

# Clinical and morphological investigation in cryptosporidium enteritis in calves

**Candidate:** Shane Roger Ryan

**Department:** Department of Animal Hygiene, Herd-health  
and Veterinary Ethology

**University:** University of Veterinary Medicine, Budapest

**Supervisor:** Viktor Jurkovich, Senior Research Fellow,  
University of Veterinary Medicine, Center for Animal  
Protection Law, Analysis and Methodology

## Abstract

*Cryptosporidium* is an enteric protozoan parasite, which causes gastrointestinal infection in animals and humans. It has a worldwide prevalence. The most notable strain of the species is *Cryptosporidium Parvum*, which is the leading cause of cryptosporidiosis in preweaned calves. A systematic review of the literature review was performed to investigate the clinical and morphological investigation in cryptosporidium enteritis in calves. Searches were completed initially used the PubMed and MedLine search engines, with an aim to identifying studies of *Cryptosporidium* across multiple continents to explore its presentation and spread in calves. The review then explored methods of identification for the genus, including distinguishing between species. The results correlate with results from the individual papers with respect to transmission, infection progression and the use of molecular identification methods to differentiate between species.

## Összefoglaló

A *Cryptosporidium* egy bélben oldódó protozoa parazita, amely gastrointestinalis fertőzést okoz állatokban és emberekben. Világszerte elterjedt. A faj legjelentősebb törzse a *Cryptosporidium Parvum*, amely az elválasztott borjak cryptosporidiosisának vezető oka. A szakirodalmi áttekintés szisztematikus áttekintését a borjak cryptosporidium enteritisében végzett klinikai és morfológiai vizsgálat vizsgálatára végezték. A kereséseket kezdetben a PubMed keresőmotor segítségével fejezték be, azzal a céllal, hogy azonosítsák a *Cryptosporidium* több kontinensen végzett tanulmányait, hogy feltárják a borjakban való megjelenését befolyásoló változókat. A felülvizsgálat ezután feltárta a nemzetség azonosításának módszereit, beleértve a fajok megkülönböztetését is. Az eredmények korrelálnak az egyes tanulmányok eredményeivel az átvitel, a fertőzés progressziója és a fajok megkülönböztetésére szolgáló molekuláris azonosítási módszerek alkalmazása tekintetében.

# Contents

Abstract .....	1
Összefoglaló.....	1
Abbreviations .....	3
Introduction.....	3
Literature review .....	4
Cryptosporidium lifecycle.....	4
Species of Cryptosporidium.....	6
Methods of Diagnosis .....	6
Enzyme immune assays .....	6
Molecular detection of cryptosporidium.....	7
Infective Dose and transmission .....	7
Materials and Methods.....	8
Results .....	10
Parklands Vets Dungannon .....	11
Discussion .....	13
Summary .....	15
Bibliography.....	17
Acknowledgements.....	20
Statements .....	20

## Abbreviations

C. : Cryptosporidium

DNA: Deoxyribonucleic Acid

EIA: Enzyme Immunoassay

PCR: Polymerase Chain Reaction

RFLP: Restriction Fragment Length Polymorphism

## Introduction

Cryptosporidium is a protozoal parasite of worldwide prevalence affecting the gastrointestinal tract of multiple animals, particularly pre-weaned calves. A 2022 paper by Adkins, P in the *Veterinary Clinics of North America: Food Animal practice* (Adkins et al, 2022) estimates that there are forty named species of Cryptosporidium. Four of these species affect cattle – *C. Andersoni* primarily affects adult cattle, host-specific species *C. bovis* and *C. Ryanae*, are most prevalent in weaned calves, and *C. Parvum*, a zoonotic species is the primary strain causing clinical disease in neonatal calves. The parasite replicates in parasitophorous vacuoles within intestinal epithelial cells. The most common gastrointestinal presentation of cryptosporidium in neonatal calves is diarrhoea, which can progress to severe illness and even death. It is estimated that 30-50% of neonatal calf diarrhoea worldwide is caused by the Cryptosporidium genus (Fayer, R 2004). Although the disease is self-limiting in many cases, the neonatal host sheds a huge number of oocytes causing the disease to spread rapidly where they can remain infective for a period of years, in favourable environmental conditions (Wells et al, 2015). This spread is particularly impactful in neonatal calves due to the repeat usage of designated calving areas by livestock owners where ingestion of oocyte containing faeces occurs. The prevalence in this subset of cattle can have significant financial impact on livestock owners, both from cost of treatment and also neonatal calf mortality (Trotz-Williams L, et al 2005). These oocytes are also very transmissible via water and therefore pose a substantial risk to human health, regularly resulting in interruptions to human water supply where they are detected. Additionally, in 2014, Smith et al identified a sub-type of *C. parvum* known to have been detected in human populations in faecal samples taken from birds – it is hypothesised that birds may inject oocytes from one farm

