Annals of Parasitology 2020, 66(3), 311–318
doi: 10.17420/ap6603.269

Original paper

Evidences of brain and lung invasion of a local water *Cryptosporidium parvum* isolate in comparison to Iowa strain: serological and immunohistochemical cytokine evaluation

Mona GABER[1], Lamia A.A. GALAL[1], Dalia HASSAN[3], Dalia M. BADARY[2], Israa M.A. MOHAMED[3], Nahed ELOSSILY[1]

[1]Department of Parasitology, Faculty of Medicine, Assiut University, Major General Ahmed Kamel Street, Al-Qasr Road, Assiut 71515, Egypt
[2]Department of Pathology, Faculty of Medicine, Assiut University, Major General Ahmed Kamel Street, Al-Qasr Road, Assiut 71515, Egypt
[3]Department of Animal Hygiene, Faculty of Veterinary Medicine, Assiut University, Major General Ahmed Kamel Street, Al-Qasr Road, Assiut 71515, Egypt

Corresponding Author: Mona GABER; e-mail: monahadea@gmail.com

ABSTRACT. *Cryptosporidium* spp. is an obligate intracellular parasite that has become a community threat. The pathological consequences of cryptosporidiosis vary not only in different *Cryptosporidium* species but even among different isolates of the same species. The present study aimed to track the serological and immunohistopathological differences between animals infected by *Cryptosporidium parvum* "Iowa isolate" (CPI) and *Cryptosporidium parvum* "water isolate" isolated from a local water supply in Assiut Governorate, Egypt (CPW). Three experimental groups were encountered: negative control group (C), the CPI group and the CPW group; each contains ten Swiss albino mice. Serum cytokine: IL10 and TNF-α were measured. Expression of *Cryptosporidium* antigen and CD3 in the intestinal, pulmonary and brain tissue were evaluated through immunohistochemical assay. IL10 and TNF-α were elevated in both infected groups, over expression of *Cryptosporidium* protein and CD3 in the intestinal, pulmonary and brain tissue in CPW infected group compared to Iowa infected one. Multi-organs affection occurred in the CPW indicating more severe pathogenicity and virulence than standard Iowa isolate. The local *C. parvum* isolate was more virulent than tested Iowa isolate as it spread extra-intestinally to reach brain tissue.

Keywords: *Cryptosporidium parvum*, isolates, immunohistochemical, interleukin 10, tumor necrosis factor

Introduction

Cryptosporidiosis is considered as a harmful, difficult to control infection of humans and many farm animals, resulting in substantial economic losses. *Cryptosporidium* species are obligate intracellular apicomplexan protozoan parasites that were reported to infect humans in 106 countries and have been found to influence more than 150 mammalian species around the world [1]. The World Health Organization has categorized *Cryptosporidium* as a reference pathogen for the evaluation of drinking water quality [2]. Some of water-associated outbreaks reported worldwide (23.7%) were due to *Cryptosporidium* spp. infection that either passed through filtered or unfiltered drinking-water systems or contaminated water distribution systems [3]. *Cryptosporidium* spp. is transmitted by the faecal-oral route [4], *via* the inhalation of oocysts [5,6], the haematogenous spread was also suggested [7].

The severity, persistence and ultimate outcome of *Cryptosporidium* infections are governed by a variety of factors concerning both host and parasite characteristics. Host factors embrace both the immune status and frequency of exposure of the infected individual [4]. The host's immune state has a strategic role in determining the susceptibility and severity of cryptosporidiosis. *Cryptosporidium* causes self-limited gastroenteritis in immuno-

competent individuals, while in immuno-compromised individuals, *Cryptosporidium* infection can be responsible for developing severe, life-threatening gastrointestinal and disseminated cryptosporidiosis in other organ systems such as pancreatic, biliary, and respiratory tract [8]. The cerebral pathology was detected in mice in a study supporting the idea of haematogenous dissemination of *Cryptosporidium* spp. [7].

Production of cells that mediate delayed hypersensitivity and limiting interferon production are mainly regulated by IL10. TNF-α, a cytokine produced by macrophage is a signify mediator of inflammatory and immunological reaction and involved in immune protective mechanisms against intracellular infections [9].

CD3 was one of the first groups of human T lymphocyte surface antigens identified using monoclonal antibodies [10]. It was subsequently shown that antibodies against CD3 could either stimulate T cells to divide or inhibit the development of effector functions such as cytotoxicity. Therefore, it was apparent that CD3 had an important role in T cell function [11].

