Cryptosporidiosis in neonatal ruminants in South Africa: a review

M.C. Marufu

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X4, Onderstepoort 0110, Pretoria, South Africa.
Telephone: +27 12 529 8227; Fax: +27 12 539 8315; Email: chris.marufu@up.ac.za

Summary

Protozoan parasites of the species *Cryptosporidium* are leading causes of diarrhoea in ruminants and humans and as such cryptosporidiosis is an important and emerging protozoal disease of public health significance. In South Africa, the organism has gained importance by causing large outbreaks in neonatal ruminants in different farming systems. The prevalence of cryptosporidiosis and risk factors associated with transmission of the disease among neonatal ruminants in South Africa are not understood and warrant investigation. The genetic characterisation of *Cryptosporidium* isolates in neonatal ruminant systems also requires elucidation.

Key words: *Cryptosporidium parvum*; neonatal diarrhea; small ruminants; oocysts

1. Introduction

Organisms of the genus *Cryptosporidium*, first described by Tyzzer in the early 1900s in mice (Tyzzer, 1907; 1912), were only recognised to be of veterinary importance in the mid-1950s through a *C. meleagridis* outbreak in turkeys (Slavin 1955). Since then, *C. parvum* has gained prominence as a leading cause of diarrhoea in neonatal ruminants and humans, worldwide. In South Africa specifically, the organism has gained importance in the country by causing large outbreaks in neonatal ruminants in different farming systems. The organism has been ranked as the fifth most important food-borne parasite globally by the World Health Organization (Odeniran & Ademola, 2019). It is against this background that the current literature review discusses cryptosporidiosis in ruminants and proposes to investigate its prevalence, distribution and associated risk factors.

2. Current knowledge on *Cryptosporidium*

The protozoan parasite *Cryptosporidium* belongs to the family *Cryptosporidiidae*, suborder *Eimeriina*, order *Eucoccidiida*, subclass *Coccidia*, class *Sporozoa*, phylum Apicomplexa (Carmena, 2010). This parasite affects a large variety of hosts globally, including humans (Robertson, 2014). Ruminants are susceptible to and also considered sources of infection particularly of *C. parvum* and *C. ubiquitum*, which have been reported to have public health significance (Xiao, 2010). Infection with *Cryptosporidium* species results in gastroenteritis that manifests as diarrhoea of varying severity depending on the host's immunity (Mahfouz et al., 2014). In adult ruminants and immunocompetent hosts, the diarrhoea is mild and self-limiting, whereas severe, chronic and life-threatening diarrhoea often occurs in neonatal ruminants and immunocompromised hosts (Samra et al., 2016). The disease has a negative impact on animal production, causing severe economic losses associated with decreased growth rate and mortality particularly in infected neonatal ruminants (Santin, 2020).

In young animals, children and immunocompromised people (such as human immunodeficiency virus (HIV) patients), the disease causes chronic diarrhoea and can often be fatal (Chalmar & Davies, 2010). With the high prevalence rate of HIV in South Africa, the contact between susceptible humans and infected animals particularly in ruminant farming communities can be an important transmission mode and source of infection for humans. No

drug or drug combination effectively treats cryptosporidiosis in humans and animals (Chako et al., 2010). Nitazoxanide has been shown to be partially effective against cryptosporidiosis in humans, but it is largely ineffective in immunocompromised patients, who remain vulnerable to infection (Abubakar et al., 2007). In animals, halofuginone can be used for the treatment of cryptosporidial diarrhoea in calves, and has been recently registered for use in food animals in South Africa (MSD, 2020). Its efficacy in reducing oocyst shedding, however, has not been clearly demonstrated (Chako et al., 2010). The prevention of cryptosporidiosis should therefore be the mainstay of control of the parasite, guided by sound epidemiological data.

Very few prevalence studies have been conducted on *Cryptosporidium* in livestock in South Africa (Samra et al., 2013, Samra et al., 2016, Samie et al., 2017). In the earlier studies by Samra and colleagues (2013; 2016) in Mpumalanga Province, focus was given to cattle and wildlife and not to sheep and goats. The study by Samie and colleagues (2017) in Limpopo Province, focused on cattle, chicken and goats, but only included four sheep. From these studies, it has been observed that the prevalence of cryptosporidiosis varies with geographic location and species, and has been shown to be high, particularly in goats in Limpopo Province (Samie et al., 2017), and low in cattle in Mpumalanga Province (Samra et al., 2016). The development of molecular methods to diagnose cryptosporidiosis has led to a more in-depth understanding of the genetic diversity of *Cryptosporidium*, which plays an important role in the diverse clinical presentations of cryptosporidiosis (Santin, 2013). Application of these molecular methods in prevalence studies in South Africa has been limited and molecular genotyping of *Cryptosporidium* infections remains a challenge.

