Indian Farmer
Volume 9, Issue 07, 2022, Pp. 323-328.
Available online at: www.indianfarmer.net
ISSN: 2394-1227 (Online)

ORIGINAL PAPER

Bovine Cryptosporidiosis: an overview

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Article Received: 19 July 2022 *Published Date: 23 July 2022*

INTRODUCTION

Cryptosporidium is a ubiquitous enteric protozoan parasite affecting mammals including human, reptiles, fish and birds. At present, more than 39 different species of the parasite are recognized as valid, along with several unrecognized genotypes reported throughout the world (Zhao *et al.,* 2020). Different bovine species such as cattle, water buffalo, yaks etc. are susceptible to *C. parvum, C. andersoni, C. bovis* and *C. ryanae*. Its infection is generally self-limiting in immunocompetent hosts but causes life-threatening acute or chronic diarrhoea in immunocompromised hosts. Affected animals suffer from prolonged illness, malabsorption, dehydration leading to severe economic losses associated with retarded growth rate, decreased productivity, treatment cost and mortality (Bhat *et al.,* 2014). Cryptosporidiosis is a water-borne zoonosis where *C. parvum* is the most important species with zoonotic potential. In human, it is second only to rotavirus as a leading cause of diarrhoea among neonatal children. The magnitude and significance of the problem is even amplified in the absence of vaccines and availability of very limited treatment options, leaving us with no tools for interrupting its often-dramatic course especially in young or susceptible host. This article briefly summarizes about cryptosporidiosis in bovines, the most important animal reservoir for human transmissions.

Historical background of bovine cryptosporidiosis

Though *Cryptosporidium* was first reported in 1907 by Tyzzer in laboratory mouse, its clinical importance was not fully regarded in medical and veterinary field for another 70 years (Tzipori and Ward, 2002). Bovine cryptosporidiosis was first reported about half-a century ago during the histological examination of jejunum of an 8 months old

heifer showing chronic diarrhoea (Panciera *et al.,* 1971). Then, the parasite was not considered as the cause of diarrhoea due to concurrent pathogenic bacterial or viral infections. Later, in early 1980s, the natural and experimental infection studies have revealed its role as primary etiological agent in bovine neonatal diarrhoea (Tzipori *et al.,* 1980). Currently, bovine cryptosporidiosis is recognized as endemic across the globe and is most important pathogen causing neonatal calf diarrhoea (Mosier and Oberst, 2000).

cryptosporidium species affecting bovines

There are four main species of *Cryptosporidium* affecting cattle. These are *C. parvum, C. andersoni* (previously known as *C. muris*), *C. bovis* (previously known as bovine genotype B) and *C. ryanae* (previously known as deer-like genotype). In addition to these, *C. suis, C. hominis, C. felis* and *C. canis* have also been reported sporadically. Among these, only *C. parvum* is associated with clinical disease in calves while adults are asymptomatic carriers. The parasite has age related distribution in bovines where *C. parvum* infects the intestine of neonatal calves especially less than three weeks of age. *C. bovis* and *C. ryanae* infect the small intestine of 2-11 months old dairy calves while *C. andersoni* infects the abomasum of adults and juvenile animals older than 2 months of age.

Life cycle

The parasite has a homoxenous life cycle and a capability to reproduce in masses and disseminate widely in various animal hosts (Wegayehu *et al.,* 2013).