Concerning the role of parasite characteristic in the outcome of *Cryptosporidium* infection, several studies were reported different degrees of pathogenicity and virulence among *Cryptosporidium* species and isolates of the same species [2]. The study conducted by Sayed et al. [7] clarified that *C. parvum* isolated from the local water supply was more virulent than the IWA strain. Virulent nature of new local isolate had been manifested by the fast and 100% animal death in the course of the pre-experimental infection. Post-mortem pictures revealed degenerative changes, vasculitis in the brain parenchyma and emphysematous deviations in the lungs [7].

The present study, aims to validate the supposed virulent nature of that new local isolate versus the standard IWA isolate by testing the presence of the parasite in different tissues and assessment of the host response on the level of CD4 regulatory function (TNFα), inhibitory function (IL10) and TCR (CD3).

Materials and Methods

Based on the previous experiment of Sayed et al. [7] the current study was conducted as follows:

The experimental animals. Thirty pathogens free bred Swiss albino male mice were used. The animals were eight weeks old and their average weight was approximately 25–35 grams each. All maintained under optimal laboratory conditions [12].

***Cryptosporidium parvum* isolates. First isolate:** *C. parvum* oocysts of Iowa isolate of bovine faeces source passed once in mouse (CPI), they were provided from Waterborne (P102M, Waterborne^{TM}, USA). The parasite was suspended in PBS, antibiotics (penicillin, streptomycin, gentamicin, amphotericin B to kill any contaminating bacteria) and 0.01% Tween 20 and stored at 4ºC until used. **Second isolate:** *C. parvum* oocysts were isolated from Assiut city, Egypt; tape drinking water samples proved to be positive by flow cytometry, 5 positive samples (collected from houses, hospitals and animals farm). Water samples were completely filtered using special filter apparatus [13]. Samples then were isolated and purified through discontinuous sucrose gradient flotation [14].

Experimental infection. A minimum of 10 mice in 3 experimental conditions. **Control group**: it was including 10 mice, did not receive any infection. **CPI groups:** it was including 10 mice, all mice in this group were infected orally with CPI isolate. The amount given to each mouse was attuned to contain approximately 1.5×10^5 oocysts according to the previous recommendation of Suresh and Rehg [14]. **CPW groups:** it was including 10 mice, all mice in this group were infected orally with CPW isolate. The amount given to each mouse was adjusted to contain approximately 600 oocysts agreeing to the recommendation of Sayed et al. [7].

Evaluation of experimental conditions. Serological evaluation of IL10 and TNF-α among groups: concentrations of TNF-α and IL10 were determined in a serum sample from animals of the different groups at the end of the experiment (14 days of infection). An enzyme-linked immunosorbent assay (ELISA) technique was employed with antibody pairs from Bio Legend, Inc. (San Diego, USA). The technique was developed according to the manufacturer protocol. **Immunohistochemical examination:** all animals were followed up throughout the 14 days of the experiment. All survivors were killed by neck dislocation under anaesthesia with ether. After animals were sacrificed, tissue samples were collected from the terminal ileum, lung and brain [15]. **Detection of *Cryptosporidium* antigen and CD3 by immuno-histochemistry:** tissue sections (4 mm-thick) were transferred to a 10 mmol/l citrate buffer solution at a

Table 1. IL10 difference among groups compared to control

Groups	No. of samples	Mean	SD
control	10	213.6	23.29099
CPI	10	1575.8**	625.09256
CPW	10	1208.6**	459.81589

Explanations: * - P value is significant ($P \leq 0.05$); ** – P value is highly significant ($P \leq 0.001$); SD – standard deviation; CPI – group infected with *C. parvum* "Iowa isolate"; CPW – group infected with *C. parvum* "water isolate" isolated from a local water supply.

pH of 6.0 and heated at 80şC for 30 min. in the microwave to retrieve antigens for *Cryptosporidium* antibody and CD3 staining. After washing in water, 3.0% H_2O_2 in methanol was applied for 20 min., in order to block endogenous peroxidase activity. The slides were incubated for 20 min. with normal goat serum at room temperature to avoid non-specific staining. Mouse monoclonal anti-*Cryptosporidium* antibody (1:50 Novus Biologicals, USA, Catalogue No: NB100 65674UV) and rabbit polyclonal anti-CD3 (1:100) were added for 1h at room temperature. Negative control sections were similarly treated. The biotinylated secondary antibody, streptavidin-horseradish peroxidase-conjugated tertiary antibody, and diaminobenzidine were applied according to the manufacturer's instructions. Sections were then counterstained with Mayer's Haematoxylin.