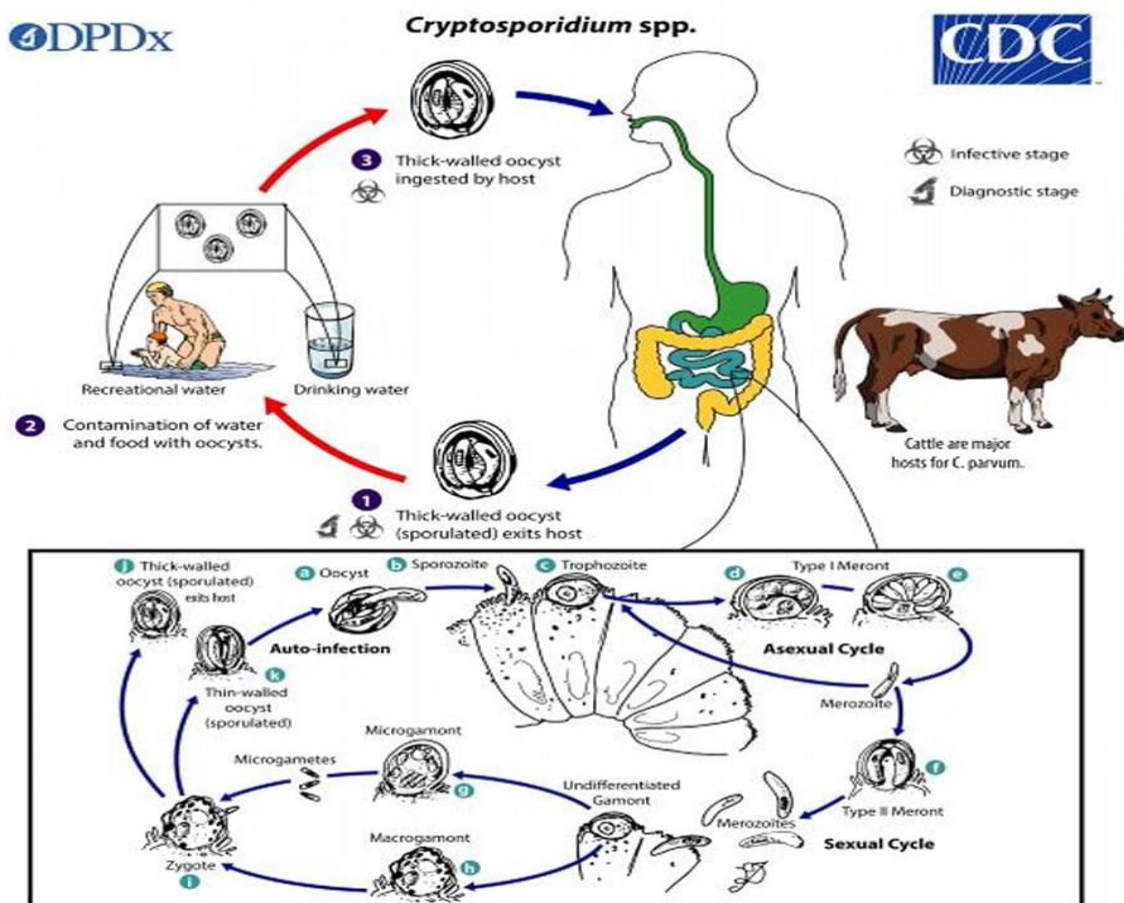
and transfer to other farms, or to non-farm areas which humans may interact with (*Smith R et al, 2014*) In humans, cryptosporidiosis is presently identified as the most frequent zoonotic cause of parasitic diarrhoea, which has proven to be particularly severe in immunocompromised individuals and infants in both developing and developed countries (*Razakandrainibe, R et al, 2018*) By understanding both the clinical and morphological presentation, the presence of cryptosporidium species in a neonatal calf can result in a) more efficient treatment reducing incidence of mortality, b) more rapid identification of locations where the oocytes are present (e.g. calving areas) and reduction of contamination of water preventing transmission to humans (*Xaio Peng et al, 1997*).

