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# The Mucosal Innate Immune Response to *Cryptosporidium parvum*, a Global One Health Issue

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*Cryptosporidium parvum* is an apicomplexan parasite that infects the intestinal epithelium of humans and livestock animals worldwide. Cryptosporidiosis is a leading cause of diarrheal-related deaths in young children and a major cause of economic loss in cattle operations. The disease is especially dangerous to infants and immunocompromised individuals, for which there is no effective treatment or vaccination. As human-to-human, animal-to-animal and animal-to-human transmission play a role in cryptosporidiosis disease ecology, a holistic 'One Health' approach is required for disease control. Upon infection, the host's innate immune response restricts parasite growth and initiates the adaptive immune response, which is necessary for parasite clearance and recovery. The innate immune response involves a complex communicative interplay between epithelial and specialized innate immune cells. Traditional models have been used to study innate immune responses to *C. parvum* but cannot fully recapitulate natural host-pathogen interactions. Recent shifts to human and bovine organoid cultures are enabling deeper understanding of host-specific innate immunity response to infection. This review examines recent advances and highlights research gaps in our understanding of the host-specific innate immune response to *C. parvum*. Furthermore, we discuss evolving research models used in the field and potential developments on the horizon.

**Keywords:** *Cryptosporidium parvum*, innate immune response, One Health, intestinal parasite, intestinal epithelium

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## INTRODUCTION

*Cryptosporidium parvum* is an apicomplexan parasite that causes potentially life-threatening infectious diarrhea in infants and neonate calves with no available FDA-approved vaccine (Abrahamsen et al., 2004; Abubakar et al., 2007). Nitazoxanide is approved for use in adult, immunocompetent patients, but is not effective or approved for use in the most vulnerable populations: infants and immunocompromised patients (Abubakar et al., 2007). Infected hosts (humans and cows) shed billions of highly infectious and environmentally stable parasites (Nydam et al., 2001; Zambriski et al., 2013). The parasite is transmitted zoonotically and between humans worldwide (Bouzid et al., 2013; Ryan et al., 2016; Hatam-Nahavandi et al., 2019), and it contaminates drinking water sources (Chique et al., 2020), recreational swimming sites (Li et al., 2019), soils (Nag et al., 2020), and aquaculture environments (Marquis et al., 2015). Wildlife is further impacted by cryptosporidiosis (Ziegler et al., 2007). As such, a comprehensive and

interdisciplinary research approach is required to eliminate this significant source of global disease burden. Cryptosporidiosis is one of three etiologies responsible for the most global diarrheal deaths in children younger than five years of age (Checkley et al., 1997; Ong et al., 2005; Gormley et al., 2011; Kotloff et al., 2013; Khalil et al., 2018). In the U.S. *C. parvum* caused the largest waterborne pathogen outbreak in American history (Corso et al., 2003), and 444 outbreaks of cryptosporidiosis were reported from 2009-2017, leading to an estimated 750,000 individual cases per year (Scallan et al., 2011; Gharpure et al., 2019). *Cryptosporidium* has even been included as a relevant biological threat agent by the CDC (Rotz et al., 2002).

While the role of CD4<sup>+</sup> T cells in clearing infection is well studied, the role of the innate immune response within the parasite's natural hosts (i.e. human and cattle) is not fully understood. We will succinctly review current advances in our understanding of the mucosal innate immune response to *C. parvum*, and innovative models that have the potential to elucidate such responses within clinically relevant hosts. We will highlight key knowledge gaps and future research opportunities.

## INNATE IMMUNE RESPONSE

The intestinal innate immune system, comprised of the gut epithelium and specialized innate immune cells, is the first line of defense against *C. parvum* infection. Innate immunity restricts the expansion and growth of the parasite and initiates the adaptive response. Understanding the innate immune response to *C. parvum* infection in its native hosts is critical in building a 'One Health' strategy to limit *Cryptosporidium's* devastating impact on global health, agriculture, and the environment (Ziegler et al., 2007; Laurent and Lacroix-Lamande, 2017; Ivanova et al., 2019).

### Intestinal Epithelial Cells

Intestinal epithelial cells that line the gut epithelium create a physical barrier between luminal content and internal tissues.

Because *C. parvum* infects intestinal epithelial cells and does not invade deeper tissues, the epithelium is particularly important regarding the immune response to *C. parvum*.

Primary bovine intestinal epithelial cell infection by *C. parvum* leads to activation of the inflammatory transcription factor NF- $\kappa$ B; this increases expression of the long noncoding RNA NR\_045064 (Li et al., 2018) and induces the transcription of numerous inflammatory mediators, primarily CXCL8 (aka IL-8) and TNF $\alpha$ , a response primarily mediated by Toll-like receptor-2 (TLR2) and TLR4 (Yang et al., 2015). *C. parvum* infection induces an increase in TLR4 expression, regulated by suppression of the noncoding miRNA, *let-7i* (Chen et al., 2007). TLR2 and TLR4 activation by *C. parvum* and subsequent NF- $\kappa$ B nuclear translocation induces the release of antimicrobial peptides LL-37 and  $\beta$ -defensin-2 (Chen et al., 2005). Despite this, TLR2 and TLR4 deficiency did not increase parasite load in neonatal mice; however, direct comparisons are difficult given the different models and experimental designs (Lantier et al., 2014).

Evidence suggests that intracellular recognition of *C. parvum* via NOD-like receptors (NLR) and subsequent activation of the inflammasome complex is an important innate response to infection. IL-18, a product of the inflammasome complex, is elevated in human epithelial cell lines following *C. parvum* infection (McDonald et al., 2006); moreover, IL-18 knockout and inflammasome components caspase-1 or ASC knockout mice are more susceptible to *Cryptosporidium* infection than control mice (Ehigiator et al., 2005; McNair et al., 2018; Sateriale et al., 2021). IL-1 $\beta$ , the second key product of inflammasome activation, was not increased post-infection, nor was there an effect on infection susceptibility in IL-1 $\beta$  knockout mice (McNair et al., 2018). The latter findings are corroborated by the fact that parasite shedding was strongly increased in mice lacking NLRP6, which induces IL-18 secretion, but not in mice lacking other inflammasome-forming NLRs including NLRP3, NLRP1b, Aim2, and NLRc4 that primarily induce IL-1 $\beta$  secretion (Sateriale et al., 2021).

Antimicrobial peptides include small positively-charged polypeptides that elicit antimicrobial effects against a variety of pathogens including bacteria, fungi, viruses, and protozoan parasites (Mahlapuu et al., 2016). Phospholipases (Carrin et al., 2012) and the antimicrobial peptides  $\beta$ -defensin-1,  $\beta$ -defensin-2, and LL-37 can kill *C. parvum* (Giacometti et al., 1999). Part of the TLR signal response by epithelial cells includes the release of LL-37 and  $\beta$ -defensin-2, and these antimicrobial peptides bind to free *C. parvum* to directly enact their effects (Chen et al., 2005). LL-37 and  $\alpha$ -defensin-2 are increased in response to the rise in the inflammasome product, IL-18, in human cell lines (McDonald et al., 2006). However, *C. parvum* influences epithelial cells by inhibiting the production of other antimicrobial peptides including  $\beta$ -defensin-1 by an undiscovered mechanism (Zaalouk et al., 2004), and CCL20 by a *C. parvum*-induced rise in miR21 (Guesdon et al., 2015).

