# Overview of current knowledge on cryptosporidiosis: impact and therapeutic solutions

Fabrice Laurent

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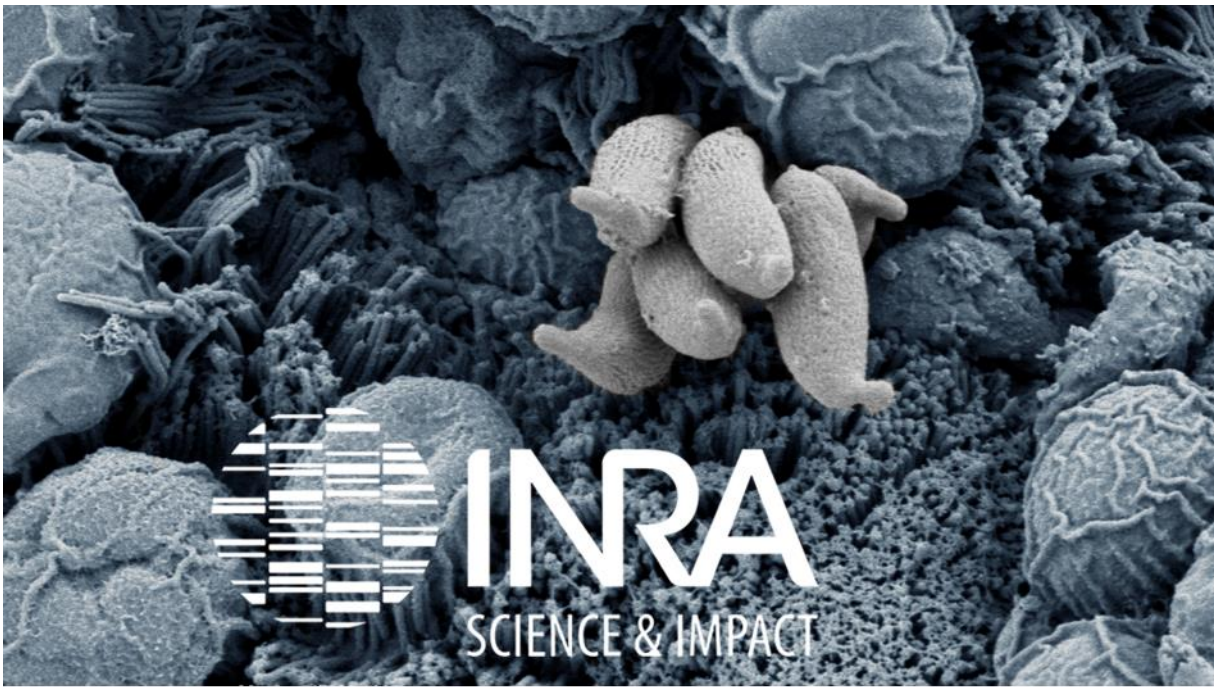


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**Cryptosporidiosis a major zoonotic disease of young ruminants**:

An estimated 56% of health problems in early life relate to diarrhea, making it a number one health issue for newborn calves. It accounts for 52.2% of mortality of unweaned calves, and is also a major cause of poor growth, increased labour requirements and increased costs (www.biomin.net source). In severe cases, an outbreak of scour can kill up to a third of affected calves. The cost per sick calf is estimated at 44 £ (excluding labour). Outbreaks of infection causing calf diarrhea are often rapid and multifactorial in nature. Major enteric pathogens known to cause calf diarrhea are protozoan parasites (*Cryptosporidium parvum*), viruses (bovine rotavirus, bovine coronavirus), and bacteria (E. Coli K99). Rapid *in vitro* diagnostic tests help to identify the infectious agent(s) responsible for the disease. Early detection is an important issue since the large amount of parasites excreted by young calves quickly contaminate the congeners. Depending of the European country considered, epidemiological surveys indicated that *Cryptosporidium* is the first or second leading cause responsible for calf diarrhea with prevalence almost equivalent to that of rotavirus infection. In a recent research program performed in France (Britany and Normandy) on Holstein and Charolais calves we observed using the Speed-V-Diar Kit that Cryptosporidium was by far the most prevalent infectious agent. Cryptosporidiosis is also a major One-health problem with severe health consequences for very young, malnourished children living in endemic areas and for immunocompromised individuals.

Cryptosporidia are particularly resistant parasites in the external environment where they can survive more than a year. In practice, for a good disinfection (soils, walls and equipment in contact with calves),

livestock buildings must be entirely cleaned, and disinfected with hot steam or treated with chemical disinfectants with a contact time of several hours.