## Literature review

### Cryptosporidium lifecycle

Cryptosporidium has the ability to complete its entire lifecycle within a single host, which makes it a monoexenous organism. It exists in the environment protected as thick walled oocytes. This makes them resistant to several environmental factors e.g. high/low temperature and are therefore well adapted to multiple conditions. This means they can remain effective for months/years. Once an oocyte is ingested by a host organism it experiences its "ideal" conditions, and is comfortable to burst releasing four sporozoites, which attach themselves to the epithelial cells of the host's gastrointestinal tract via the villi tips. According to research carried out by MSD – the eggs of cryptosporidium parasites that are transmitted from host are transmitted through contact with contaminated water and sometimes food. Transmission and scanning microscopies of the ileal mucosa from twelve calves infected with Cryptosporidium showed sporozoites free in lumen and adhered to the epithelium. Many stages of cryptosporidium exist, including mezoites, trophozoites, schizonts, gametes and oocytes and all stages have been seen attached to villi tips within a host's gastrointestinal tract. Evidence of attachment and development of cryptosporidium on the villi were seen in a number of presentations including absence, disintegration at micro villi or disorganisation of the terminal web. Once the sporozoites attach to the host's epithelial cells at the ileocecal junction, they invade and become engulfed by the cells forming a vacuole. A unique feature of cryptosporidium is that it also forms a feeder organelle. When this feeder organelle is created, the sporozoite becomes more nourished, more spherical in shape and progresses to the next development stage, trophozoite. The

parasite can now replicate and reproduce by asexual reproduction through the formation of merozoites. Two types of meronts exist – Type One, which releases six merozoites and can immediately re-infect by invading neighbouring cells, and Type Two which release four merozoites which start off the sexual reproduction pathway. The merozoites released from this pathway invade host cells and differentiate into either macrogamonts or microgamonts. Differentiations between these using the electron microscope is very difficult, as all resemble the merogametes. Reproduction occurs via meiosis and the zygote divides into four sporozoites and the oocysts that are developed may be released and re-infect the host. Some of the parasite’s membranes had spike like protrusions when a cross-section of the oocyst was examined. A comparative investigation carried out concluded that cryptosporidium seen in calves and in guinea pigs display similar morphology. Using a scanning electron microscope, the presence of Cryptosporidium, atrophy of the fingerlike villi can be seen. Many Cryptosporidium of various sizes adhere to the surface as white nodules. Black circles are craters left when the parasites leave the structures.



**Figure 1.** Image from the Centers for Disease Control and Prevention, Global Health, Division of Parasitic Disease and Malaria.

## Species of Cryptosporidium

There are twenty-eight known species of cryptosporidium. There have been many genotypes identified, many of which are zoonotic. While many of the species are host specific, there are certain ones which are adaptable to many hosts e.g., *C. parvum* and *C. ubiquitum*. The four most commonly found species in cattle are *C. parvum*, *C. bovis*, *C. ryanae* and *C. andresi*. *C. parvum* is a common cause of diarrhoea in calves and cryptosporidial oocytes have been detected in faeces of 70% of one- to three-week-old dairy calves. It is a zoonotic parasite and investigations have been carried out on the possible transmission from direct contact between infected calves and humans. Extensive sub-typing of *C. parvum* found in infected dairy calves has been performed in Israel. *C. parvum* and *C. bovis* are responsible for over 90% of bovine infections (Feng et al, 2018, Vidmer Et al, 2020). Upon the infection of *Cryptosporidium parvum*, particularly in neonatal calves, intra-herd prevalence extends up to 100%. *C. bovis* and *C. ryanae* are primarily found in post-weaned calves while *C. andresi* is found in adult cattle. Human cryptosporidium infection is primarily caused by *C. parvum* and *C. hominis* (*C. hominis* which can also potentially affect cattle). Both calves with active diarrhoea and healing calves are considered major reservoirs for human infections. It is very difficult to differentiate between oocyte species using a microscope as they are all very similar in shape and size. With regards to sheep and goats, these are predominantly infected by *C. parvum* (Xiao Peng et al, 1997), *C. bovis* and *C. ubiquitum*.

## Methods of Diagnosis

### Enzyme immune assays

Enzyme immune assays are very commonly used in the United Kingdom (UK) for the detection of *Cryptosporidium* and is very sensitive in the detection of *C. parvum* (Xiao Peng Et al, 1997). The enzyme immunoassay is based on a monoclonal antibody to a genus specific *Cryptosporidium* antigen for detecting *Cryptosporidium* in faecal samples. Enzyme immunoassay (EIA) uses rabbits anti-cryptosporidium polyclonal antibody to capture the *Cryptosporidium* antigen from the faecal sample.

## Molecular detection of cryptosporidium

The species of *Cryptosporidium* can be differentiated using molecular methods. Firstly, the DNA is extracted from the oocyte and the sample then undergoes a PCR and the genes of interest are amplified. There are two types of PCR available, standard and Real-time PCR. In the standard PCR, only one pair of forward and reverse primers are used to amplify a gene or region of gene. The amplified regions are then separated using gene electrophoresis on an agarose gel and are visualised by staining with a stain such as bromide. This stain will bind to the nucleic acid which can then be viewed under UV light. Restriction Fragment Length Polymorphism (RFL-P) is a technology which is used to identify the species of cryptosporidium. It is based on the ability of the restriction enzymes to digest the PCR products into fragments. The fragments are then visible on agarose gel. This produces varying band patterns and from this species identification can be attempted (Spano et al, 1997, Sulaimon et all 1999). Real-time PCR is also used and is considered the gold standard for the detection of cryptosporidium. It is the most accurate in detecting a small number of oocytes at a given time, and is reported to be able to detect as few as two oocytes.