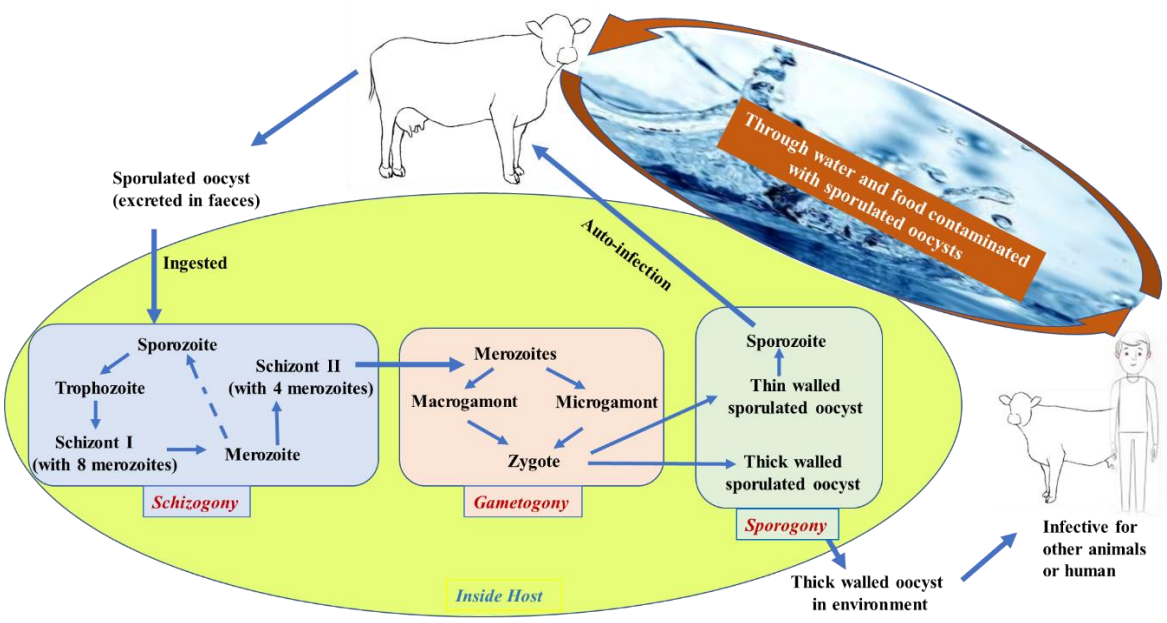

Fig 1: Diagrammatic representation of *Cryptosporidium parvum* life cycle

The life cycle is divided into three developmental phases: schizogony (also called as merogony), gametogony and sporogony having three major developmental stages namely schizonts (or meronts), gamonts and oocysts. The parasite multiplies intracellularly and extra-cytoplasmically in the intestinal epithelial cells. The oocysts are released from the infective host and are immediately infective for other hosts in the surrounding without the need of any developmental time. There are two types of oocyst, thin walled: which rupture within the infected host leading to autoinfection (re-infection of the same host from within the host body, increasing the severity of the disease pathogenesis) and thick walled which come out of the host and are infecting other hosts. Oocysts are very sturdy and are resistant to common disinfectants including chlorine-based disinfectants.

Disease transmission

This protozoan parasite can be transmitted via fecal oral route from one animal to another or even from animals to human and vice-versa with water or food contaminated with viable sporulated oocysts (Abu-Madi *et al.,* 2011). Various factors favour disease transmission, like small size of oocysts, their high resistance to chlorine and acids, their fully sporulated and immediately infectious nature, zoonotic potential with least host specificity. Other factors like age of host, hygienic conditions, feed and water sources, diarrhoea and climate etc. also affects the disease transmission (Odeniran and Ademola, 2019).

Disease pathology in bovines

Bovine cryptosporidiosis is associated with acute or chronic gastro-intestinal disturbances resulting in anorexia, weight loss, retarded growth, decreased milk production and mortality (Brook *et al*., 2008). Most of the calves infected with *C. parvum* show clinical symptoms within 3-5 days of birth which includes profuse watery diarrhoea, inappetence, gastrointestinal discomfort, abdominal tension, nausea, lethargy and dehydration in severe cases (during prepatent period). These clinical symptoms last for 4-17 days (during patent period). Mortality rates are usually low. Calves continue to shed oocysts after the clinical signs disappear.

Detection and diagnosis

The detection and diagnosis of *Cryptosporidium* species is challenging and leads to its under-reporting. The consistency of faecal sample (diarrhoeic/non-diarrhoeic), study design (point prevalence studies may underestimate the prevalence), diagnostic technique employed for examination, all these significantly affect the diagnosis results. Age of animal at the time of sampling is also very important as calves <6 weeks of age are most likely to be shedding *C. parvum* while older animals may shed other species. The clinical parasitology laboratories still rely on microscopic examination of MZN-stained faecal smears for the detection of *Cryptosporidium* species. The microscopy based methods lack sensitivity and specificity and are not able to differentiate various species of the parasite properly. To address this issue, molecular diagnostics are used where PCR-RFLP of 18S rRNA gene based nested PCR product and its sequence analysis

is used to differentiate different species of the parasite. Sero-diagnostics like ELISA, immuno-chromatographic tests are other diagnostic options which are under development.