Statistical analysis. Data analysis was conducted using SPSS program version 16. Chi-square test was used to compare qualitative variables, while the independent test was used in comparisons between quantitative variables. Qualitative data were presented in form of frequencies and proportions while mean and standard deviation were used to express quantitative data. Statistically, significance was considered when P value less than 0.05 and 95% confidence intervals did not overlap.

Table 2. TNF-α difference among groups in comparision to control

Groups	No. of samples	Mean	SD
control	10	139.4	2.26241
CPI	10	749.84**	318.52469
CPW	10	928.92**	169.45119

Explanations: see Table 1

Ethics aspects. The Ethics Committee of the Assiut University approved this study as it meets the International Guiding Principles for Biomedical Research Involving Animals as issued by the Council for International Organizations of Medical Sciences (approval no.17300369).

Results

IL10 difference among groups in comparison to control (Table 1)

IL10 serum level in CPW and CPI significantly increased than control (Fig. 1), while the difference between IL10 serum level in CPI and CPW was significant.

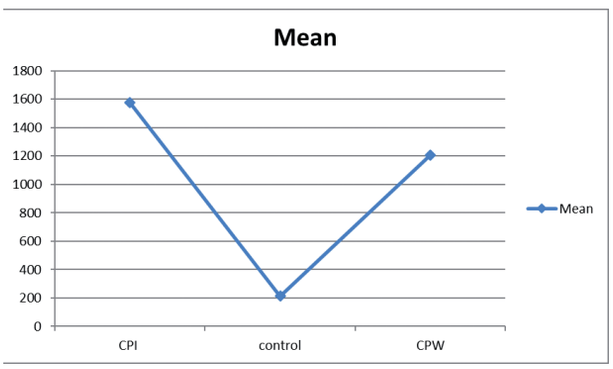

Figure 1. Means of IL10 difference among groups in comparison to control
Explanations: CPI - group infected with *C. parvum* "Iowa isolate"; CPW - group infected with *C. parvum* "water isolate" isolated from a local water supply.

TNF-α difference among groups in comparison to control (Table 2)

TNF serum level significantly increased in both in CPW and CPI groups than the control. There is no significant difference between TNF serum level in CPW and CPI (Fig. 2).

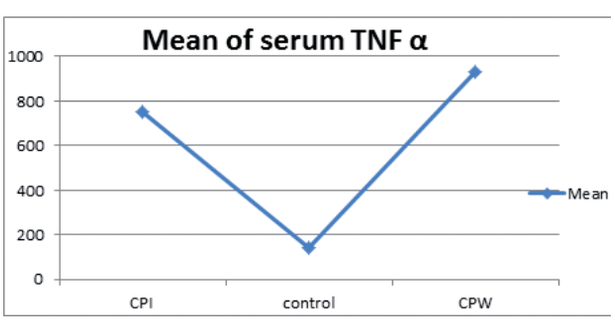

Figure 2. Means of TNF-α difference among groups in comparison to the control
Explanations: see Figure 1

Expression of *Cryptosporidium* antigen protein in the intestinal, pulmonary and brain tissue

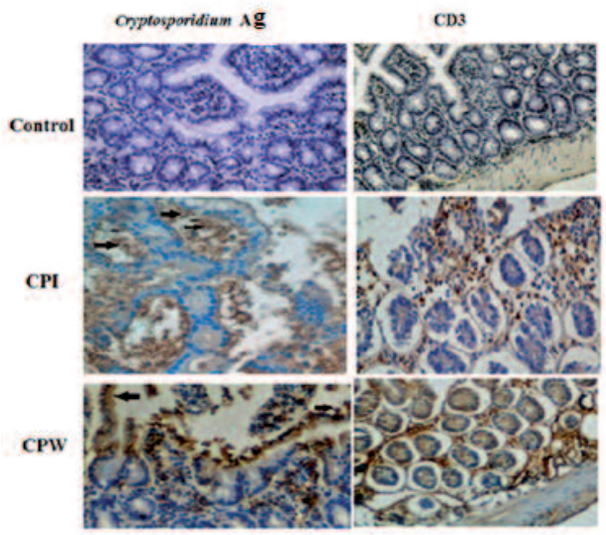

Figure 3. Immunohistochemical analysis of *Cryptosporidium* antigen and CD3 expression in intestinal mice tissues
Explanations: *Cryptosporidium* Ag – *Cryptosporidium* antigen; CPI – group infected with *C. parvum* "Iowa isolate"; CPW – group infected with *C. parvum* "water isolate" isolated from a local water supply.