*C. parvum* infection is restricted to a parasitophorous vacuole on the apical side of the intestinal epithelium, therefore chemokine and cytokine release from infected epithelial cells is critical in the recruitment of specialized immune cells that facilitate parasite clearance (Laurent et al., 1999). Activation of TLRs by *C. parvum* induces the NF- $\kappa$ B signaling pathway causing the basolateral release of Growth Regulated Oncogene- $\alpha$  (GRO- $\alpha$ ) (Yang et al., 2015) and CXCL8, which are key neutrophil chemoattractant molecules (Laurent et al., 1997). Additionally, in the neonatal mouse model, several chemokines including CCL2, CCL5, CXCL10, and CXCL9 are released, which recruit various immune cells to the infection site (Lacroix-Lamande et al., 2002; Auray et al., 2007; Lantier et al., 2013). Chemokine-induced immune cell recruitment is critical in the response to *C. parvum*, as evidenced by the increased susceptibility to infection of mice deficient in chemokine receptors, even in spite of redundancy in immune cell recruitment processes (Lacroix-Lamande et al., 2008; Lantier et al., 2013).

Another defense against intracellular pathogens is apoptosis of the host cell, and infection by *C. parvum* initiates apoptosis of infected and surrounding epithelial cells through Fas and Fas-L

interactions (Chen et al., 1999). However, within hours post-infection, *C. parvum* in one life stage, the trophozoite, inhibits apoptosis, likely to facilitate growth within the host cell, by inducing the production of anti-apoptotic factors BCL-2 (Mele et al., 2004), survivin (Liu et al., 2009), and osteoprotegerin (McCole et al., 2000; Castellanos-Gonzalez et al., 2008). Later in infection, in a different part of the *C. parvum* life cycle known as the sporozoite and merozoite life stages, inhibition is removed and apoptosis of the host cell is promoted (Mele et al., 2004; Liu et al., 2009).

## Interferons (IFNs)

IFNs are an essential component to the host response to *C. parvum*. The importance of IFN- $\gamma$  is shown by an increased susceptibility to *C. parvum* infection in IFN- $\gamma$ <sup>-/-</sup> mice (Mead and You, 1998; Lacroix-Lamande et al., 2002) and wild-type neonate mice treated with anti-IFN- $\gamma$ -antibodies (McDonald et al., 2013). Adult mice with a disrupted IFN- $\gamma$  gene shed more parasites, experience extensive damage to the intestinal mucosa, and die within weeks of infection (Theodos et al., 1997). Severe combined immunodeficiency (SCID) mice, which are deficient in T and B cells, experience reduced *C. parvum* infection compared to SCID IFN- $\gamma$ <sup>-/-</sup> mice, showing that protective IFN- $\gamma$  during *C. parvum* infection is derived, at least in part, from non-T or B cells (Hayward et al., 2000). In addition to increased IFN- $\gamma$ , *in vivo* piglet infection and *in vitro* experiments show that intestinal epithelial cells secrete abundant IFN- $\lambda$ 3 (a type-III IFN) independently of specialized immune cells (Ferguson et al., 2019). Historically, type III IFNs have been associated with local epithelial defense from viruses (Zhou et al., 2018). More recently, IFN- $\lambda$  was shown to mediate the gut epithelium defense against non-viral pathogens via TLRs (Odendall et al., 2017). Neutralization of IFN- $\lambda$ 3 leads to increased villus blunting and fecal shedding of infective *C. parvum* in neonate mice, and when intestinal epithelial cells are primed with recombinant IFN- $\lambda$ 3 they show reduced barrier disruption and increased cellular defense against *C. parvum* (Ferguson et al., 2019).

## Specialized Immune Cells

### Natural Killer (NK) Cells

NK cells contribute to the innate immune response to *C. parvum* through IFN- $\gamma$  production and cytolysis of infected epithelial cells. *In vivo*, treating immunocompetent or immunodeficient mice with the NK cell activator, IL-12, leads to a protective effect against *C. parvum* associated with a concomitant rise in intestinal IFN- $\gamma$  (Urban et al., 1996); *in vitro*, human NK cells lyse infected intestinal epithelial cells in response to IL-15 and presentation of MHC class I-related protein A and B (Dann et al., 2005). Mice lacking NK cells experience increased severity of infection and excrete more oocysts compared to mice with NK cells, but when treated with anti-IFN- $\gamma$  antibodies the infection of NK positive mice was heavily exacerbated, thus implying a protective role of NK cells that is connected to IFN- $\gamma$  (Barakat et al., 2009). Despite the increased morbidity in mice without NK cells, they produced IFN- $\gamma$  after infection, meaning that NK cells are one, but not the only source of IFN- $\gamma$  in response to *C.*

*parvum*. The number of NK cells localized in the gut is increased within days following *C. parvum* exposure in lambs (Olsen et al., 2015). Activation of the NK cell receptor, NKG2D, is involved in NK cell-mediated protection, via its ligand, MICA, which is upregulated in the intestinal epithelium of infected humans (Dann et al., 2005). The role that other innate-like lymphocytes play during *C. parvum* infection is poorly understood and future investigations are warranted.

### Dendritic Cells (DCs)

DCs exposed to *C. parvum* secrete numerous cytokines including IL-6, IL-1 $\beta$ , IL-12, IL-18, TNF $\alpha$ , and type I interferons via TLR4 receptor activation (Barakat et al., 2009; Bedi and Mead, 2012; Perez-Cordon et al., 2014). DCs also capture *C. parvum* antigens in the gut mucosa and migrate to draining lymph nodes where they present these antigens and facilitate the adaptive immune response (Auray et al., 2007; Perez-Cordon et al., 2014). DCs may acquire such antigens by directly capturing luminal organisms or phagocytizing apoptotic infected epithelial cells (Farache et al., 2013). Macrophages may further engulf free *C. parvum* and transfer the parasite to DCs for migration (Marcial and Madara, 1986). One hypothesis for the increased infection susceptibility of neonatal mice compared to adults is that neonates have fewer intestinal DCs, and injecting neonates with Flt3L – which induces DC differentiation from progenitor cells – increases the number of DCs as well as resistance to infection (Lantier et al., 2013). Furthermore, adult mice devoid of DCs are more susceptible to infection and excrete more parasites, and adoptive transfer of DCs pre-exposed to *C. parvum* reduces the parasite load (Bedi et al., 2014).

### Macrophages

Macrophages develop from the same bone marrow precursor cells as DCs and are found in most organ systems and epithelial barriers, including the gut (Verschoor et al., 2012; Kumar, 2019). Following *C. parvum* infection in neonatal mice, macrophages accumulate in the lamina propria (de Sablet et al., 2016) and are associated with intact and digested parasites in Payer's patches in guinea pigs (Marcial and Madara, 1986). Macrophages' contribution to *C. parvum* clearance appears to be primarily as a secondary source of IFN- $\gamma$ . Infected Rag2<sup>-/-</sup> $\gamma$ c<sup>-/-</sup> mice, which lack T and B lymphocytes and NK cells, still produce IFN- $\gamma$ , suggesting an IFN- $\gamma$  source alternative to T cells and NK cells (Barakat et al., 2009). When treated with clodronate-liposomes to deplete macrophages, the mice were less resistant to *C. parvum* and could not produce IFN- $\gamma$  (Barakat et al., 2009). IFN- $\gamma$  production by macrophages is promoted by IL-18 when Rag2<sup>-/-</sup> $\gamma$ c<sup>-/-</sup> mice are infected by *C. parvum* (Choudhry et al., 2012), and IFN- $\gamma$ <sup>-/-</sup> mice have fewer macrophages and T cells recruited to the gut accompanying an inability to recover from infection (Lacroix-Lamande et al., 2002).

### Neutrophils

Neutrophils infiltrate the intestinal mucosa during *C. parvum* infection (Goodgame et al., 1995), and preventing mucosal recruitment of neutrophils increases *C. parvum*-related barrier

dysfunction as measured by transepithelial electrical resistance (Zadrozny et al., 2006). Inhibiting neutrophil recruitment does not influence mortality or infection severity, nor does it affect *C. parvum*-mediated villous atrophy and diarrhea (Zadrozny et al., 2006). With no influence on mortality or infection severity, it does not appear that neutrophils are directly protective in the context of *C. parvum*.