3. Importance to relevant industry

In South Africa, prevalence data on *Cryptosporidium* in ruminant livestock still remains scant despite the importance of ruminants as a source of protein. The prevalence of *Cryptosporidium* spp. has been determined mostly in cattle (Samra et al., 2013, Samra et al., 2016, Samie et al., 2017), and few studies have focused on sheep and goats (Samie et al. 2017), which are often farmed simultaneously with cattle and can be important sources of infection for humans. Microscopy and serodiagnostic tools have been used in these studies and yet these techniques lack sensitivity and specificity. The prevalence of *Cryptosporidium* infection and the various species of cryptosporidiosis responsible for infection in cattle, sheep and goats in South Africa therefore remains largely unknown. An increase in reports of clinical outbreaks and the zoonotic potential of these outbreaks of cryptosporidiosis in neonatal ruminants in South Africa make it imperative to conduct a more detailed and widespread study of the epidemiology of *Cryptosporidium* infection with the aim of understanding the prevalence. It is essential to employ molecular tools which have greater sensitivity and specificity to enable detection of the important *Cryptosporidium* species that affect ruminants.

Genetic characterisation of *Cryptosporidium* in ruminants in South Africa is still lacking and molecular studies have not been conducted. Genetic characterisation of the species of *Cryptosporidium* affecting different ruminant species in South Africa will be important in understanding species diversity of the pathogen and host species specificity in ruminants as well as determine the zoonotic importance of the *Cryptosporidium* spp. The risk factors associated with cryptosporidiosis are largely unknown in neonatal ruminant species in the country. A study of the risk factors associated with cryptosporidiosis in neonatal ruminants in South Africa is required as this will enable formulation of strategies to counter these risks and reduce the disease burden in ruminants, and lessen risk of human infections.

4. Knowledge gaps that need to be addressed

Cryptosporidium andersoni and *C. bovis* were reported to occur in calves 3-12 months of age at a wildlife-livestock-human interface in South Africa (Samra et al., 2013). Another study by Samra et al. (2016) however, only detected *C. parvum* and *C. bovis* in calves less than four months old in the same study area. The inclusion of juvenile cattle in the earlier study may explain the presence of *C. andersoni*, which was not detected in the latter study involving calves less than four months old, since this *Cryptosporidium* species has been shown to affect juvenile and adult cattle (Santin, 2020). Samie et al. (2017) observed *C. parvum* in calves and adult cattle and *C. andersoni* in cows in Limpopo Province. Interestingly, age related distribution of *Cryptosporidium* species in cattle has been reported to be not fully consistent (Diaz et al., 2021). More work is required on the prevalence of *Cryptosporidium* species in cattle of different age groups in South Africa as this will allow determination of those age classes posing a higher risk to public and animal health. The use of molecular methods to identify and sequence the *Cryptosporidium* species affecting different age groups of cattle will be a requisite. Studies on the effects of age-related differences in *Cryptosporidium* species should be conducted in different cattle production systems occurring in South Africa.

Cryptosporidiosis has major negative impacts to the cattle industry through calve mortalities, diagnosis and treatment costs, and extra husbandry costs for calves to reach market weight (Olson et al., 2004). *Cryptosporidium* infections have been reported to negatively impact cattle production, causing a reduction in milk yield in dairy cattle, as well as weight loss in beef and feedlot animals (Anderson, 1998; Esteban & Anderson, 1995; Ralston et al., 2010; Ralston et al., 2003; Shivley et al., 2018). In South Africa, there is a dearth of studies that have determined the factors associated with oocyst shedding and impact of cryptosporidiosis in different cattle production systems. The factors associated with oocyst shedding in the different cattle production systems in South Africa thus merit investigation. It is also essential to determine the impact of cryptosporidiosis on the different cattle production systems in the country.