Immunohistological evaluation of the formalin fixed paraffin embedded intestinal**,** pulmonary and brain tissue revealed presence of *Cryptosporidium* antigen in intestinal**,** pulmonary and brain tissue of the CPW, while in CPI it was detected only in intestinal tissues compared to their absence in the healthy controls (Fig. 3–5).

Over-expression of CD3 protein in the intestinal, pulmonary and brain tissue

Immunohistological evaluation of the formalin fixed paraffin embedded intestinal**,** pulmonary and brain tissue revealed overexpression of CD3 protein in lymphocytes infiltrate the intestinal**,** pulmonary and brain tissue of the CPI and CPW groups as compared to the healthy controls (Fig. 3–5).

Discussion

Interestingly**,** the local water *C. parvum* (CPW) isolate was previously approved as more infective and virulent than the imported *C. parvum* Iowa (CPI) isolate on different parameters lacking immunological ones [7]. The current work put their propositions under the microscope of science by testing both effects on local and systemic

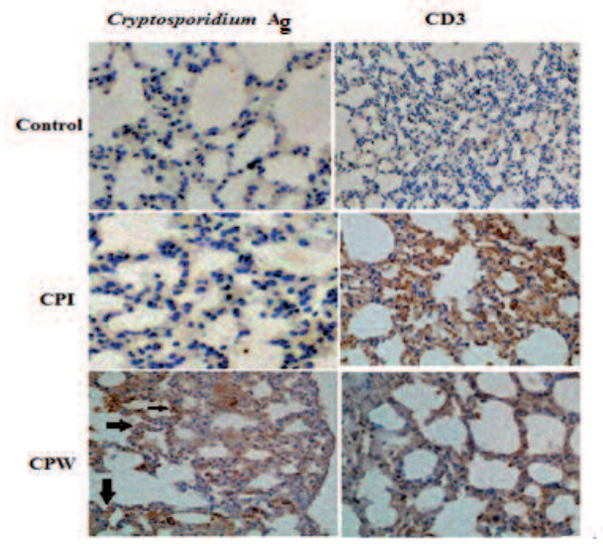

Figure 4. Immunohistochemical analysis of *Cryptosporidium* antigen and CD3 expression in mice lung tissues
Explanations: see Figure 3

immunological response.

Cryptosporidium spp. oocysts isolated from local water samples (CPW) were identified by molecular characterization as *C. parvum*. Both isolates were experimentally evaluated, aiming to study virulence of tested local CPW isolate and imported *C. parvum* Iowa (CPI) isolate.

Walking on the guide of our previous study [7], both isolates infectious dose accomplished 100% infections in all infected mice. Infection was insured by the detection of oocysts in faeces of challenged mice 36 hours or later after experimentation started [2].

By the immunohistological evaluation of formalin fixed paraffin embedded intestinal, pulmonary and brain tissues, the data revealed the presence of *Cryptosporidium* antigen in intestinal and pulmonary and brain tissues in CPW infected group, while in CPI infected mice, the organisms protein detected in intestinal tissues only, this proved the virulent nature of local water isolate.

The pathogenesis of pulmonary cryptosporidiosis has not been fully clarified. According to previous studies reported that respiratory cryptosporidiosis may occur commonly in immune-competent and immune-deficient individuals [16]. Similar results of establishment of *C. parvum* various epicellular stages and forms of oocysts within lung organoids was recorded before [17].

The possible mechanisms of pulmonary infection proposed to be *via* oocyst inhalation [18,19]. The

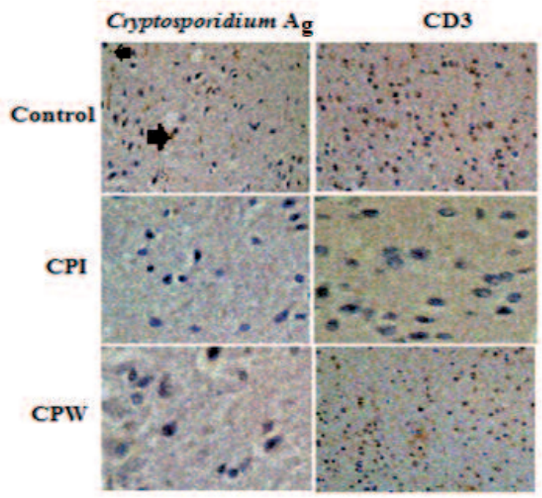

Figure 5. Immunohistochemical analysis of *Cryptosporidium* antigen and CD3 expression in mice brain tissues
Explanations: see Figure 3

most grounded proof for individual-to-individual respiratory transmission of cryptosporidiosis in immunocompetent children was expressed when 35% of Ugandan tested kids determined to have intestinal cryptosporidiosis and cough; had *Cryptosporidium* DNA in their sputum tests resulting in the suggesting of the presence of organisms in the respiratory tract [20]. Another possible rout of respiratory infection is the aspiration of gastrointestinal contents during vomiting episode in patients with intestinal involvement [18,21,22].