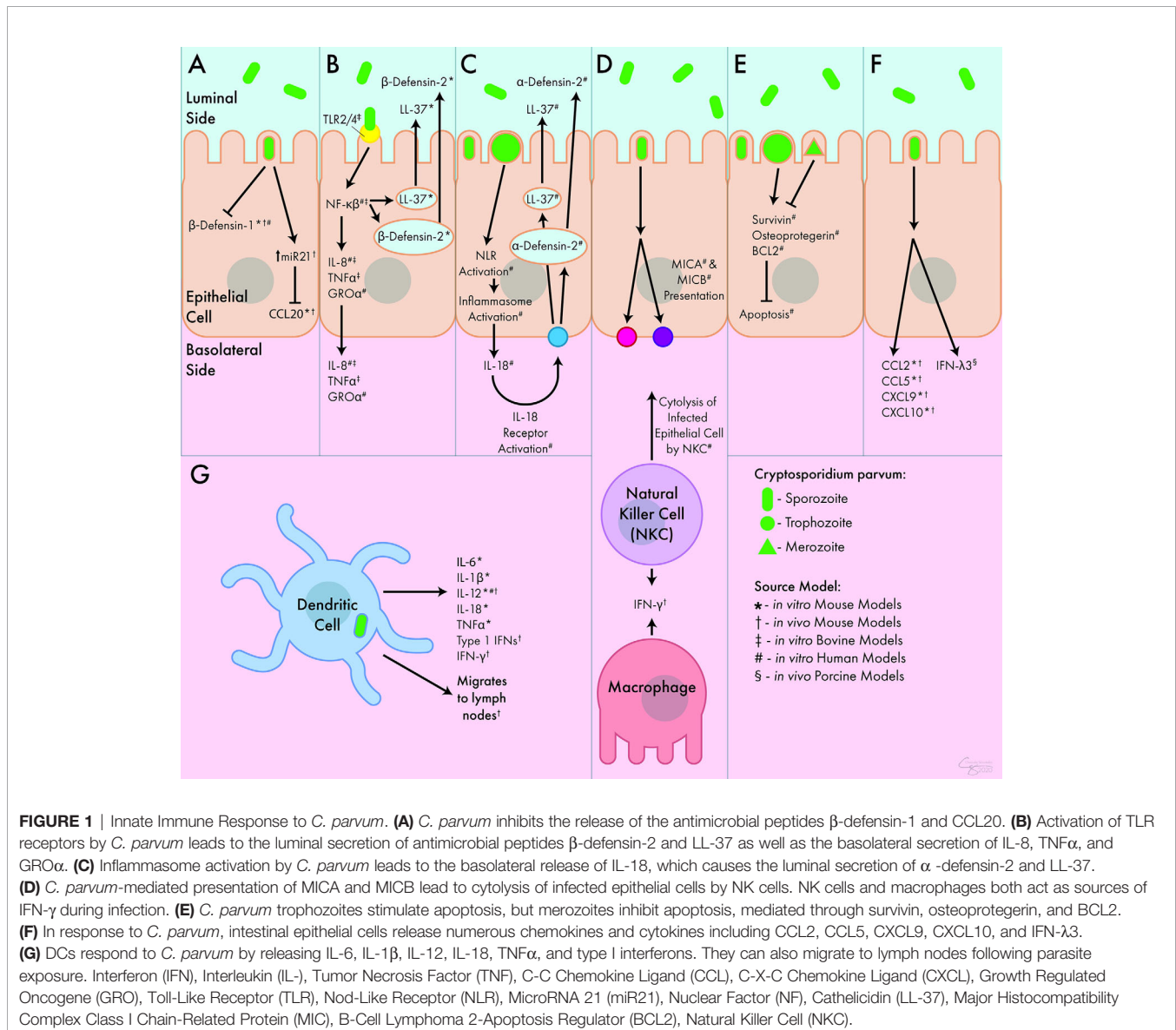
As research models advance, the multi-dimensional innate immune response grows more complex but better understood (Figure 1). However, questions regarding the relevancy of these data to the natural hosts of *C. parvum* remain, provided the use of models that do not fully recapitulate the environment of human or ruminant intestines. New biotechnological advances, such as the development of bovine and human organoids, may provide the models necessary to confirm what is currently inferred about the innate immune response to *C. parvum* in these hosts.

## CRYPTOSPORIDIUM RESEARCH MODELS

The potential to fully understand *C. parvum*'s pathogenesis and develop therapeutics is dependent on the models used to research the host-pathogen interactions it induces within its natural and clinically relevant hosts (i.e. human and cattle). Traditional *in vitro* *C. parvum* infection models can only be maintained for several days at a time and do not fully recapitulate native intestinal tissue, and *In vivo* mouse models are sub-optimal, as mice are not a natural host of *C. parvum*. Innovative models and advancing technologies are necessary to advance this field.

### In Vivo Models

*In vivo* animal models are foundational to host-pathogen interaction research, but the nature of *C. parvum* complicates the application of traditional animal models. The natural and clinically





relevant hosts for *C. parvum* are humans and ruminants; mice can sustain *C. parvum* infection but only when severely immunocompromised (Mead et al., 1991; Petry et al., 1995; Griffiths et al., 1998). Given that humans and ruminants are the primary natural hosts, calves, lambs, and non-human primates have been used to investigate cryptosporidiosis in naturally infected species (Tzipori, 1998). However, housing and maintaining large animal species requires significant funds and specialized facilities, equipment, and training. Moreover, many of the genetic and molecular research tools that are available for mice models are not available for large animal models such as cows or sheep.

Adult mice, a preferred animal model in terms of costs and availability of reagents, are resistant to *C. parvum* but are susceptible to infection by the related species, *C. muris*; however, *C. muris* differs from *C. parvum* in phylogeny, biochemical nature of infection, and infection site (*C. muris* infects the stomach mucosa) (Sateriale et al., 2019). Mice can become susceptible to *C. parvum* through chemical or genetic immunosuppression, such as the previously discussed SCID (Mead et al., 1991), IFN- $\gamma$ <sup>-/-</sup> (Griffiths et al., 1998), and Rag2<sup>-/-</sup> mice, which, in addition to neonate mice, have provided established murine platforms for *C. parvum* research (Petry et al., 1995). Unfortunately, mouse models have limited translatability for natural hosts such as humans and cattle. This has been elucidated through bovine-specific responses to *C. parvum* that are absent in mice, such as differences in NK cell receptor activation (Allan et al., 2015), recruitment of  $\gamma\delta$  T cells (Guzman et al., 2012), and developed resistance in adulthood (Sateriale et al., 2019). More recently, *C. tyzzeri* was identified as a natural mouse pathogen that mirrors aspects of *C. parvum*'s pathogenesis and host response in mice (Sateriale et al., 2019).

## In Vitro Models

The allure of primary intestinal epithelium cells lies in the morphological and species-specific accuracy compared to immortalized cell lines. Primary human (Castellanos-Gonzalez et al., 2013) and bovine intestinal epithelial cells have been successfully infected with *C. parvum* (Hashim et al., 2006). Unfortunately, primary intestinal cells have limitations involving their availability, obsolescence, and difficulty in long-term propagation (Varughese et al., 2014).