## Infective Dose and transmission

The dose of *Cryptosporidium* required to cause infection is dependent on the immune status of the host and on the strain or species of *Cryptosporidium* (Zintl A, Mulcahy G et al, 2006). The infective dose is deemed to be low due to the rapid cycle of the parasite and it's ability to auto-infect host cells. A study performed on 29 healthy volunteers reported a median value of 132 oocytes (DuPont HL, Chappell CL et al, 1995), however mathematical models suggest that as few as one oocyte is sufficient to trigger an infection in some cases (Haas CN, Rose JB et al, 1994). One study in calves infected with *C. parvum* found that the infective dose for oocyte shedding only was 5.8 oocytes, scour only was 9.7 oocytes and for calves with a combination of both was 16.6 oocytes. (Zambriski et al, 2013).

Infected hosts shed a large number of oocytes, which are immediately infective, which means transmission is quick, especially in young calves, where farms have dedicated birthing areas. Depending on the infective dose, calves will start releasing oocytes in feces



four to nine days after initial infection (*Faubert et al, 2000*). Shedding oocytes continues for approximately six to fourteen days, a time period which stays consistent regardless of infective dose (*Zambriski et al, 2013*). The quantity of oocytes shed during this period, however, will vary, with younger calves and more infected calves shedding more than others during the shedding period.

Transmission to humans has been reported following interaction of humans with infected livestock e.g. veterinarians treating animals on a farm or persons visiting a petting farm. This is deemed zoonotic transmission.

Transmission can also occur from contaminated water sources – this is seen particularly where there is a high agricultural presence near a water supply. Transmission of *C. hominis*, which has been detected in animals, including calves, but with a very low prevalence is also commonly water borne. Contamination of water with *Cryptosporidium* is difficult to eradicate using traditional water control methods e.g., temperature, filtration or chlorination due to the small, durable nature of *Cryptosporidium*.

## Materials and Methods

In this study, PubMed and Medline were searched for relevant articles published between January 1, 2013 and October 31, 2023. The referenced articles in the selected papers were also reviewed, and if deemed relevant, were included in the review. Only results written in the English language were selected. Studies were required to have a minimum sample size of thirty to be considered for inclusion. Studies selected were those based on calves, as they are the animal in scope of this literature review. Studies which did not differentiate a) the species of *Cryptosporidium* identified and b) the method of DNA isolation and analysis were also excluded, but may be referenced in this review if used in gathering supporting information.

According to a study carried out in North-Western Spain, all farms in this study were located in the Galicia area. The study was carried out here due to its extensive farming nature along with having the highest number of dairy cattle in the country. A total of 594 faecal samples were collected from a combination of 86 dairy herds and 60 beef herds. The herds that were chosen for the examination were those that were included in the Cattle

health and defence associations so therefore were implemented in a sanitary programme. The samples were taken by veterinarian and it only included farms where no diarrhetic outbreaks were registered in suckling calves in the 12 months prior to sampling and no preventative treatments were used against cryptosporidium. All animals tested appeared healthy and samples were taken directly from rectum. All of the individual samples collected were scored according to the Ireland-Perry R.L Scale (*Ireland-Perry R.L., Stallings C.C 1993*). All samples appeared to be firm and non-diarrhetic. All of the animals ages were recorded as per official documentation and ranked from 2 days to 16.8 years. A series of five groups were formed according to age and were numbered accordingly. 108 calves less than one-month which will be referred to as group one, 62 pre weaned calves aged 2-12 months. Which will be referred to as group 2. A total of 96 post weaned calves from 2-12 months which will be referred to as group 3. A total of 116 yearlings and 212 older than two years which will be referred to as group 4 and group 5 respectively. All faecal samples that were collected were stored at 4 degrees Celsius and tested within 48 hours after collection (*Castro-Hermida J.A., García-Prevedo I, 2011*)

The samples were firstly concentrated from 2 g of the faecal sample using a diaphasic sedimentation technique. Three cycles of freeze thaw were carried out at -196 degrees Celsius for one minute and 100 degrees Celsius for 7 mins. From this they were able to extract cryptosporidium DNA using a commercial kit and the purified DNA was stored at minus 20 degrees Celsius.