Infected neonatal calves are considered a high risk and dangerous source of infection as they can shed up to 107 oocysts per gram of faeces (de Graaf et al., 1999; Garro et al., 2016). Seasonal variation in the prevalence of cryptosporidiosis in cattle has been observed (Brankston et al., 2018; Szonyi et al., 2010), though there were differences in the season when the organism is most likely to occur. Poor hygiene, communal housing, overcrowded conditions and soiling of the dam's udder by faeces all increase the risk of infection of calves (de Graaf et al., 1999). However, shedding of oocysts in faeces was not demonstrated in pre-weaned calves of native cattle breeds (Maikai et al., 2011), suggesting a reduced importance in the transmission of cryptosporidiosis from native breeds to other livestock and humans.

The risk factors associated with cryptosporidiosis in cattle in South Africa have not been fully elucidated and thus need to be investigated. Management practices, seasonal trends, and the presence of oocyst shedding calves, all warrant investigation as they could potentially influence the prevalence of cryptosporidiosis in cattle in the country. There is a need to further investigate the breed effect on shedding of *Cryptosporidium* oocysts, and comparisons of exotic, composite and indigenous cattle breeds will be important in this regard. Since differences in regional and managemental factors have been observed, there is thus a need to perform regional risk factor surveys to identify the most decisive variables in a particular cattle population (Diaz et al., 2021).

In sheep, the most commonly detected *Cryptosporidium* species are *C. ubiquitum*, *C. xiaoi* and *C. parvum* (Bordes et al., 2020). The distribution of *Cryptosporidium* species in sheep is not

clearly associated with age unlike in cattle (Santin, 2020). It has, however, been reported that *C. parvum* or *C. xiaoi* are the predominant species in lambs younger than 1 month old, while *C. ubiquitum* was reported to be most common species in older animals (Guo et al., 2021; Mueller-Doblies et al., 2008; Wang et al., 2009; Ye et al., 2013). Other studies have however, found *C. ubiquitum* to be similarly distributed in lambs and adults (Santín et al., 2007; Wang et al., 2009). There are also geographic differences in the distribution of the three dominant species in sheep (Ryan et al., 2014); *C. parvum* is the dominant species in Europe, *C. xiaoi* is dominant in Australia, whereas *C. ubiquitum* appears to dominate in the Americas and Asia (Ye et al. 2013). Other species isolated in sheep worldwide have been reviewed by Hatam-Nahavandi et al. (2019) and include *C. hominis*, *C. andersoni*, *C. bovis*, *C. fayeri*, *C. scrofarum*, *C. suis* and *C. bovis*.

In goats, *C. ubiquitum*, *C. parvum* and *C. xiaoi* have been commonly reported to cause clinical disease or asymptomatic infection (Santin, 2020). Other species identified in goats albeit only sporadically have been reviewed, including *C. hominis*, *C. baileyi*, *C andersoni*, and rat genotype II (Hatam-Nahavandi et al., 2019). In South Africa, there appears to be no studies reporting the predominant *Cryptosporidium* species in sheep and goats and therefore such studies are warranted. Moreover, there are no studies which have investigated age-related *Cryptosporidium* species infection in neonatal sheep and goats in South Africa and this warrants investigation.

The transmission of *Cryptosporidium* occurs via the faecal-oral route with animals and people being infected from consuming oocyst-contaminated drinking water, feed or pastures (Innes et al., 2020). Contact with contaminated animals or people can be a route of infection (Dumaine et al., 2019). Water can harbour infective oocysts for long periods and is an important medium of transmission for *Cryptosporidium* species (Ojuromi & Ashafa, 2018). In countries like South Africa, the sharing of water bodies by humans and domesticated and wild animals, greatly increases the risk of surface water contamination by *Cryptosporidium* species shed from these sources (Aldeyarbi et al., 2016). Despite the many studies describing the transmission dynamics of *Cryptosporidium* in different parts of the world, in South Africa, however, these still remain relatively unexplored. It is important to identify all potential sources of infection in South Africa, as this may have a bearing on the prevention and control of cryptosporidiosis especially amongst communities involved in ruminant livestock production. In particular, the presence of *Cryptosporidium* species in dam water on ruminant livestock farms needs to be investigated as these have been shown to be a source of animal-animal transmission (Zahedi et al., 2020).