Most *in vitro* models for *C. parvum* host-pathogen interaction research include cancer-derived transformed or immortalized human cell lines including HCT-8, Caco-2, and HT29 cells, which are all derived from colorectal adenocarcinomas (Karanis and Aldeyarbi, 2011). Other non-colorectal cancer cell lines have also been used: RL95-2 (human endometrial carcinoma) (Rasmussen et al., 1993), Madin-Darby bovine kidney cells (Upton et al., 1994), MRC-5 (lung fibroblast) (Dawson et al., 2004), FHs 74 Int cells (non-cancer, immortalized human small intestinal epithelium) (Varughese et al., 2014), and BS-C-1 (African green monkey kidney) cells (Deng and Cliver, 1998). None of these lines maintained infection longer than six days except for HT29 cells, which could maintain infection for thirteen days but only for the asexual life stages of *C. parvum*. One non-intestinal cell line, COLO-680N, is human esophageal squamous carcinoma-derived and can propagate infective

parasites continually for eight weeks, but applications to host-pathogen interaction are questionable given that the esophagus is not the natural niche for *C. parvum* (Miller et al., 2018).

Early attempts to utilize three-dimensional structures for *C. parvum* research involved low-shear microgravity cultures where HCT-8 cells seeded onto submucosa grafts formed structures that maintained *C. parvum* infection; however, parasites decreased after 48 hours (Alcantara Warren et al., 2008). Later, a hollow fiber bioreactor system was used to infect three-dimensional HCT-8 cell structures for over six months, which is far longer than two-dimensional HCT-8 infection, while producing significantly more oocysts/day/mL (Morada et al., 2016). Silk fiber scaffolding has also been utilized to induce three-dimensional culture of Caco-2 and HT29 cells, maintaining infection for two weeks (DeCicco RePass et al., 2017).

While these cell lines are useful tools, they are susceptible to genetic variation, most cannot maintain all phases of the *C. parvum* life cycle, and most cannot maintain and propagate *C. parvum* infection for extended periods of time (Bhalchandra et al., 2018). This, in addition to the fact that these cell lines do not recapitulate the native intestinal epithelial tissue of *C. parvum* hosts, encourages the search for increasingly accurate models of study.

## Enteroids

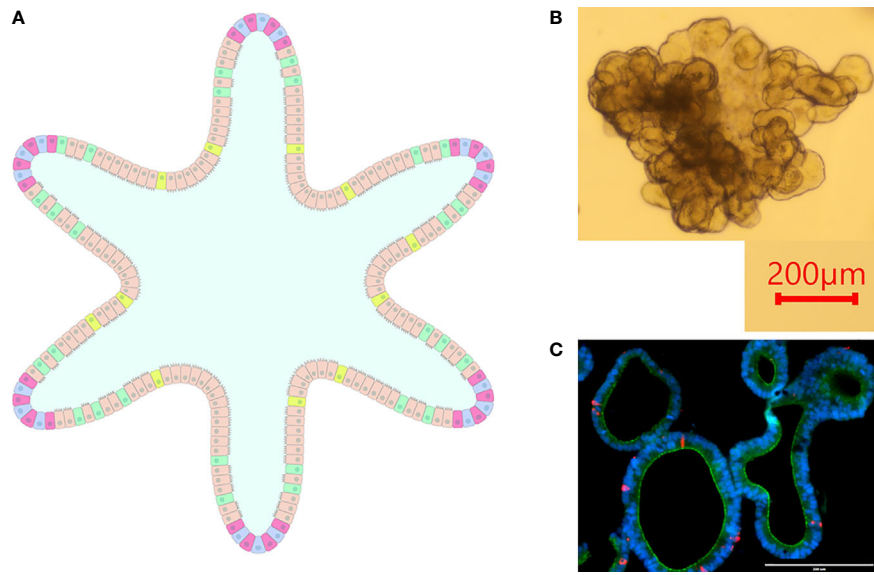
Intestinal organoids (aka enteroids) circumvent shortcomings exhibited by cell lines and primary epithelial cells while also introducing a three-dimensional culture model. Enteroids are composed of a polarized single layer of epithelium with crypt and villus domains containing the various intestinal epithelial cells such as stem cells, enterocytes, enteroendocrine cells, goblet cells, etc., thus recapitulating the microanatomy and functionality of native intestinal epithelial tissue (Figure 2) (Zachos et al., 2016).

Stem cell-derived organoids allow long-term three-dimensional culture while maintaining the morphological relevance of native tissue. Isolated crypts from neonatal and immunocompromised mice were exposed to *C. parvum* upon plating, resulting in inhibited organoid propagation and budding, decreased expression of intestinal stem cell markers, and increased cell senescence (Zhang et al., 2016). In another study, human enteroids were infected with *C. parvum* by microinjection and the parasite was able to complete its entire life cycle within these organoids (Heo et al., 2018). Though bovine enteroids have been described, they have not yet been used to study *C. parvum* infection (Powell and Behnke, 2017; Hamilton et al., 2018; Derricott et al., 2019).

Organoid technology for *C. parvum* research is in its relative infancy, but the benefits of the culture model are enticing and allow questions that were not possible to investigate with previous models.

## DISCUSSION

*C. parvum* is a parasite of international clinical importance across human and animal healthcare. Because of its high infectivity, resistance to water treatment, and the danger it poses to immunocompromised individuals, understanding the responses it induces in its host is a high priority endeavor to allow the creation of



**FIGURE 2** | Intestinal Organoids. **(A)** graphical representation of an intestinal organoid. The inside of the organoid corresponds to the luminal side, and the outside of the organoid corresponds to the basolateral side. Blue: intestinal stem cells, Red: Paneth cells, Tan: enterocytes, Green: goblet cells, Yellow: enteroendocrine cells. **(B)** Bovine intestinal organoid 6 days post-plating. Numerous folds and budding structures are noted, indicating crypt and villi-like domains. **(C)** Sectioned ovine intestinal organoid illustrating nuclei (DAPI), apical junctional protein ZO-1 (green), and chromogranin-A (red) indicating enteroendocrine cell differentiation.

effective preventative measures and therapies. The innate immune response is multifaceted and involves the intestinal epithelium, innate immune cells, and a complex interplay of cytokine signaling. To discover this, various models have been utilized. These include natural host species such as calves as well as more specialized models like immunocompromised mice, and *in vitro* models such as primary cell explants and immortalized cell lines. A relatively recent shift to three-dimensional cultures and the expanding use of organoids opens new avenues to study the parasite and its host-pathogen interaction.

As research in the field continues, attention must be brought to handling *C. parvum* from a ‘One Health’ perspective. New models must increase the relevant understanding of the parasite in bovine and human hosts and drive the discovery of innate mechanisms of resistance that can be utilized for management. Improved knowledge of the innate defenses against *C. parvum* in both ruminant and human hosts will hopefully lead to treatments to augment host natural innate defense and act as transient preventative measures to reduce environmental transmission of *C. parvum* between and within host species.

## REFERENCES

- Abrahamsen M. S., Templeton T. J., Enomoto S., Abrahante J. E., Zhu G., Lancto C. A., et al. (2004). Complete Genome Sequence of the Apicomplexan, *Cryptosporidium Parvum*. *Science* 304 (5669), 441–445. doi: 10.1126/science.1094786
- Abubakar I., Aliyu S. H., Arumugam C., Hunter P. R., and Usman N. K. (2007). Prevention and Treatment of Cryptosporidiosis in Immunocompromised Patients. *Cochrane Database Syst. Rev.* 1 (1), CD004932. doi: 10.1002/14651858.CD004932.pub2
- Alcantara Warren C., Destura R. V., Sevilleja J. E., Barroso L. F., Carvalho H., Barrett L. J., et al. (2008). Detection of Epithelial-Cell Injury, and Quantification of Infection,

## AUTHOR CONTRIBUTIONS

CC performed writing – original draft preparation. CC and AK performed writing – review and editing. AK performed project administration and supervision. All authors contributed to the article and approved the submitted version.