Nested PCR techniques was then used to detect the presence of cryptosporidium DNA. The *Cryptosporidium parvum* was characterised at the GP 60 gene.

In a study by Atwal H.K et al in 2022, four different assays for detecting *Cryptosporidium parvum*, a protozoan parasite responsible for diarrhea in calves were assessed (*Atwal H.K et al, 2022*). The parasite spreads through contaminated water and food, posing a risk to both cattle and humans. The study aimed to assess the agreement, ease of procedure, and cost-effectiveness of Kinyoun acid-fast stain direct smear, Crypt-a-Glo fluorescent antibody test, Xpect lateral flow immunoassay, and PCR methods. Results from 62 out of 74 samples were in agreement across all tests. The Kinyoun acid-fast stain direct smear emerged as the most efficient and cost-effective method for routine *Cryptosporidium* testing in calves. (*Santín M, et al, 2020*)

The study analyzed 74 fecal samples from calves using Kinyoun acid-fast stain direct

smear, Crypt-a-Glo fluorescent antibody test, Xpect lateral flow immunoassay, and PCR. The Fleiss kappa value was calculated to determine the agreement among tests. The cost and hands-on time per test were also assessed.

## Results

A total of 3000 articles were identified from preliminary screenings of articles and their referenced articles on PubMed and Medline. 84 Relevant results were selected from PubMed and 39 results were selected from Medline. Duplicates were identified between the search engines. These were counted in the PubMed results, and excluded from the Medline results. Preliminary screening involved reading the title, and if not immediately obvious, the abstract of the journal article in order to ascertain its relevance. Only research papers published in journals were included, items including but not limited to letters were excluded from this review. While there was a large variance in the prevalence of *Cryptosporidium* in the calf populations of different countries, there was consistency in the species of *Cryptosporidium* seen in calves when analysed by age groups across various geographical regions with *C. parvum* the predominant species in neonatal calves with *C. bovine* and *C. ryanae* more prevalent in older calves. The search period was short in order to reflect the use of the most cutting-edge identification methods to differentiate between the morphological presentation of the species as several older papers noted difficulties difficulty differentiating the oocytes in their samples.

From the study carried out in Galacia, cryptosporidium DNA was detected in 99 of the samples from a total 594 samples. It was an interesting study as it allowed for the identification of 6 different species of cryptosporidium. *Cryptosporidium parvum* and *Cryptosporidium bovis* were the most prevalent with 42 out of 99 and 36 out of 99 being found. Among the other species being found were *C. ryanae* (10 found) , *C. occultus* (7), *C. andersoni* (2) and finally *C. Xiao* (1)

This was a very interesting study as it showed that the level of cryptosporidium that effected bovine species decreased with age. In this study it stated that 28.7 percent of the positive cases were discovered in the calves examined that were aged less than one month old. It was also discovered that *C. parvum*, *C. bovis* and *C.ryanae* were found in all ages but the *C.parvum* was predominant in the youngest calves that were examined. To summarise the

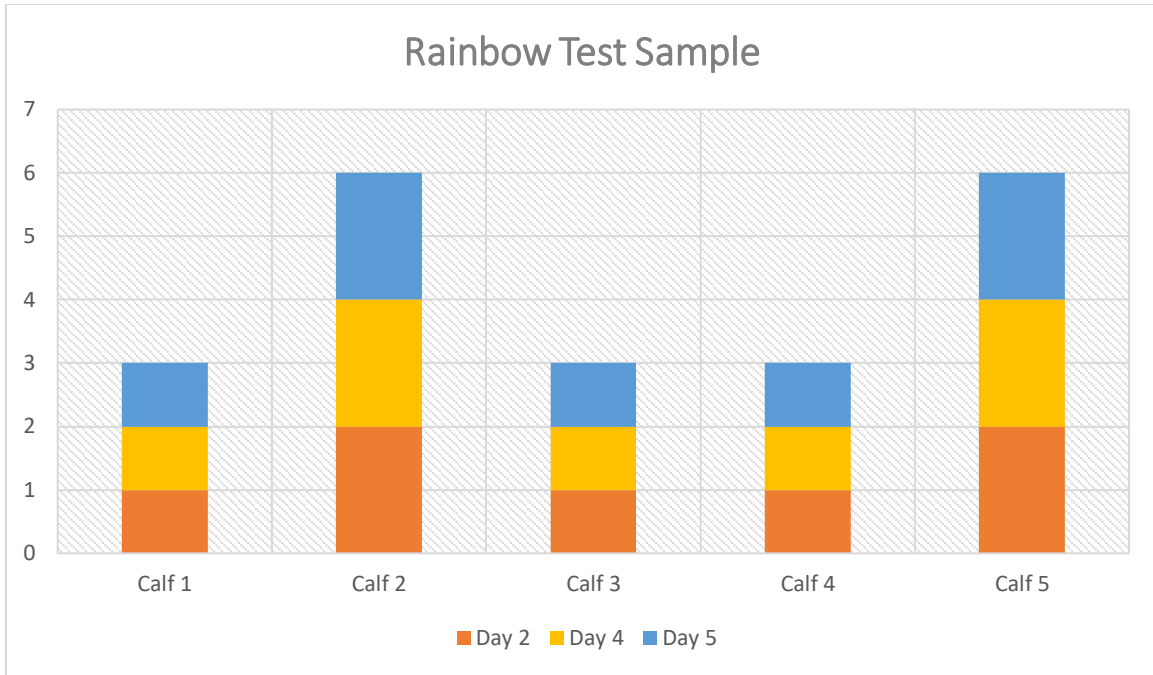
results, it was discovered that suckling calves were mostly affected by cryptosporidium parvum whilst *C. bovis* was predominately affecting calves aged from 1 to 24 months. The species *Cryptosporidium Andersoni* was detected in adult cattle only and only in sporadic occasions (*Ma J, Li P et al, 2015*).