There was a little evidence supported the haematogenous spread of *C. parvum* from the intestinal tract to extraintestinal tissues which suggested to be via the circulating phagocytes [23]. The speculation is upheld by the presence of *Cryptosporidium* spp. within macrophages, prompting diminished phagocytic capacity. In addition, this parasite can multiply within macrophages *in vitro*. As well as the presence of the parasite within the blood vessels in the intestinal and pulmonary sub-mucosa revealed by autopsy studies [19,24,25].

The detected *C. parvum* protein in the brain tissue of experimentally infected mice with CPW comes in agreement with a previous finding which recorded degenerative changes and vasculitis in brain parenchyma on post-mortem histopathological examination of mice infected by CPW strain [7].

Higher virulence of the CPW strain than CPI strain might be the main factor of the recorded cerebral affections as a result of probable haematogenous spread of *C. parvum*. This finding could be supported by the discoveries of past investigations as *Cryptosporidium* parasite has been identified in; sinuses [26]; conjunctiva of birds [27,28] and in the pancreatic ducts of a child with severe combined immune deficiency distributed cryptosporidiosis that was found at autopsy [2].

Additionally, cerebral affection could be explained by the presence of an enterotoxin secreted by the parasite getting to the central nervous system. However, there is no evidence for a toxin-mediated pathology had been reported, in spite of the previous efforts to recognize such a toxin [29].

The concept of disseminated cryptosporidiosis is imposing itself to the discussion; the previous finding of parasite in the bile, liver, lung and brain support the simultaneous spread assumption theory [2,7,12,30].

Cryptosporidium infection does not normally cause systemic infection or penetrate deep tissue, possible diverse genetic structures, different hosts; the age of oocysts; vehicle of transmission (stool and water) and exposure to environmental conditions could explain infectivity and virulence differences detected among the two tested isolates. Another cause attributed to the high virulence and infectivity of water isolate is the absence of hosts where oocysts can propagate in [2].

Different patterns of colonization along the host's tissues reported in different species and strains of the parasite [30–32], even though the same isolate (Iowa), has shown different patterns of colonization along the gut in experimentally infected animal models [33]. All can explain the variable pathogenicity detected between the two tested *Cryptosporidium* isolates.

The infection result is ultimately determined by pathogen-host interaction. The integrity of the intestinal tract, as well as mucosal immunity, may be responsible for infection outcome unpredictability. Such an association supported by evidence comes from cryptosporidiosis in immunocompromised subjects, where illness is much more persistent and severe than in normal immunocompetent subjects [34].

Cryptosporidium parvum usually establishes itself in a membrane-bound compartment on the apical surface of the intestinal epithelium, leading to dropping local mucosal immunity through the effects of inflammatory cells and cytokines employed at the site of infection [32].

The systemic pro-inflammatory cytokine TNF-α

is significantly increased among the experimentally infected mice by both CPW and CPI strains compared with the non-infected control group. This result comes inconsistent with the findings of many studies that demonstrated that TNF-α is elevated in association with the inflammatory changes stimulated by *Cryptosporidium* infection both in animal and cell lines and in human xenograft models [35,36]. The role of TNF-α in the control of infection has already been demonstrated as it plays an important role in a variety of parasitic diseases [37] such *Leishmania* infection [38].

In the present study, the IL10 serum level in both CPW and CPI infected groups was significantly increased in comparison with control groups. This finding comes in agreement with the result obtained by the study conducted by Lacroix et al. [35] who found that mRNA expression levels for both IL4 and IL10 increased during *Cryptosporidium* infection. The levels of IL10 in human ileocecal adenocarcinoma (HCT-8) cells increased significantly during infection with *C. hominis* and *C. parvum* [39]. However, increased expression of IL10 has never been observed in murine or bovine mucosa infected by *C. parvum*, and its role in protection has not been demonstrated [35].