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in the HCT-8 Organoid Model of Cryptosporidiosis. *J. Infect. Dis.* 198 (1), 143–149. doi: 10.1086/588819

- Allan A. J., Sanderson N. D., Gubbins S., Ellis S. A., and Hammond J. A. (2015). Cattle NK Cell Heterogeneity and the Influence of MHC Class I. *J. Immunol.* 195 (5), 2199–2206. doi: 10.4049/jimmunol.1500227
- Auray G., Lacroix-Lamande S., Mancassola R., Dimier-Poisson I., and Laurent F. (2007). Involvement of Intestinal Epithelial Cells in Dendritic Cell Recruitment During *C. Parvum* Infection. *Microbes Infect.* 9 (5), 574–582. doi: 10.1016/j.micinf.2007.01.026
- Barakat F. M., McDonald V., Di Santo J. P., and Korbel D. S. (2009). Roles for NK Cells and an NK Cell-Independent Source of Intestinal Gamma Interferon for

- Innate Immunity to *Cryptosporidium Parvum* Infection. *Infect. Immun.* 77 (11), 5044–5049. doi: 10.1128/IAI.00377-09
- Barakat F. M., McDonald V., Foster G. R., Tovey M. G., and Korbel D. S. (2009). *Cryptosporidium Parvum* Infection Rapidly Induces a Protective Innate Immune Response Involving Type I Interferon. *J. Infect. Dis.* 200 (10), 1548–1555. doi: 10.1086/644601
- Bedi B., McNair N. N., and Mead J. R. (2014). Dendritic Cells Play a Role in Host Susceptibility to *Cryptosporidium Parvum* Infection. *Immunol. Lett.* 158 (1–2), 42–51. doi: 10.1016/j.imlet.2013.11.015
- Bedi B., and Mead J. R. (2012). *Cryptosporidium Parvum* Antigens Induce Mouse and Human Dendritic Cells to Generate Th1-enhancing Cytokines. *Parasite Immunol.* 34 (10), 473–485. doi: 10.1111/j.1365-3024.2012.01382.x
- Bhalchandra S., Cardenas D., and Ward H. D. (2018). Recent Breakthroughs and Ongoing Limitations in *Cryptosporidium* Research. *F1000Res* 7, 1–9. doi: 10.12688/f1000research.15333.1
- Bouziid M., Hunter P. R., Chalmers R. M., and Tyler K. M. (2013). *Cryptosporidium* Pathogenicity and Virulence. *Clin. Microbiol. Rev.* 26 (1), 115–134. doi: 10.1128/CMR.00076-12
- Carryn S., Schaefer D. A., Imboden M., Homan E. J., Bremel R. D., and Riggs M. W. (2012). Phospholipases and Cationic Peptides Inhibit *Cryptosporidium Parvum* Sporozoite Infectivity by Parasitocidal and non-Parasitocidal Mechanisms. *J. Parasitol.* 98 (1), 199–204. doi: 10.1645/GE-2822.1
- Castellanos-Gonzalez A., Cabada M. M., Nichols J., Gomez G., and White A. C. Jr. (2013). Human Primary Intestinal Epithelial Cells as an Improved In Vitro Model for *Cryptosporidium Parvum* Infection. *Infect. Immun.* 81 (6), 1996–2001. doi: 10.1128/IAI.01131-12
- Castellanos-Gonzalez A., Yancey L. S., Wang H. C., Pantenburg B., Liscum K. R., Lewis D. E., et al. (2008). *Cryptosporidium* Infection of Human Intestinal Epithelial Cells Increases Expression of Osteoprotegerin: A Novel Mechanism for Evasion of Host Defenses. *J. Infect. Dis.* 197 (6), 916–923. doi: 10.1086/528374
- Checkley W., Gilman R. H., Epstein L. D., Suarez M., Diaz J. F., Cabrera L., et al. (1997). Asymptomatic and Symptomatic *Cryptosporidiosis*: Their Acute Effect on Weight Gain in Peruvian Children. *Am. J. Epidemiol.* 145 (2), 156–163. doi: 10.1093/oxfordjournals.aje.a009086
- Chen X. M., Gores G. J., Paya C. V., and LaRusso N. F. (1999). *Cryptosporidium Parvum* Induces Apoptosis in Biliary Epithelia by a Fas/Fas Ligand-Dependent Mechanism. *Am. J. Physiol.* 277 (3), G599–G608. doi: 10.1152/ajpgi.1999.277.3.G599
- Chen X. M., O'Hara S. P., Nelson J. B., Splinter P. L., Small A. J., Tietz P. S., et al. (2005). Multiple TLRs are Expressed in Human Cholangiocytes and Mediate Host Epithelial Defense Responses to *Cryptosporidium Parvum* Via Activation of NF- $\kappa$ B. *J. Immunol.* 175 (11), 7447–7456. doi: 10.4049/jimmunol.175.11.7447
- Chen X. M., Splinter P. L., O'Hara S. P., and LaRusso N. F. (2007). A Cellular micro-RNA, let-7i, Regulates Toll-like Receptor 4 Expression and Contributes to Cholangiocyte Immune Responses Against *Cryptosporidium Parvum* Infection. *J. Biol. Chem.* 282 (39), 28929–28938. doi: 10.1074/jbc.M702633200
- Chique C., Hynds P. D., Andrade L., Burke L., Morris D., Ryan M. P., et al. (2020). *Cryptosporidium* Spp. in Groundwater Supplies Intended for Human Consumption - A Descriptive Review of Global Prevalence, Risk Factors and Knowledge Gaps. *Water Res.* 176:115726. doi: 10.1016/j.watres.2020.115726
- Choudhry N., Petry F., van Rooijen N., and McDonald V. (2012). A Protective Role for Interleukin 18 in Interferon Gamma-Mediated Innate Immunity to *Cryptosporidium Parvum* That is Independent of Natural Killer Cells. *J. Infect. Dis.* 206 (1), 117–124. doi: 10.1093/infdis/jis300
- Corso P. S., Kramer M. H., Blair K. A., Addiss D. G., Davis J. P., and Haddix A. C. (2003). Cost of Illness in the 1993 Waterborne *Cryptosporidium* Outbreak, Milwaukee, Wisconsin. *Emerg. Infect. Dis.* 9 (4), 426–431. doi: 10.3201/eid0904.020417
- Dann S. M., Wang H. C., Gambarin K. J., Actor J. K., Robinson P., Lewis D. E., et al. (2005). Interleukin-15 Activates Human Natural Killer Cells to Clear the Intestinal Protozoan *Cryptosporidium*. *J. Infect. Dis.* 192 (7), 1294–1302. doi: 10.1086/444393