In the test comparing of assays - Results from 62 samples were consistent across all assays, indicating a high level of agreement. The Kinyoun acid-fast stain direct smear was found to be the most reliable and cost-effective method, with advantages such as simplicity and low cost (*Murray PR, et al, 2003*). The Xpect lateral flow immunoassay offered quick results but at a higher cost. Crypt-a-Glo and PCR methods were relatively more time-consuming and less cost-effective (*Quinn PJ, et al, 1994*)

### Parklands Veterinary Group, Dungannon

While undertaking the systematic review of the literature, an opportunity presented to perform cryptosporidium testing in partnership with the Parklands Veterinary group, based in Dungannon in Northern Ireland.

Faecal samples from dairy calves were collected and examined. These samples collected directly from the rectum of calves avoiding any side contamination. These samples were collected in a sterile test pot provided by the veterinary practice. A series of 5 samples were taken from each calf from a sample of 10 dairy bred calves over a period of 5 days. The Rainbow Calf Scour Diagnostic Faecal Test Kit supplied by MSD was used in the investigation. This is a universal test that is designed to detect Rotavirus, Coronavirus, *E. Coli* F5, *Cryptosporidium Parvum* in the subject's stool. The sample was diluted in the sample tube and homogenised. A period of 10 minutes was required for each calf's test.



**Figure 2:** Graphical representation of Positive Rainbow scour tests.

As per the results- a faecal sample was taken from each calf for the 5 Days of the test. Out of the sample calf 2 and calf 5 sample tested positive on all occasions for Cryptosporidium.

Calf 1.	Standing and looked alert	Complete feed	solid
Calf 2.	Cannot stand – High Temperature	Animal refuses to feed.	liquid
Calf 3.	Listless – less alert than calf 1.	Partial feed	Semi-solid
Calf 4.	Unsteady on feet	Refused to eat	Semi-solid
Calf 5.	Needed assistance to rise	Partial feed	Liquid

**Figure 3:** Scoring system used to score calves demeanour daily

## Discussion

This research comprised of a literature review of journal articles about *Cryptosporidium* infection in calves, both the clinical presentation (including transmission and multiplication of the species in host) and the morphological presentation (including methods of identification and characterisation). The results speak to a consistent pattern with respect to *Cryptosporidium* infection, particularly which species will affect which age group of bovines. *C. parvum* the most likely to affect young calves, and *C. bovis* and *C. ryanae* in older calves.

In reviewing the available literature, some potential risks to summaries presented have been identified. Firstly, while this literature review aimed to explore a large geographical spread by selecting articles from multiple countries and continents, it was noted that most studies themselves are location specific – this is likely due to the nature of sample collection (faeces) and the location of large farms in relation to laboratory testing locations (as farms are generally rural). While the findings of the country specific studies are aligned, it is noted that differences in DNA extraction, sample collection, storage or handling may mean that a like for like comparison may have flaws.

Secondly, as referred to earlier, a large subset of studies reported that due to the similar morphological presentations of the different *cryptosporidium* species, it is likely that underreporting/overreporting of oocytes of different species occurred. This literature review period reduced the time span for study inclusion to offset this effect, but it is acknowledged that molecular identification, while significantly better than microscopy alone, is still evolving.

Infection with *cryptosporidium* is common in young calves (*de Graaf et al. 1999*). From my research it is evident that calves become infected with *cryptosporidium* from a very young age with most detrimental effects seen in those calves infected immediately at birth.