In the present study, there is overexpression of CD3 proteins in lymphocytes infiltrate the intestinal, pulmonary and brain tissues. This result come inconsistently with previous study which demonstrated that the vast majority of CD cells in gut sections were $CD3^+$ in neonatal lambs inoculated with *Cryptosporidium parvum* at birth [40]. The role of $CD3^+/CD4^+$ lymphocytes is proved to be pivotal for cryptosporidiosis recovery [41].

The local *C. parvum* isolate was more virulent than tested Iowa isolate as it spread extra-intestinally to reach brain tissue. Serum and tissue cytokine elevation affirm the massive tissue damage property of the local isolate.

Acknowledgements

We would like to thank Prof. Mohomed Essa Merghany, Department of Parasitology, Faculty of Medicine, Assiut University for his technical comments that greatly improved the manuscript.

References

[1] Checkley W., White A.C.Jr., Jaganath D., Arrowood M.J., Chalmers R.M., Chen X.M., Fayer R., Griffiths J.K., Guerrant R.L., Hedstrom L., Huston C.D., Kotloff K.L., Kang G., Mead J.R., Miller M., Petri W.A. Jr., Priest J.W., Roos D.S., Striepen B., Thompson R.C.A., Ward H.D., Van Voorhis W.A., Xiao L., Zhu G., Houpt E.R. 2015. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. *The Lancet Infectious Diseases* 15: 85-94. doi:10.1016/S1473-3099(14)70772-8

[2] Medema G., Teunis P., Blokker M., Deere D., Davison A., Charles P., Loret J.F. 2006. WHO guidelines for drinking water quality: *Cryptosporidium.* 3rd ed., EHC Cryptosporidium draft 2, WHO, Geneva, Switzerland.

[3] Karanis P., Kourenti C., Smith H. 2007. Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. *Journal of Water and Health* 5: 1-38. doi:10.2166/wh.2006.002

[4] Meinhardt P.L., Casemore D.P., Miller K.B. 1996. Epidemiologic aspects of human cryptosporidiosis and the role of waterborne transmission. *Epidemiologic Reviews* 18: 118-136. doi:10.1093/oxfordjournals.epirev.a017920

[5] Harari M.D., West B., Dwyer B. 1986. *Cryptosporidium* as cause of laryngotracheitis in an infant. *The Lancet* 327: 1207. doi:10.1016/s0140-6736(86)91181-5

[6] Tzipori S., Ward H. 2002. Cryptosporidiosis: biology, pathogenesis and disease. *Microbes and Infection* 4: 1047-1058. doi:10.1016/s1286-4579(02)01629-5

[7] Sayed F.G., Hamza A.I., Galal L.A., Sayed D.M., Gaber M. 2016. Virulence of geographically different *Cryptosporidium parvum* isolates in experimental animal model. *Annals of Parasitology* 62: 221-232. doi:10.17420/ap6203.56

[8] Chalmers R.M., Davies A.P. 2010. Minireview: clinical cryptosporidiosis. *Experimental Parasitology* 124:138-146. doi:10.1016/j.exppara.2009.02.003

[9] Beutler B., Cerami A. 1989. The biology of cachectin/TNF – a primary mediator of the host response. *Annual Reviews* 7: 625-655. doi:10.1146/annurev.iy.07.040189.003205

[10] Kung P., Goldstein G., Reinherz E.L., Schlossman S.F. 1979. Monoclonal antibodies defining distinctive human T cell surface antigens. *Science* 206: 347-349. doi:10.1126/science.314668

[11] Mizoguchi A., Andoh A. 2013. Animal models of inflammatory bowel disease for drug discovery. In: *Animal models for the study of human disease*. (Ed. P.M. Conn). Academic Press, London: 499-527. doi:10.1016/B978-0-12-415894-8.00022-1

[12] Abdou A.G., Harba N.M., Afifi A.F., Elnaidany N.F. 2013. Assessment of *Cryptosporidium parvum* infection in immunocompetent and immunocompromised mice and its role in triggering intestinal dysplasia. *International Journal of Infectious Diseases* 17: e593-e600.

doi:10.1016/j.ijid.2012.11.023

[13] Hassan D., Farghali M., Eldeek H., Gaber M., Elossily N., Ismail T. 2019. Antiprotozoal activity of silver nanoparticles against *Cryptosporidium parvum* oocysts: new insights on their feasibility as a water disinfectant. *Journal of Microbiological Methods* 165: 105698. doi:10.1016/j.mimet.2019.105698

[14] Suresh P., Rehg J.E. 1996. Comparative evaluation of several techniques for purification of *Cryptosporidium parvum* oocysts from rat feces. *Journal of Clinical Microbiology* 34: 38-40. https://jcm.asm.org/content/ jcm/34/1/38.full.pdf