- Dawson D. J., Samuel C. M., Scranage V., and Atherton C. J. (2004). Survival of *Cryptosporidium* Species in Environments Relevant to Foods and Beverages. *J. Appl. Microbiol.* 96 (6), 1222–1229. doi: 10.1111/j.1365-2672.2004.02281.x
- DeCicco RePass M. A., Chen Y., Lin Y., Zhou W., Kaplan D. L., and Ward H. D. (2017). Novel Bioengineered Three-Dimensional Human Intestinal Model for Long-Term Infection of *Cryptosporidium Parvum*. *Infect. Immun.* 85 (3), 1–11. doi: 10.1128/IAI.00731-16
- Deng M. Q., and Cliver D. O. (1998). *Cryptosporidium Parvum* Development in the BS-C-1 Cell Line. *J. Parasitol.* 84 (1), 8–15. doi: 10.2307/3284519
- Derricott H., Luu L., Fong W. Y., Hartley C. S., Johnston L. J., Armstrong S. D., et al. (2019). Developing a 3D Intestinal Epithelium Model for Livestock Species. *Cell Tissue Res.* 375 (2), 409–424. doi: 10.1007/s00441-018-2924-9
- de Sablet T., Potiron L., Marquis M., Bussiere F. I., Lacroix-Lamande S., and Laurent F. (2016). *Cryptosporidium Parvum* Increases Intestinal Permeability Through Interaction With Epithelial Cells and IL-1 $\beta$  and TNF $\alpha$  Released by Inflammatory Monocytes. *Cell Microbiol.* 18 (12), 1871–1880. doi: 10.1111/cmi.12632
- Ehigiator H. N., Romagnoli P., Borgelt K., Fernandez M., McNair N., Secor W. E., et al. (2005). Mucosal Cytokine and Antigen-Specific Responses to *Cryptosporidium Parvum* in IL-12p40 KO Mice. *Parasite Immunol.* 27 (1–2), 17–28. doi: 10.1111/j.1365-3024.2005.00736.x
- Farache J., Koren I., Milo I., Gurevich I., Kim K. W., Zigmund E., et al. (2013). Luminal Bacteria Recruit CD103+ Dendritic Cells Into the Intestinal Epithelium to Sample Bacterial Antigens for Presentation. *Immunity* 38 (3), 581–595. doi: 10.1016/j.immuni.2013.01.009
- Ferguson S. H., Foster D. M., Sherry B., Magness S. T., Nielsen D. M., and Gookin J. L. (2019). Interferon-Lambda3 Promotes Epithelial Defense and Barrier Function Against *Cryptosporidium Parvum* Infection. *Cell Mol. Gastroenterol. Hepatol.* 8 (1), 1–20. doi: 10.1016/j.jcmgh.2019.02.007
- Gharpure R., Perez A., Miller A. D., Wikswo M. E., Silver R., and Hlavsa M. C. (2019). *Cryptosporidiosis* Outbreaks - United States, 2009–2017. *MMWR Morb Mortal Wkly Rep.* 68 (25), 568–572. doi: 10.15585/mmwr.mm6825a3
- Giacometti A., Cirioni O., Barchiesi F., Caselli F., and Scalise G. (1999). In-Vitro Activity of Polycationic Peptides Against *Cryptosporidium Parvum*, *Pneumocystis Carinii* and Yeast Clinical Isolates. *J. Antimicrob. Chemother.* 44 (3), 403–406. doi: 10.1093/jac/44.3.403
- Goodgame R. W., Kimball K., Ou C. N., White A. C. Jr., Genta R. M., Lifschitz C. H., et al. (1995). Intestinal Function and Injury in Acquired Immunodeficiency Syndrome-Related *Cryptosporidiosis*. *Gastroenterology* 108 (4), 1075–1082. doi: 10.1016/0016-5085(95)90205-8
- Gormley F. J., Little C. L., Chalmers R. M., Rawal N., and Adak G. K. (2011). Zoonotic *Cryptosporidiosis* From Petting Farms, England and Wales, 1992–2009. *Emerg. Infect. Dis.* 17 (1), 151–152. doi: 10.3201/eid1701.100902
- Griffiths J. K., Theodos C., Paris M., and Tzipori S. (1998). The Gamma Interferon Gene Knockout Mouse: A Highly Sensitive Model for Evaluation of Therapeutic Agents Against *Cryptosporidium Parvum*. *J. Clin. Microbiol.* 36 (9), 2503–2508. doi: 10.1128/JCM.36.9.2503-2508.1998
- Guesdon W., Auray G., Pezier T., Bussiere F. I., Drouet F., Le Vern Y., et al. (2015). Ccl20 Displays Antimicrobial Activity Against *Cryptosporidium Parvum*, But Its Expression Is Reduced During Infection in the Intestine of Neonatal Mice. *J. Infect. Dis.* 212 (8), 1332–1340. doi: 10.1093/infdis/jiv206
- Guzman E., Price S., Poulosom H., and Hope J. (2012). Bovine Gammadelta T Cells: Cells With Multiple Functions and Important Roles in Immunity. *Vet. Immunol. Immunopathol.* 148 (1–2), 161–167. doi: 10.1016/j.vetimm.2011.03.013
- Hamilton C. A., Young R., Jayaraman S., Sehgal A., Paxton E., Thomson S., et al. (2018). Development of In Vitro Enteroids Derived From Bovine Small Intestinal Crypts. *Vet. Res.* 49 (1):54. doi: 10.1186/s13567-018-0547-5
- Hashim A., Mulcahy G., Bourke B., and Clyne M. (2006). Interaction of *Cryptosporidium Hominis* and *Cryptosporidium Parvum* With Primary Human and Bovine Intestinal Cells. *Infect. Immun.* 74 (1), 99–107. doi: 10.1128/IAI.74.1.99-107.2006
- Hatam-Nahavandi K., Ahmadpour E., Carmena D., Spotin A., Bangoura B., and Xiao L. (2019). *Cryptosporidium* Infections in Terrestrial Ungulates With Focus on Livestock: A Systematic Review and Meta-Analysis. *Parasit Vectors* 12 (1), 453. doi: 10.1186/s13071-019-3704-4
- Hayward A. R., Chmura K., and Cosyns M. (2000). Interferon-Gamma is Required for Innate Immunity to *Cryptosporidium Parvum* in Mice. *J. Infect. Dis.* 182 (3), 1001–1004. doi: 10.1086/315802
- Heo I., Dutta D., Schaefer D. A., Iakobachvili N., Artegiani B., Sachs N., et al. (2018). Modelling *Cryptosporidium* Infection in Human Small Intestinal and Lung Organoids. *Nat. Microbiol.* 3 (7), 814–823. doi: 10.1038/s41564-018-0177-8