Long term consequences of the infection:

There are growing evidences that *C. parvum* infection can induce long-term consequences on gut health long after the recovery of the infection. Epidemiological studies have reported that after resolution of *C. parvum* infection, patients still suffer for abdominal pain. In our team we noticed that long after recovery, mice presented an alteration of immune cell composition and microbiota associated with an increased visceral susceptibility. In addition, mice infected with *C. parvum* at young age presented an increased susceptibility to salmonella infection at adult age thus highlighting the detrimental role of early infection by *Cryptosporidium* on long-term gut health and susceptibility to enteric diseases (S. Lamandé, unpublished). With an immunosuppressed mouse model (adult SCID-mice treated with dexamethasone), the presence of digestive adenocarcinoma were observed following *C. parvum* infection (Certad *et al.* Int J Parasitol. 2010). Since enteric infection in early life alter intestinal immune development and microbiota establishment that can result in long-term consequences on gut health, it is therefore of major importance insure health in the vulnerable newborn calves.

**A quick expanding and powerful toolbox to investigate host-cryptosporidium interaction and evaluate control methods**:

For long time, numerous technical difficulties to study *Cryptosporidium* have limited progress. However, recent advances have been obtained regarding genetic manipulation (Vinayak et al., 2015), cryo-preservation of viable parasites (Jaskiewicz et al., 2018), a complete parasite life cycle with production of infectious oocyst within intestinal organoid cultures (Heo et al., 2018) or air-liquid interface cultivation system (Wilke et al. Cell Host Microbe 2019). All these major improvements will facilitate the understanding of host-cryptosporidium interactions, host immune response and drug screening. Indeed the ability to force the parasite to express reporter genes (fluorescence, luminescence etc) allows its detection and precise stage specific quantification

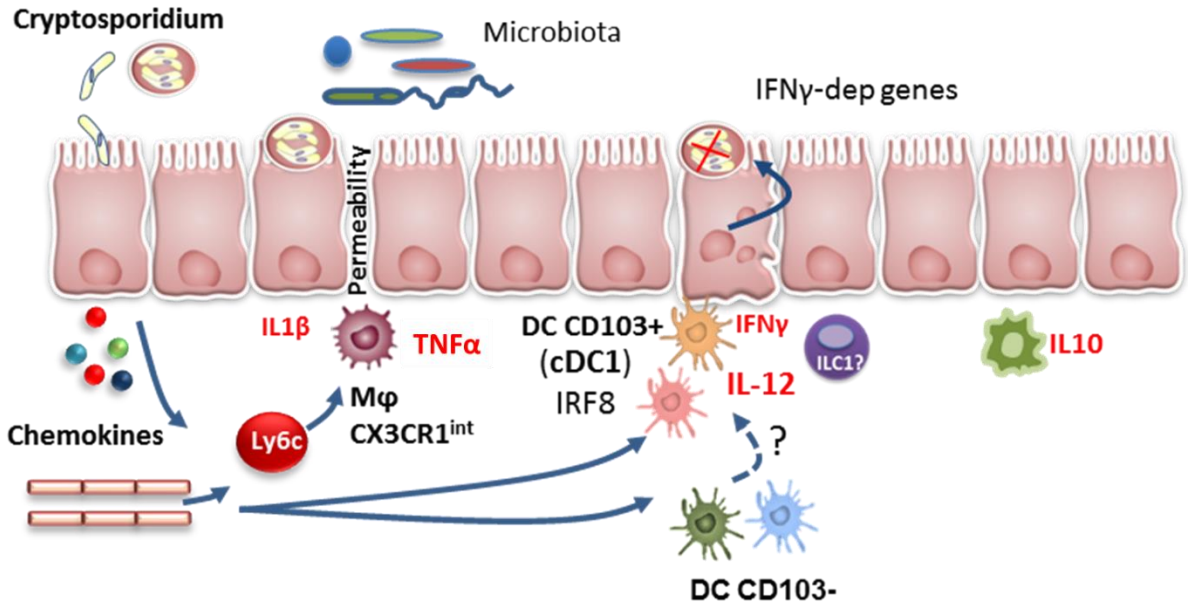
Drug treatments against *Cryptosporidium*: Halocur Intervet international/MSD; HUVEPHARMA PAROFOR

Currently, the halofuginone lactate is the only drug whose efficacy in the prevention and reduction of neonatal diarrhea caused by *C. parvum* in calves has been demonstrated and recognized by the authorities in France. Its indication is for diarrhea that has been seen for less than 24 hours, once a day for 7 days. Given the mixed nature of these infections, most often, the veterinarian completes his prescription with rehydration, antibiotics and digestive dressings. Recently, an antibiotic, historically used in farms facing cryptosporidiosis, has obtained a marketing authorization with the indication "treatment of intestinal infections with E. coli". It is an antibiotic of the aminoglycoside family. This medicinal product may only be used in the indication "cryptosporidiosis" after a declaration with the National Agency for Veterinary Medicinal Products (ANMV) of the ineffectiveness of the reference treatment. Several laboratories have engaged throughout the world researches on new therapeutics against Cryptosporidium sometime with large funding of the B&M gates foundation. Some efficient compounds against cryptosporidium have been identified to date such as : BKI (U Washington), tRNA synthetase MetRs, lysyl-tRNA synthetase (U Washington, Baragana et al. PNAS 2019, Buckner AAC 2019 ) and LysRS (Anacor), KDU 731 (Novartis, Manjunatha Nature 2017), LDH inhibitors (U Illinois, Li