5. Accepted methodologies to be used

Procedures have been developed for the isolation and enrichment of *Cryptosporidium* genomic DNA directly from stool specimens (Guo et al., 2015; Li et al., 2014). This has been achieved by oocyst purification using a combination of sucrose flotation and immunomagnetic separation [IMS]. This was aided by removal of residual contaminants by hypochloride treatment of purified oocysts, DNA extraction and whole genome amplification (WGA) of the extracted DNA using commercial kits, and assessment of *Cryptosporidium* DNA quantity using qPCR analysis of WGA products (Guo et al., 2015). Comparative genomic analyses and genetic manipulation tools have been developed and are available for *Cryptosporidium* species (Fan et al. 2019; Vinayak 2020). Such tools have made it possible to characterise *Cryptosporidium* isolates and elucidation of the biological functions of candidate genes and proteins. These methodologies will improve understanding of the prevalence and transmission

of *Cryptosporidium* spp. in neonatal ruminants reared in various management systems and zoonotic transmission of *C. parvum* and *C. ubiquitum* in different geographic locations.

References

Abubakar, I., Aliyu, S. H., Arumugam, C., Usman, N. K., & Hunter, P. R. (2007). Treatment of cryptosporidiosis in immunocompromised individuals: systematic review and meta-analysis. *British Journal of Clinical Pharmacology*, 387-393.

Adams, R. B., Guerrant, R. L., Zu, S., Fang, G., & Roche, J. K. (1994). Cryptosporidium parvum infection of intestinal epithelium: morphologic and functional studies in an in vitro model. . *Journal of Infectious Diseases*, 170-177.

Aldeyarbi, H. M., Abu El-Ezz, N. T., & Karanis, P. (2016). Cryptosporidium and cryptosporidiosis: the African perspective. *Environmental Science and Pollution Research volume*, 13811–13821.

Anderson, B. C. (1998). Cryptosporidiosis in bovine and human health. *Journal of Dairy Sciences*, 3036-3041.

Bouzid, M., Hunter, P. R., Chalmers, R. M., & Tyler, K. (2013). Cryptosporidium Pathogenicity and Virulence. *Clinical Microbiology Reviews*, 115-134.

Carmena, D. (2010). Waterborne transmission of Cryptosporidium and Giardia: detection, surveillance and implications for public health . *Current Research Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, 3-14.

Castro-Hermida, J. A., Gonzalez-Waleta, M., & Mezo, M. (2007). Natural infection by Cryptosporidium parvum and Giardia duodenalis in sheep and goats in Galicia (NW Spain). *Small Ruminant Reseach*, 96-100.

Chako, C. Z., Tyler, J. W., Schultz, L. G., Chiguma, L., & Beerntsen, B. T. (2010). Cryptosporidiosis in People: It's Not Just About the Cows. *Journal of Veterinary Internal Medicine*, 37-43.

Chalmar, R. M., & Davies, A. P. (2010). Minireview: clinical cryptosporidiosis. *Experimental Parasitology*, 138-146.

Diaz, P., Navarro, E., Remesar, S., Garcia-Dios, D., Martinez-Calaibuig, N., Prieto, A., . . . Morondo, P. (2021). The Age-Related Cryptosporidium Species Distribution in Asymptomatic Cattle from North-Western Spain. *Animals*, 256.

Dinler, C., & Ulutas, B. (2017). Cryptosporidiosis in Ruminants: Update and Current Therapeutic Approaches. *American Journal of Animal and Veterinary Sciences*, 96-103.

Dumaine, J. E., Tandel, J., & Striepen, B. (2019). Cryptosporidium parvum. *Trends in Parasitology*, 485-486.

Esteban, E., & Anderson, B. C. (1995). Crypfosporldium murls: Prevalence, Persistency, and Detrimental Effect on Milk Production in a Drylot Dairy. *Journal of Dairy Science*, 1068-1072.

Foster, D. M., & Smith, G. W. (2009). Pathophysiology of Diarrhea in Calves. *Veterinary Clinics of North America: Food Animal Practice*, 13-36.

Gunasekera, S., Zahedi , A., O'Dea, M., King, B., Monis, P., Thierry, B., . . . Ryan, U. (2020). Organoids and Bioengineered Intestinal Models: Potential Solutions to the Cryptosporidium Culturing Dilemma. *Microorganisms*, 715.

Hatam-Nahavandi, K., Ahmadpour, E., Carmena, D., Spotin, A., Bangoura, B., & Xiao, L. (2019). Cryptosporidium infections in terrestrial ungulates with focus on livestock: a systematic review and meta-analysis. *Parasites & Vectors*, 453.