From the study carried out in Galicia, the prevalence of *cryptosporidium* both symptomatically and asymptotically is high. The prevalence of *cryptosporidium* is significantly high in calves under one month old and decreases with age. This protozoa infection is very common to infect younger calves and this is due to their immune system being more easily infected due to its naïve nature (*Ares-Mazás M.E et al, 1999*). This study

also makes the hypothesis that the ability of the protozoa cryptosporidium to affect the younger calves more commonly maybe due to the build-up of immunity from being exposed to different strains of cryptosporidium at lesser pathogenic levels in the early period of the calf's life. It also makes the claim that a number of other studies have been performed that have found that in the USA, Brazil, China and Japan, the occurrence of cryptosporidium in healthy cattle over 2 years old is less than 10 percent (*Fayer R et al, 2007*) Prevalence of Cryptosporidium species and genotypes in mature dairy cattle on farms in eastern United States compared with younger cattle from the same locations.

There is also a level of public health implications as in some periods it has been proven that some cryptosporidium species detected in cattle have been found in human's samples too. The species *C. parvum* is said to be the most important crypto species in humans (*Ryan U et al, 2014, Chalmers R.M et al, 2009*). *C. andersoni* has also been detected in humans but it is considered to be of minor significance compared to that of *C. parvum*. (*Šlapeta J. et al, 2013, Santín M. et al, 2020*). Taking all of this into account, asymptomatic cattle in particular the calves under one month old could be the zoonotic link of cryptosporidium in humans. Therefore, people in close contact with cattle should do so with extreme caution taking all hygienic measure to avoid getting infected.

To comment on the results of the comparison of assays study, The high agreement among tests suggests their reliability in detecting *Cryptosporidium parvum* in calf fecal samples. The Kinyoun acid-fast stain direct smear stood out due to its simplicity, low cost, and reasonable accuracy. However, the subjective nature of interpretation in some assays calls for caution. Considering the lack of a gold standard test, this comparative analysis provides valuable insights for choosing an appropriate detection method.

The study emphasizes the importance of accurate and cost-effective detection methods for *Cryptosporidium parvum* in calves. Among the tested assays, the Kinyoun acid-fast stain direct smear proves to be the most reliable choice for routine testing. This research provides valuable guidance for veterinarians and producers, promoting effective parasite control measures in cattle farming and minimizing the risk of zoonotic transmission to humans.

With regards to the study carried out in Parklands Vets, it must be noted that this investigation only identified whether cryptosporidium was present or not. This testing method used is most sensitive for cryptosporidium parvum – seemingly ignoring other strains of the disease to a degree. Also, the sample size that was used was quite small but this was the maximum number of calves available to test on the farm in question. Other than this, the test appeared to be consistent with other research carried out in other studies indicating the calves in the neonatal period of their life are most sensitive to becoming infected to the disease.

## Summary

Calves from day 5-35 are susceptible to infection with *C. Parvum*, however it is most prevalent in calves in their second week of life. The clinical presentation of this infection is persistent diarrhoea, which is extremely difficult to manage. Internally, the infection causes damage to the lining of the gastrointestinal wall which limits the calf's ability to absorb nutrients. This takes a significant toll on the calf, resulting in lethargy and reduced liquid intake, resulting in dehydration. (*O'Connor, 2017*). In some cases, a fever may be present. Appetite of the calf varies according to the severity of the illness. Faeces varies from pasty to watery in consistency, pale yellow, yellow or greenish in colour and may contain blood and/or mucus (*F Mahmoud et al, 2016*). In 2020, Lichtmannsperger et al documented a correlation between liquid/watery diarrhoea and young age (mean 14.4 days old, median 10 old) with the presence of *C. parvum* (Lichtmannsperger et al, 2020). While the majority of cases self-resolve, the combination of malnutrition and diarrhoea result in weight loss and adverse financial impact for livestock owners.

The morphology of the *Cryptosporidium* genus proves invaluable to methods of its identification, however it must be acknowledged that distinguishing species of the genus proves challenging due to the strong similarities, particularly at microscopic level (*Ryan et al, 2014*). However, continuous advances in molecular identification have been critical for species identification. In 2019, Lichtmannsperger et al documented a process for DNA extraction from oocytes present in faeces of calves (*Lichtmannsperger et al, 2019*). In 2020, Polymerase Chain Reaction (PCR) is one such molecular identification test available. The 18S PCR assay, a cryptosporidium specific assay and the gp60 PCR assay specifically for



*C. Parvum* were utilised with a high level of success by Lichtmannsperger et al in 2022 (Lichtmannsperger et al, 2020). The categorisation is based on differences in the sequence in the non-repeat region of the gp60 gene, which can be done using Basic Local Alignment Search Tool (BLAST) search where variation in the trinucleotide repeat region can be counted (Enbom, T et al, 2023). The author notes, that gp60, while effective in the identification of *C. Parvum* likely results in under-reporting of the presence of other species in the Cryptosporidium family, particularly *C. ryanae* and *C. bovis*. There is consistency in the literature with respect to the subtypes of *C. Parvum* identified, with IIaA15G2R1 being the most commonly reported, and IIaA17G1R1 also appearing regularly (Smith R et al, 2014, Chalmers et al, 2011). Correlations have been identified between a specific sub-type and a geographical location.