[15] Surl C.G., Kim H.C. 2006. Concurrent response to challenge infection with *Cryptosporidium parvum* in immunosuppressed C57BL/6N mice. *Journal of Veterinary Science* 7: 47-51. doi:10.4142/jvs.2006.7.1.47

[16] Sponseller J.K., Griffiths J.K., Tzipori S. 2014. The evolution of respiratory cryptosporidiosis: evidence for transmission by inhalation. *Clinical Microbiology Review* 27: 575-586. doi:10.1128/CMR.00115-13

[17] Heo I., Dutta D., Schaefer D.A., Iakobachvili N., Artegiani B., Sachs N., Boonekamp K.E., Bowden G., Hendrickx A.P.A., Willems R.J.L., Peters P.J., Riggs M.W., O'Connor R., Clevers H. 2018. Modelling *Cryptosporidium* infection in human small intestinal and lung organoids. *Nature Microbiology* 3: 814-823. doi:10.1038/s41564-018-0177-8

[18] Kocoshis S.A., Cibull M.L., Davis T.E., Hinton J.T., Seip M., Banwell J.G. 1984. Intestinal and pulmonary cryptosporidiosis in an infant with severe combined immune deficiency. *Journal of Pediatric Gastroenterology and Nutrition* 3: 149-157. doi:10.1097/00005176-198401000-00028

[19] Ma P., Villanueva T.G., Kaufman D., Gillooley J.F. 1984. Respiratory cryptosporidiosis in the acquired immune deficiency syndrome. Use of modified cold Kinyoun and hemacolor stains for rapid diagnoses. *JAMA* 252: 1298-1301. doi:10.1001/jama.1984.03350100028024

[20] Mor S.M., Tumwine J.K., Ndeezi G., Srinivasan M.G., Kaddu-Mulindwa D.H., Tzipori S., Griffiths, J.K. 2010. Respiratory cryptosporidiosis in HIV-seronegative children in Uganda: potential for respiratory transmission. *Clinical Infectious Diseases* 50: 1366-1372. doi:10.1086/652140

[21] Corti M., Villafañe M.F., Muzzio E., Bava J., Abuín J.C., Palmieri O.J. 2008. Criptosporidiosis broncopulmonar en pacientes infectados por el virus de la inmunodeficiencia humana [Pulmonary cryptosporidiosis in AIDS patients]. *Revista Argentina de Microbiologia* 40: 106-108 (in Spanish with summary in English).

[22] Vohra P., Sharma M., Chaudhary U.A. 2012. A comprehensive review of diagnostic techniques for detection of *Cryptosporidium parvum* in stool samples. *IOSR Journal of Pharmacy* 2: 15-26. doi:10.9790/3013-25701526

[23] Gentile G., Baldassarri L., Caprioli A., Donelli G., Venditti M., Avvisati G., Martino P. 1987. Colonic vascular invasion as a possible route of extraintestinal cryptosporidiosis. *The American Journal of Medicine* 82: 574-575. doi:10.1016/0002-9343(87)90474-8

[24] Dupont C., Bougnoux M.E., Turner L., Rouveix E., Dorra M. 1996. Microbiological findings about pulmonary cryptosporidiosis in two AIDS patients. *Journal of Clinical Microbiology* 34: 227-229. https://jcm.asm.org/content/ jcm/34/1/227.full.pdf

[25] Albuquerque Y.M. Silva M.C., Lima A.L., Magalhães V., Lima A., Magalhães V. 2012. Pulmonary cryptosporidiosis in AIDS patients, an underdiagnosed disease. *Jornal Brasileiro de Pneumologia* 38: 530-532. doi:10.1590/s1806-37132012000400017

[26] Glisson J.R., Brown T.P., Brugh M., Page R.K., Kleven S.H., Davis R.B. 1984. Sinusitis in turkeys associated with respiratory cryptosporidiosis. *Avian Diseases* 28: 783-790. doi:10.2307/1590250

[27] Mason R.W, Hartley W.J. 1980. Respiratory cryptosporidiosis in a peacock chick. *Avian Diseases* 24: 771-776. doi:10.2307/1589814

[28] Molina-López R.A., Ramis A., Martín-Vázquez S., Gómez-Couso H., Ares-Mazás E., Cacciò S.M., Leiva M., Darwich L. 2010. *Cryptosporidium baileyi* infection associated with an outbreak of ocular and respiratory disease in otus owls (*Otus scops*) in a rehabilitation centre. *Avian Pathology* 39: 171-176. doi:10.1080/03079451003717589