Innes, E. A., Chalmers, R. M., Wells, B., & Pawlowic, M. C. (2020). A One Health Approach to Tackle Cryptosporidiosis. *Trends in Parasitology, 36*(3), 290-202.

Mahfouz, M. E., Mira, N., & Amer, S. (2014). Prevalence and Genotyping of Cryptosporidium spp. in Farm Animals in Egypt. *The Journal of Veterinary Medical Science*, 1569-1575.

Ojuromi, O. T., & Ashafa, A. O. (2018). Cryptosporidiosis in Southern Africa: Review of prevalence and molecular epidemiology of a neglected disease. *Annals of Tropical Medicine and Public Health, 11*(4), 108-118.

Olson, M. E., O'Handley, R. M., Ralston, B. J., McAllister, T. A., & Thompson, R. A. (2004). Update on Cryptosporidium and Giardia infections in cattle. *Trends in Parasitology*, 185-191.

Paraud, C., & Chartier, C. (2012). Cryptosporidiosis in small ruminants. *Small Ruminant Research*, 93-97.

Ralston , B. J., McAllister, T. A., & Olson, M. E. (2010). Prevalence and infection pattern of naturally acquired giardiasis and cryptosporidiosis in range beef calves and their dams. *Veterinary Parasitology*, 113-122.

Robertson, L. J. (2014). Cryptosporidium as a Foodborne Pathogen. *SpringerBriefs in Food, Health and Nutrition*, 1-95.

Ryan, U., Fayer, R., & Xiao, L. (2014). Cryptosporidium species in humans and animals:current understanding and research needs. *Parasitology*, 1667-1685.

Samie, A., Hlungwani, A. H., & Mbati, P. A. (2017). Prevalence and risk factors of Cryptosporidium species among domestic animals in rural communities in Northern South Africa. *Tropical Biomedicine* , 636–647.

Samra, N. A., Jori, F., Caccio, S. M., Frean, J., Poonsamy, B., & Thompson, P. (2016). Cryptosporidium genotypes in children and calves living at the wildlife or livestock interface of the Kruger National Park, South Africa. *Onderstepoort Journal of Veterinary Research*, a1024.

Samra, N. A., Thompson, P. N., Jori, F., Frean, J., Poonsamy, B., du Plessis, D., . . . Xiao, L. (2013). Genetic Characterization of Cryptosporidium spp. in Diarrhoeic Children from Four Provinces in South Africa. *Zoonoses and Public Health*, 154-159.

Santin, M. (2020). Cryptosporidium and Giardia in Ruminants. *Veterinary Clinics: Food Animal Practice*, 223–238.

Smith, R. P., Clifton-Hadley, F. A., Cheney, T., & Giles, M. (2014). Prevalence and molecular typing of Cryptosporidium in dairy cattle in England and Wales and examination of potential on-farm transmission routes. *Veterinary Parasitology*, 111-119.

Thompson, 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* , 42.

Widmer, G., Klein, P., & Bonilla, R. (2007). Adaptation of Cryptosporidium oocysts to different excystation conditions. *Parasitology*, 1583-1588.

Wyatt, C. R., Riggs, M. W., & Fayer, R. (2010). Cryptosporidiosis in Neonatal Calves. *Veterinary Clinics of North America: Food Animal Practice*, 89-103.

Xiao, L. (2010). Molecular epidemiology of cryptosporidiosis: an update. *Experimental Parasitology*, 80-89.

Xiao, L., Herd, R. P., & McClure, K. E. (1994). Periparturient rise in the excretion of Giardia sp. cysts and Cryptosporidium parvum oocysts as a source of infection for lambs. *The Journal of parasitology*, 55–59.

Zahedi , A., Paparini, A., Jian, F., Robertson, I., & Ryan, U. (2016). Public health significance of zoonotic Cryptosporidium species in wildlife: Critical insights into better drinking water management. *International Journal of Parasitology. Parasites and Wildlife*, 88-109.

297 Zahedi, A., Ryan, U., Rawlings, V., Greay, T., Hancock, S., Bruce, M., & Jacobson, C. (2020).
298 Cryptosporidium and Giardia in dam water on sheep farms – An important source of
299 transmission? *Veterinary Parasitology*, 109281.
300