressed patient. Gastroenterol. 1976;70:1156-60.
10. CDC. Cryptosporidiosis: assessment of chemotherapy of males with acquired immune deficiency syndrome (AIDS). MMWR 1982;31:589-92.
11. Kaftandjiev I. New protozoan diseases. Infectology. 2010;47(2):12-7.
12. Velev V, Georgieva K. [Cryptosporidiosis. Medical Review]. 2017;53(6):5-9.Bulgarian
13. Fayer R. Cryptosporidium and Cryptosporidiosisл 1997, CRC Press, LL.C.
14. MacKenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blair KA, Peterson DE et al. A massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply. N. Engl. J. Med. 1994;331:161-7.
15. Cox FE. Hystory of human parasitology. Clin Microbiol Rev. 2002 Oct;15(4):595-61291.
16. McDonald V. Cryptosporidiosis.F. E. G. Cox (ed.), The Welcome Trust illustrated history of tropical diseases. London, United Kingdom. The Wellcome Trust :1996. p257-65
17. 17. Dubey JP, Speer CA, Fayer R (ed.). Cryptosporidiosis of man and animals. CRC Press, Inc., Boca Raton, Fla. 1990
18. Halacheva M. Belchev D. Detection of Cryptosporidium sp. oocysts in calves. Veterinary Journal. 1984;10:30-31.
19. Kurdova R. [Diarhoeal syndrome in tropical regions. Cryptosporidiosis. In: Tropical

diseases.] Iliev B, editor. Pleven, Bulgaria: Medical University – Pleven; 2001.p.200-3. Bulgarian
20. Boeva-Bangyozova B. Cryptosporidiosis. Medical practice. 2007;3:23-30.
21. Boeva-Bangyozova B, Rainova I. Summer threats: Cryptosporidiosis. Forum Medicus. Issue 23, 11.07.2016, LXXI:6-7.
22. Beyer TV, Sidorenko NV, Anatskaiya ON. Intestinal Cryptosporidiosis in early childhood and its sequelae. Medical parasitology and parasitic diseases. 2006;1:3-7.
23. Kamburov P. Cryptosporidiosis (Cryptosporidiosis), Veterinary-medical parasitology, Agroprogress. 1994:321-3.
24. Ma P. Cryptosporidiosis and immune enteropathy: a review. Curr Clin Top Infect Dis. 1987; 8:99-153.
25. Atlas RM. Bioterrorism: From Threat to Reality. Ann Rev Microbiol. 2002;56:167-85.
26. Ryan U, Fayer R, Xiao L. Cryptosporidium species in humans and animals: current understanding and research needs. Parsitol. 2014;141(13):1667-85.
27. Shrivastava A.K, Kumar S, Smith WA, Sahu PS. Revisiting the global problem of cryptosporidiosis and recommendation Trop. Parasitol. 2017;7(1):8-17.
28. O`Donoghhue PJ. Cryptosporidium and cryptosporidiosis in man and animals. Int. J. Parasitol. 1995; 25(2):139-95.
29. Ghazy A, Abdel-Shafy S, Shaapan R, Shaapan M. Cryptosporidiosis in Animals and Man:1. Taxonomic Classification. Life Cycle, Epidemiology and Zoonotic Improtance. Asian J Epidemiol. 2015;8(3):48-63.
30. Li X, das Graças Cabral Pereira M, Larsen R, Xiao C, Phillips R, Striby K, McCowan B, Atwilla E. Cryptosporidium rubeyi n. sp. (Apicomplexa: Cryptosporidiidae) in multiple Spermophilus ground squirrel species. Int J Parasitol Parasites Wildl. 2015 Dec;4(3):343-50.
31. Bouzid M, Hunter PR, Chalmers RM, Tyler KM. Cryptosporidium pathogenicity and virulence. Clin Microbiol Rev. 2013 Jan. 26(1):115-34.
32. Ryan U, Hijjawi N. New developments in Cryptosporidium research. Int. J. Parasitol. 2015. 45:367-73.
33. Zahedi A, Paparini A, Jian F, Robertson I, Ryan U. Public health significance of zoonotic Cryptosporidium species: Critical insights into drinking water management. Int J Parasitol Widlife. 2016;5(1):88-109.
34. Elwin K, Hadfield SJ, Robinson G, Crouch ND, Chalmers RM. Cryptosporidium viatorum n. sp. (Apicomplexa: Cryptosporidiidae) among travellers returning to Great Britain from the Indian subcontinent, 2007-2011. Int J Parasitol. 2012;42:675–82.
35. Ng-Hublin JS, Combs B, Mackenzie B, Ryan U. Human cryptosporidiosis diagnosed in Western Australia: a mixed infection with Cryptosporidium meleagridis, the Cryptosporidium mink genotype, and an unknown Cryptosporidium species. J Clin Microbiol. 2013;51:2463–5.
36. Aldeyarbi HM, Karanis P. The Ultra-Structural Similarities between Cryptosporidium Gregarines. J Eukaryot Microbiol.2016;63(1):79-85.
37. Thompson RC, Ash A. Molecular epidemiology of Giardia and Cryptosporidium infections. Infect Genet Evol. 2016;40:315-23.
38. Borowski H, Thompson RC, Amstrong T, Clode PL. Morphological characterization of Cryptosporidium parvum life-cycle stages in an in vitro model system. Parasitol. 2010;137(1):13-26.
39. Kurdova R. [Cryptosporidiosis, Clinical parasitology and tropical medicine.] Sofia:Iztok-Zapad. 2016. Bulgarian
40. Current WL, Gracia LS. Cryptosporidiosis. Clinic. Microbiol. Rev. 1991;4:325-58.
41. Putignani L, Menichella D. Global Distribution, Public Health and Clinical Impact of the Protozoan Pathogen Cryptosporidium. Interdiscip Perspect Infect Dis. 2010, Article ID 753512.
42. Fayer R, Ungar BL. Cryptosporidium spp. and cryptosporidiosis. Microbiol Rev. 1986;50(4):458-83.
43. Fayer R, Xiao L. Review of "Cryptosporidium spp. and cryptosporidiosis". Parasit Vectors.2008;1:47
44. Markell EK, Joh DT, Krotoski WA. Medical Parasitology. USA: W.B. Saunders company;1999.
45. 45. White AC Jr. Cryptosporidiosis (Cryptosporidium species). Bennett JE, Dolin R, Blaser MK, eds. Principles and Practice of Infectious Diseases. 8th ed. Philadelphia, Pa: Elsevier Churchill Livingstone; 2015.p 3173-83.
46. Mazurie AJ, Alves JM, Ozaki LS, Zhou S, Schwartz DC, Buck GA. Comparative Genomics of Cryptosporidium. Int J Genom. 2013, Article ID 832756.
47. Yushchuka ND, Vengerova Y. Cryptosporidiosis, Infectios diseases. Moscow: National Guidelines, GEOTAR-Media Publishing Group, 2009.
48. Laurent F, McCole D, Eckmann L, Kagnoff MF. Pathogenesis of Cryptosporidium parvum

infection. Microbes Infect. 1999 Feb;1(2):141-8.
49. Chen W, Harp JA, Harmsen AG. Cryptosporidium parvum infection in gene-targeted B cell-deficient mice. J. Parasitol. 2003;89(2):391–3.
50. Kenneth R, George C. Sherris Medical Microbiology: An Introduction to Infectious Disease (4th ed.). New York: McGraw-Hill;2004.
51. Clark Douglas P. New Insights into Human Cryptosporidiosis. Clin Microbiol Rev. 1999;12(4):554–63.