[29] Hannahs G. 1997. *Cryptosporidium parvum*: an emerging pathogen. Kenyon College, Gamgier, Ohio. USA. http://biology.kenyon.edu/slonc/bio38/hannahs/crypto.htm

[30] Certad G., Ngouanesavanh T., Guyot K., Gantois N., Chassat T., Mouray A., Fleurisse L., Pinon A., Cailliez J.C., Dei-Cas E., Creusy C. 2007. *Cryptosporidium parvum,* a potential cause of colic adenocarcinoma. *Infectious Agents and Cancer* 2: 22. doi:10.1186/1750-9378-2-22

[31] Okhuysen P.C., Rich S.M., Chappell C.L., Grimes K.A., Widmer G., Feng X., Tzipori S. 2002. Infectivity of a *Cryptosporidium parvum* Isolate of cervine origin for healthy adults and interferon γ knockout mice. *Journal of Infectious Diseases* 185: 1320-1325. doi:10.1086/340132

[32] Okhuysen P.C., Chappell C.L. 2002. *Cryptosporidium* virulence determinants – are we there yet? *International Journal for Parasitology* 32: 517-525. doi:10.1016/S0020-7519(01)00356-3

[33] Xiao L., Fayer R., Ryan U., Upton S.J. 2004. *Cryptosporidium* taxonomy: recent advances and implications for public health. *Clinical Microbiology* Review 17: 72-97. doi:10.1128/cmr.17.1.72-97.2004

[34] Bouzid M., Hunter P.R., Chalmers R.M., Tyler K.M. 2013. *Cryptosporidium* pathogenicity and virulence.

Clinical Microbiology Review 26: 115-134. doi:10.1128/CMR.00076-12

[35] Lacroix S., Mancassola R., Naciri M., Laurent F. 2001. *Cryptosporidium parvum* – specific mucosal immune response in C57BL/6 neonatal and gamma interferon-deficient mice: role of tumor necrosis factor alpha in protection. *Infection and Immunity* 69: 1635-1642. doi:10.1128/IAI.69.3.1635-1642.2001

[36] Steiner T.S., Nataro J.P., Poteet-smith C.E., Smith J.A., Guerrant R.L. 2000. Enteroaggregative *Escherichia coli* expresses a novel flagellin that causes IL-8 release from intestinal epithelial cells. *Journal of Clinical Investigation* 105: 1769-1777. doi:10.1172/JCI8892

[37] Ogra P.L., Mestecky J., Lamm M.E., Strober W., McGhee J.R., Bienenstock J. 1994. Handbook of mucosal immunology. 1st ed., Academic Press Inc., San Diego, California, USA.

[38] Thomson S., Hamilton C.A., Hope J.C., Katzer F., Mabbott N.A., Morrison L.J., Innes E.A. 2017. Bovine cryptosporidiosis: impact, host–parasite interaction and control strategies. *Veterinary Research* 48: 42. doi.org/10.1186/s13567-017-0447-0

[39] Stratakos A.C., Sima F., Ward P., Linton M., Kelly C., Pinkerton L., Stef L., Pet I., Iancu T., Pircalabioru G., Corcionivoschi N. 2017. The *in vitro* and *ex vivo* effect of Auranta 3001 in preventing *Cryptosporidium hominis* and *Cryptosporidium parvum* infection. *Gut Pathogen* 9: 49. doi:10.1186/s13099-017-0192-y

[40] Olsen L., Ĺkesson C.P., Storset A.K., Lacroix-Lamandé S., Boysen P., Metton C., Connelley T., Espenes A., Laurent F., Drouet F. 2015. The early intestinal immune response in experimental neonatal ovine cryptosporidiosis is characterized by an increased frequency of perforin expressing $NCR1^{+}$ NK cells and by $NCR1^{-}$ $CD8^{+}$ cell recruitment. *Veterinary Research* 46: 28. doi:10.1186/s13567-014-0136-1

[41] White J. 2010. Cryptosporidiosis (*Cryptosporidium hominis*, *Cryptosporidium parvum*, and other species). In: *Mandell, Douglas and Bennett's infectious disease essentials (principles and practice of infectious diseases).* (Eds. J.E. Bennett, R. Dolin, M.J. Blaser). Elsevier. Churchill Livingstone, Philadelphia, Pennsylvania: 3547-3560.

Received 14 March 2020
Accepted 08 June 2020