*et al.* PLoS Pathogen 2019). A suite of phenotypic assays to ensure pipeline diversity when prioritizing drug-like *Cryptosporidium* growth inhibitors as recently been published (Jumani *et al.* Nat commun. 2019). Seeking for more efficient compounds against *Cryptosporidium*, a collaboration was set-up between M.H Hakimi team (INSERM U1209, CNRS UMR 5309, University Grenoble-Alpes) and my team (AIM team, UMR1282-ISP-AIM INRA-Univ de Tours) bringing together complementary expertise. Benzoxaboroles are boron-containing compounds that had previously demonstrated efficacy in a number of clinical indications in recent years. For example, Hakimi's team reported that benzoxaborole AN3661 had potent *in vitro* activity against *T. gondii* Parasites selected to be resistant to AN3661 had mutations in *TgCPSF3*, which encodes a homologue of cleavage and polyadenylation specificity factor subunit 3, an endonuclease involved in mRNA processing in eukaryotes. In another study, it was shown that AN3661 is also active against the human malaria parasite *P. falciparum* (Sonoiki *et al*, 2017). This compound was therefore evaluated against Cryptoporidium and we found that oxaborole-mediated inhibition of CPSF3 reduced intestinal parasite burden in both immunocompromised and neonatal mouse models with far greater efficacy than nitazoxanide (Swale *et al* in press). We generated Cryptosporidium crystal structures revealing a mechanism of action whereby the mRNA processing activity of CPSF3 is efficiently blocked by the binding of the oxaborole group at the metal-dependent catalytic center. Compared to Cryptosporidium CPSF3, human CPSF3 adopts a different conformation, which is not compatible with the binding of the drug. This structural difference that typifies apicomplexan contributes to the selectivity of AN3661 toward CPSF3 (IS> 600) and might explain the lack of toxicity of this oxaborole. Our data identified CPSF3 as one of the most promising primary target for chemotherapy and provide insights to accelerate the development of next-generation anti-*Cryptosporidium* therapeutics.

Innate immunity is crucial for protection in neonates :

To decipher immune responses, mouse models were extensively used. We and others determined that innate immunity was sufficient to control the acute phase of the infection. We extensively investigated the role of mononuclear phagocytes during the infection and determined that dendritic cells were key in the protection process (Lantier *et al.* 2013). We continued this work and demonstrated that the subset expressing the markers CD11c + ClassII + Batf3+ also called conventional dendritic cells 1 (cDC1) were the most important effector cells. We recently characterized intestinal cDC1 cells in ruminants which will allow us to confirm their function during infection in a natural host. Some other mononuclear phagocytes such as inflammatory monocytes are recruited in the infected epithelium. These cells do not contribute to protection but were involved in the loss of transepithelial permeability (De Sablet *et al.* 2016). Therefore a selective activation of cDC1 will be the most efficient strategy to control cryptosporidiosis.




Laurent & Lamandé, 2017.

Vaccination a possible strategy to control ruminants cryptosporidiosis?:

Recently, a natural model of cryptosporidium infection in mice was used to investigate the importance of adaptive immunity. Immunocompetent adult mice present a self-limited infection to *Cryptosporidium tizzeri* (Sateriale et al. 2019). Once infected, mice are protected against a homologous challenge reviving the interest of developing a vaccine to prevent or limit the severity of cryptosporidiosis. However, at the present time, a vaccination for young calves infected within the very first days of life is difficult to imagine and vaccination appears more appropriate for young children that are at high risk until 5 years. For young calves, one possible strategy will be to vaccinate the mother during gestation to induce a maternal transmitted immunity to the offspring. To date, experiments using this strategy presented limited to no efficacy to control cryptosporidiosis. However, despite the fact that several publications demonstrated that antibodies play minor to no role in the control of cryptosporidiosis, sufficient colostrum administration is however strongly recommended to prevent neonatal diseases in general.

Immunostimulation a way to strengthen neonatal immune system ?:

Stimulation of the innate immune system of neonates is an alternative to vaccination. We first determined that neonates (neonatal mice, goat kids and lambs) were highly responsive to Toll-like receptors stimulation. By strengthening innate immune mechanism with toll-like receptors agonists we were able to strongly reduce parasite development. Some agonists are dependent on the presence of the microbiota while others do not need any external synergy to be efficient (Lantier *et al*, 2014). In every case, MyD88 signaling pathway and dendritic cells were required to induce a sufficient protection confirming their key role in the elimination of infected cells. Natural products with immunostimulant properties that can be added to colostrum is therefore an elegant alternative to reduce or prevent infections. This strategy is also compatible with further efficient molecules able to cure an established infection.