- Ivanova D. L., Denton S. L., Fettel K. D., Sondgeroth K. S., Munoz Gutierrez J., Bangoura B., et al. (2019). Innate Lymphoid Cells in Protection, Pathology, and Adaptive Immunity During Apicomplexan Infection. *Front. Immunol.* 10, 196. doi: 10.3389/fimmu.2019.00196
- Karanis P., and Aldeyarbi H. M. (2011). Evolution of *Cryptosporidium* In Vitro Culture. *Int. J. Parasitol.* 41 (12), 1231–1242. doi: 10.1016/j.ijpara.2011.08.001
- Khalil I. A., Troeger C., Rao P. C., Blacker B. F., Brown A., Brewer T. G., et al. (2018). Morbidity, Mortality, and Long-Term Consequences Associated With Diarrhoea From Cryptosporidium Infection in Children Younger Than 5 Years: A Meta-Analyses Study. *Lancet Glob Health* 6 (7), e758–e768. doi: 10.1016/S2214-109X(18)30283-3
- Kotloff K. L., Nataro J. P., Blackwelder W. C., Nasrin D., Farag T. H., Panchalingam S., et al. (2013). Burden and Aetiology of Diarrhoeal Disease in Infants and Young Children in Developing Countries (the Global Enteric Multicenter Study, GEMS): A Prospective, Case-Control Study. *Lancet* 382 (9888), 209–222. doi: 10.1016/S0140-6736(13)60844-2
- Kumar V. (2019). “Macrophages: The Potent Immunoregulatory Innate Immune Cells,” in *Macrophage Activation - Biology and Disease*. Ed. K. H. Bhat (London, UK: IntechOpen).
- Lacroix-Lamande S., Mancassola R., Auray G., Bernardet N., and Laurent F. (2008). CCR5 is Involved in Controlling the Early Stage of *Cryptosporidium Parvum* Infection in Neonates But is Dispensable for Parasite Elimination. *Microbes Infect.* 10 (4), 390–395. doi: 10.1016/j.micinf.2007.12.020
- Lacroix-Lamande S., Mancassola R., Naciri M., and Laurent F. (2002). Role of Gamma Interferon in Chemokine Expression in the Ileum of Mice and in a Murine Intestinal Epithelial Cell Line After *Cryptosporidium Parvum* Infection. *Infect. Immunol.* 70 (4), 2090–2099. doi: 10.1128/iai.70.4.2090-2099.2002
- Lantier L., Drouet F., Guesdon W., Mancassola R., Metton C., Lo-Man R., et al. (2014). Poly(I:C)-Induced Protection of Neonatal Mice Against Intestinal *Cryptosporidium Parvum* Infection Requires an Additional TLR5 Signal Provided by the Gut Flora. *J. Infect. Dis.* 209 (3), 457–467. doi: 10.1093/infdis/jit432
- Lantier L., Lacroix-Lamande S., Potiron L., Metton C., Drouet F., Guesdon W., et al. (2013). Intestinal CD103+ Dendritic Cells are Key Players in the Innate Immune Control of *Cryptosporidium Parvum* Infection in Neonatal Mice. *PLoS Pathog.* 9 (12), e1003801. doi: 10.1371/journal.ppat.1003801
- Laurent F., Eckmann L., Savidge T. C., Morgan G., Theodos C., Naciri M., et al. (1997). *Cryptosporidium Parvum* Infection of Human Intestinal Epithelial Cells Induces the Polarized Secretion of C-X-C Chemokines. *Infect. Immunol.* 65 (12), 5067–5073. doi: 10.1128/IAI.65.12.5067-5073.1997
- Laurent F., and Lacroix-Lamande S. (2017). Innate Immune Responses Play a Key Role in Controlling Infection of the Intestinal Epithelium by *Cryptosporidium*. *Int. J. Parasitol.* 47 (12), 711–721. doi: 10.1016/j.ijpara.2017.08.001
- Laurent F., McCole D., Eckmann L., and Kagnoff M. F. (1999). Pathogenesis of *Cryptosporidium Parvum* Infection. *Microbes Infect.* 1 (2), 141–148. doi: 10.1016/s1286-4579(99)80005-7
- Li X., Chase J. A., Bond R. F., Lor P., Fernandez K., Nguyen T. H., et al. (2019). Microbiological Safety of Popular Recreation Swimming Sites in Central California. *Environ. Monit. Assess.* 191 (7), 456. doi: 10.1007/s10661-019-7601-2
- Li M., Gong A. Y., Zhang X. T., Wang Y., Mathy N. W., Martins G. A., et al. (2018). Induction of a Long Noncoding RNA Transcript, NR\_045064, Promotes Defense Gene Transcription and Facilitates Intestinal Epithelial Cell Responses Against *Cryptosporidium* Infection. *J. Immunol.* 201 (12), 3630–3640. doi: 10.4049/jimmunol.1800566
- Liu J., Deng M., Lancto C. A., Abrahamsen M. S., Rutherford M. S., and Enomoto S. (2009). Biphasic Modulation of Apoptotic Pathways in *Cryptosporidium Parvum*-Infected Human Intestinal Epithelial Cells. *Infect. Immunol.* 77 (2), 837–849. doi: 10.1128/IAI.00955-08
- Mahlpuu M., Hakansson J., Ringstad L., and Bjorn C. (2016). Antimicrobial Peptides: An Emerging Category of Therapeutic Agents. *Front. Cell Infect. Microbiol.* 6, 194. doi: 10.3389/fcimb.2016.00194
- Marcial M. A., and Madara J. L. (1986). *Cryptosporidium*: Cellular Localization, Structural Analysis of Absorptive Cell-Parasite Membrane-Membrane Interactions in Guinea Pigs, and Suggestion of Protozoan Transport by M Cells. *Gastroenterology* 90 (3), 583–594. doi: 10.1016/0016-5085(86)91112-1
- Marquis N. D., Record N. R., and Robledo J. A. (2015). Survey for Protozoan Parasites in Eastern Oysters (*Crassostrea Virginica*) From the Gulf of Maine Using PCR-based Assays. *Parasitol Int.* 64 (5), 299–302. doi: 10.1016/j.parint.2015.04.001
- McCole D. F., Eckmann L., Laurent F., and Kagnoff M. F. (2000). Intestinal Epithelial Cell Apoptosis Following *Cryptosporidium Parvum* Infection. *Infect. Immunol.* 68 (3), 1710–1713. doi: 10.1128/iai.68.3.1710-1713.2000
- McDonald V., Korb D. S., Barakat F. M., Choudhry N., and Petry F. (2013). Innate Immune Responses Against *Cryptosporidium Parvum* Infection. *Parasite Immunol.* 35 (2), 55–64. doi: 10.1111/pim.12020
- McDonald V., Pollok R. C., Dhaliwal W., Naik S., Farthing M. J., and Bajaj-Elliott M. (2006). A Potential Role for Interleukin-18 in Inhibition of the Development of *Cryptosporidium Parvum*. *Clin. Exp. Immunol.* 145 (3), 555–562. doi: 10.1111/j.1365-2249.2006.03159.x
- McNair N. N., Bedi C., Shayakhmetov D. M., Arrowood M. J., and Mead J. R. (2018). Inflammasome Components Caspase-1 and Adaptor Protein Apoptosis-Associated Speck-Like Proteins are Important in Resistance to *Cryptosporidium Parvum*. *Microbes Infect.* 20 (6), 369–375. doi: 10.1016/j.micinf.2018.04.006
- Mead J. R., Arrowood M. J., Sidwell R. W., and Healey M. C. (1991). Chronic *Cryptosporidium Parvum* Infections in Congenitally Immunodeficient SCID and Nude Mice. *J. Infect. Dis.* 163 (6), 1297–1304. doi: 10.1093/infdis/163.6.1297
- Mead J. R., and You X. (1998). Susceptibility Differences to *Cryptosporidium Parvum* Infection in Two Strains of Gamma Interferon Knockout Mice. *J. Parasitol.* 84 (5), 1045–1048. doi: 10.2307/3284643
- Mele R., Gomez Morales M. A., Tosini F., and Pozio E. (2004). *Cryptosporidium Parvum* At Different Developmental Stages Modulates Host Cell Apoptosis In Vitro. *Infect. Immunol.* 72 (10), 6061–6067. doi: 10.1128/IAI.72.10.6061-6067.2004
- Miller C. N., Josse L., Brown I., Blakeman B., Povey J., Yiangou L., et al. (2018). A Cell Culture Platform for *Cryptosporidium* That Enables Long-Term Cultivation and New Tools for the Systematic Investigation of its Biology. *Int. J. Parasitol.* 48 (3–4), 197–201. doi: 10.1016/j.ijpara.2017.10.001
- Morada M., Lee S., Gunther-Cummins L., Weiss L. M., Widmer G., Tzipori S., et al. (2016). Continuous Culture of *Cryptosporidium Parvum* Using Hollow Fiber Technology. *Int. J. Parasitol.* 46 (1), 21–29. doi: 10.1016/j.ijpara.2015.07.006
- Nag R., Whyte P., Markey B. K., O’Flaherty V., Bolton D., Fenton O., et al. (2020). Ranking Hazards Pertaining to Human Health Concerns From Land Application of Anaerobic Digestate. *Sci. Total Environ.* 710, 136297. doi: 10.1016/j.scitotenv.2019.136297
- Nydam D. V., Wade S. E., Schaaf S. L., and Mohammed H. O. (2001). Number of *Cryptosporidium Parvum* Oocysts or *Giardia* Spp Cysts Shed by Dairy Calves After Natural Infection. *Am. J. Vet. Res.* 62 (10), 1612–1615. doi: 10.2460/ajvr.2001.62.1612
- Odendall C., Voak A. A., and Kagan J. C. (2017). Type III Ifns Are Commonly Induced by Bacteria-Sensing Tlrs and Reinforce Epithelial Barriers During Infection. *J. Immunol.* 199 (9), 3270–3279. doi: 10.4049/jimmunol.1700250
- Olsen L., Akesson C. P., Storset A. K., Lacroix-Lamande S., Boysen P., Metton C., et al. (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. *Vet. Res.* 46, 28. doi: 10.1186/s13567-014-0136-1
- Ong C. S., Li A. S., Priest J. W., Copes R., Khan M., Fyfe M. W., et al. (2005). Enzyme Immunoassay of *Cryptosporidium*-specific Immunoglobulin G Antibodies to Assess Longitudinal Infection Trends in Six Communities in British Columbia, Canada. *Am. J. Trop. Med. Hyg.* 73 (2), 288–295. doi: 10.4269/ajtmh.2005.73.288
- Perez-Cordon G., Yang G., Zhou B., Nie W., Li S., Shi L., et al. (2014). Interaction of *Cryptosporidium Parvum* With Mouse Dendritic Cells Leads to Their Activation and Parasite Transportation to Mesenteric Lymph Nodes. *Pathog. Dis.* 70 (1), 17–27. doi: 10.1111/2049-632X.12078
- Petry F., Robinson H. A., and McDonald V. (1995). Murine Infection Model for Maintenance and Amplification of *Cryptosporidium Parvum* Oocysts. *J. Clin. Microbiol.* 33 (7), 1922–1924. doi: 10.1128/JCM.33.7.1922-1924.1995
- Powell R. H., and Behnke M. S. (2017). WRN Conditioned Media is Sufficient for In Vitro Propagation of Intestinal Organoids From Large Farm and Small Companion Animals. *Biol. Open* 6 (5), 698–705. doi: 10.1242/bio.021717
- Rasmussen K. R., Larsen N. C., and Healey M. C. (1993). Complete Development of *Cryptosporidium Parvum* in a Human Endometrial Carcinoma Cell Line. *Infect. Immunol.* 61 (4), 1482–1485. doi: 10.1128/IAI.61.4.1482-1485.1993
- Rotz L. D., Khan A. S., Lillibridge S. R., Ostroff S. M., and Hughes J. M. (2002). Public Health Assessment of Potential Biological Terrorism Agents. *Emerg. Infect. Dis.* 8 (2), 225–230. doi: 10.3201/eid0802.010164