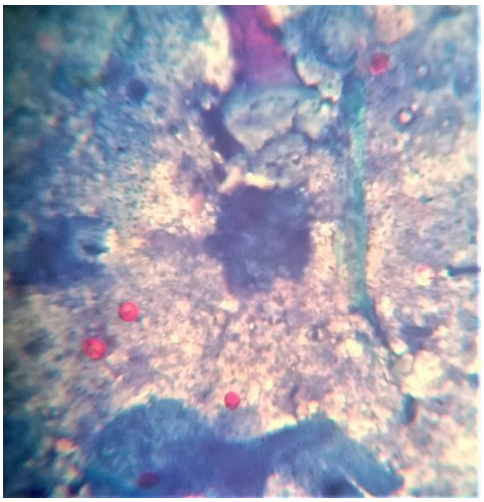

Fig 2: MZN-stained bovine faecal smear (100X) with red coloured *Cryptosporidium* oocysts

Zoonotic aspect

Farm animals are a recognized source of human infections. More than 90% of the human infections are caused by two main species, *C. hominis*, a species exclusively infecting human; and *C. parvum*, the most important zoonotic species (Chalmers and Giles, 2010). As there are morphological similarities between various *Cryptosporidium* species oocysts, the reports of *Cryptosporidium* infections employing traditional microscopy methods assume the detected species as *C. parvum* which otherwise over-estimate the potential role of bovines as reservoir for human diseases. Recently with the assistance of molecular diagnostic techniques, more than 20 species and genotypes of *Cryptosporidium* are identified in human, including two more species from bovines namely, *C. bovis* and *C. andersoni* (Xiao, 2010; Ryan *et al*., 2014; Zahedi *et al*., 2016).

Treatment

As a parasitic disease, *Cryptosporidium* is more difficult to treat than bacterial or viral infections. In animals, there is no licensed effective chemotherapeutic agent available to treat cryptosporidiosis, still halofuginone is found effective to decrease the severity of diarrhoea and limits the spread of the disease. Halofuginone is used prophylactically and should be given within 24-48 hours of birth for 7 consecutive days. It is not much effective in animals showing signs of diarrhoea for >24 hours. Supportive therapy including oral rehydration should be given to severely affected animals and they should be prevented from chilling. Moreover, in otherwise healthy calves, the clinical signs including diarrhoea usually subside within 4-7 days. In human, a single FDA approved drug for *Cryptosporidium* infections, nitazoxanide is largely ineffective in children who

are undernourished and individuals with compromised immune systems, who are most susceptible for severe illness. The disease severity urges for new therapeutics and preventive options.

Control and risk management practices based on current knowledge

Keeping in mind the limited availability of effective drugs and non-availability of vaccines, the control strategies based on the understanding of transmission routes of the parasite are the only feasible way to control the disease. Better risk management, improved sanitation and improved disease diagnosis are required. As of now, cryptosporidiosis is very difficult to control due to highly resistant and environmentally stable oocysts. These oocysts are sporulated when excreted and thus are immediately infective with very low infective doses. They are resistant to several disinfectants still through cleaning with hot water followed by drying is effective as oocysts are susceptible to extreme temperatures i.e. below -20 °C and upto 60 °C and desiccation. Other managemental practices to control this disease include:

- Quarantine the new animals for at least 7 days and diarrhoeic animals until one week after the diarrhoea has ceased.
- Lower the stocking rates in the shed.
- Known infected animals should be isolated and should be handled either by separate workers or handled after handling the healthy animals by the same workers.
- The new-borne calves should be given sufficient colostrum.
- Cows about to give birth should be thoroughly cleaned especially their teats.
- Secure your animals and their feeding areas from coming in contact with wild animals.
- Keep in mind the zoonotic potential of *Cryptosporidium*, the livestock owners and all the farm staffs should follow the necessary hygienic precautions.

CONCLUSION

Despite its worldwide prevalence and significant severity in young and immunocompromised animals, *Cryptosporidium* still remains the least explored parasite where we are facing challenges in its diagnosis, treatment as well as control. There is every chance of missing the parasite in conventional faecal microscopic examination techniques which is the main reason behind its under-reporting. Early diagnosis is the best way to fight with the infection as the available treatment options are not much effective in later stages of the disease. Moreover, the oocyst morphology is not sufficient enough to differentiate the various species of the parasite, among which many have zoonotic potential. Further, we are not sufficiently backed up with the proper treatment options and its control is also very difficult with general managemental practices and in the absence of a vaccine. All this emphasizes a need to handle cryptosporidiosis in more cautious way where everyone has a role to play. Researchers should focus on developing better diagnostic tools and speed up the process of vaccine or drug

development. Livestock owners, on the other hand should adopt proper managemental practices to control the parasite in farms and in the environment.

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