- Ryan U., Zahedi A., and Paparini A. (2016). Cryptosporidium in Humans and Animals—a One Health Approach to Prophylaxis. *Parasite Immunol.* 38 (9), 535–547. doi: 10.1111/pim.12350
- Sateriale A., Gullicksrud J. A., Engiles J. B., McLeod B. I., Kugler E. M., Henao-Mejia J., et al. (2021). The Intestinal Parasite *Cryptosporidium* is Controlled by an Enterocyte Intrinsic Inflammasome That Depends on NLRP6. *Proc. Natl. Acad. Sci. U.S.A.* 118 (2), 1–8. doi: 10.1073/pnas.2007807118
- Sateriale A., Slapeta J., Baptista R., Engiles J. B., Gullicksrud J. A., Herbert G. T., et al. (2019). A Genetically Tractable, Natural Mouse Model of Cryptosporidiosis Offers Insights Into Host Protective Immunity. *Cell Host Microbe* 26 (1), 135–146.e135. doi: 10.1016/j.chom.2019.05.006
- Scallan E., Hoekstra R. M., Angulo F. J., Tauxe R. V., Widdowson M. A., Roy S. L., et al. (2011). Foodborne Illness Acquired in the United States—Major Pathogens. *Emerg. Infect. Dis.* 17 (1), 7–15. doi: 10.3201/eid1701.P111101
- Theodos C. M., Sullivan K. L., Griffiths J. K., and Tzipori S. (1997). Profiles of Healing and Nonhealing *Cryptosporidium Parvum* Infection in C57BL/6 Mice With Functional B and T Lymphocytes: The Extent of Gamma Interferon Modulation Determines the Outcome of Infection. *Infect. Immun.* 65 (11), 4761–4769. doi: 10.1128/IAI.65.11.4761-4769.1997
- Tzipori S. (1998). Cryptosporidiosis: Laboratory Investigations and Chemotherapy. *Adv. Parasitol* 40, 187–221. doi: 10.1016/s0065-308x(08)60121-9
- Upton S. J., Tilley M., and Brillhart D. B. (1994). Comparative Development of *Cryptosporidium Parvum* (Apicomplexa) in 11 Continuous Host Cell Lines. *FEMS Microbiol. Lett.* 118 (3), 233–236. doi: 10.1111/j.1574-6968.1994.tb06833.x
- Urban J. F. Jr., Fayer R., Chen S. J., Gause W. C., Gately M. K., and Finkelman F. D. (1996). IL-12 Protects Immunocompetent and Immunodeficient Neonatal Mice Against Infection With *Cryptosporidium Parvum*. *J. Immunol.* 156 (1), 263–268.
- Varughese E. A., Bennett-Stamper C. L., Wymer L. J., and Yadav J. S. (2014). A New In Vitro Model Using Small Intestinal Epithelial Cells to Enhance Infection of *Cryptosporidium Parvum*. *J. Microbiol. Methods* 106, 47–54. doi: 10.1016/j.mimet.2014.07.017
- Verschoor C. P., Puchta A., and Bowdish D. M. (2012). The Macrophage. *Methods Mol. Biol.* 844, 139–156. doi: 10.1007/978-1-61779-527-5\_10
- Yang Z., Fu Y., Gong P., Zheng J., Liu L., Yu Y., et al. (2015). Bovine TLR2 and TLR4 Mediate *Cryptosporidium Parvum* Recognition in Bovine Intestinal Epithelial Cells. *Microb. Pathog.* 85, 29–34. doi: 10.1016/j.micpath.2015.05.009
- Zaalouk T. K., Bajaj-Elliott M., George J. T., and McDonald V. (2004). Differential Regulation of Beta-Defensin Gene Expression During *Cryptosporidium Parvum* Infection. *Infect. Immun.* 72 (5), 2772–2779. doi: 10.1128/iai.72.5.2772-2779.2004
- Zachos N. C., Kovbasnjuk O., Foulke-Abel J., In J., Blutt S. E., de Jonge H. R., et al. (2016). Human Enteroids/Colonoids and Intestinal Organoids Functionally Recapitulate Normal Intestinal Physiology and Pathophysiology. *J. Biol. Chem.* 291 (8), 3759–3766. doi: 10.1074/jbc.R114.635995
- Zadrozny L. M., Stauffer S. H., Armstrong M. U., Jones S. L., and Gookin J. L. (2006). Neutrophils do Not Mediate the Pathophysiological Sequelae of *Cryptosporidium Parvum* Infection in Neonatal Piglets. *Infect. Immun.* 74 (10), 5497–5505. doi: 10.1128/IAI.00153-06
- Zambriski J. A., Nydam D. V., Wilcox Z. J., Bowman D. D., Mohammed H. O., and Liotta J. L. (2013). *Cryptosporidium Parvum*: Determination of ID(5)0 and the Dose-Response Relationship in Experimentally Challenged Dairy Calves. *Vet. Parasitol* 197 (1-2), 104–112. doi: 10.1016/j.vetpar.2013.04.022
- Zhang X. T., Gong A. Y., Wang Y., Chen X., Lim S. S., Dolata C. E., et al. (2016). *Cryptosporidium Parvum* Infection Attenuates the Ex Vivo Propagation of Murine Intestinal Enteroids. *Physiol. Rep.* 4 (24), 1–13. doi: 10.14814/phy2.13060
- Zhou J. H., Wang Y. N., Chang Q. Y., Ma P., Hu Y., and Cao X. (2018). Type III Interferons in Viral Infection and Antiviral Immunity. *Cell Physiol. Biochem.* 51 (1), 173–185. doi: 10.1159/000495172
- Ziegler P. E., Wade S. E., Schaaf S. L., Stern D. A., Nadeski C. A., and Mohammed H. O. (2007). Prevalence of *Cryptosporidium* Species in Wildlife Populations Within a Watershed Landscape in Southeastern New York State. *Vet. Parasitol* 147 (1-2), 176–184. doi: 10.1016/j.vetpar.2007.03.024

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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