The study carried out in north-western Spain, proves that cryptosporidium proves the concept of the importance of age in correlation with the potency of cryptosporidium. The younger the calf the higher the probability of it taking infection. It also outlined the public health concern that is associated with the zoonotic importance of certain species of cryptosporidium.

## Bibliography

- BARKER, F.K.; CARBONELL, P.L.: *Cryptosporidium agni* sp.n. from lambs and *Cryptosporidium bovis* spa. from calf with observations on the oocyst. *Z Parasitenkd* 44:289- 298. 1974
- POHLENZ, J.; MOON, H.W.; CHEVILLE, N.F.; BEMRICK, W.J.: Cryptosporidiosis as a probable factor in neonatal diarrhea of calves. *J Am Vet Med Assoc* 172:452-457, 1978
- KELLEY, R.O.; DEKKER, R.A.F.; BLUEMINK, J .G.: Thiocarbohydrazide-mediated osmium binding: a technique for protecting soft biological specimens in the scanning electron microscope in *Principles and Techniques of Scanning Electron Microscope. Biological Applications*. ed. Hayst; pp. 34-44. vol. 4. Von Nostrand Reinhold. New York, 1975
- Ma J., Li P., Zhao X., Xu H., Wu W., Wang Y., Guo Y., Wang L., Feng Y., Xiao L. Occurrence and molecular characterization of *Cryptosporidium* spp. and *Enterocytozoon bieneusi* in dairy cattle, beef cattle and water buffaloes in China. *Vet. Parasitol.* 2015;207:220–227. doi: 10.1016/j.vetpar.2014.10.011
- Santín M. *Cryptosporidium* and *Giardia* in ruminants. *Vet. Clin. N. Am.–Food A.* 2020;36:223–238. doi: 10.1016/j.cvfa.2019.11.005.
- Silva F.M., Lopes R.S., Araújo-Junior J.P. Identification of *Cryptosporidium* species and genotypes in dairy cattle in Brazil. *Rev. Bras. Parasitol. Vet.* 2013;22:22–28. doi: 10.1590/S1984-29612013005000010
- Chalmers R.M., Katzer F. Looking for *Cryptosporidium*: The application of advances in detection and diagnosis. *Trends Parasitol.* 2013;29:237–251. doi: 10.1016/j.pt.2013.03.001
- Šlapeta J. Cryptosporidiosis and *Cryptosporidium* species in animals and humans: A thirty colour rainbow? *Int. J. Parasitol.* 2013;43:957–970. doi: 10.1016/j.ijpara.2013.07.005.
- Ryan U., Zahedi A., Papparini A. *Cryptosporidium* in humans and animals-a one health approach to prophylaxis. *Parasite Immunol.* 2016;38:535–547. doi: 10.1111/pim.12350
- Díaz P., Quílez J., Chalmers R.M., Panadero R., López C., Sánchez-Acedo C., Morrondo P., Díez-Baños P. Genotype and subtype analysis of *Cryptosporidium* isolates from calves and lambs in Galicia (NW Spain) *Parasitology.* 2010;137:1187–1193. doi: 10.1017/S0031182010000181
- Fayer R., Santín M., Trout J.M. Prevalence of *Cryptosporidium* species and genotypes in mature dairy cattle on farms in eastern United States compared with younger cattle from the same locations. *Vet. Parasitol.* 2007;145:260–266. doi: 10.1016/j.vetpar.2006.12.009
- Ireland-Perry R.L., Stallings C.C. Fecal Consistency as Related to Dietary Composition in Lactating Holstein Cows. *Journal of Dairy Science,* 1993 76(4): 1074-1082
- Castro-Hermida J.A., García-Presedo I., Almeida A., González-Warleta M., Correia Da Costa J.M., Mezo M. *Cryptosporidium* spp. and *Giardia duodenalis* in two areas of Galicia (NW Spain) *Sci. Total Environ.* 2011;409:2451–2459.
- Adkins, P. R., 2022. Cryptosporidiosis. *Veterinary Clinics of North America: Food Animal Practice,* 38(1), pp. 121-131.
- al, d. G. D. e., 1999. A review of the importance of cryptosporidiosis in farm animals. *Int J Parasitol,* 29(8), pp. 1269-1287.

- al, M.-A. M. e., 1999. Oocysts, IgG levels and immunoblot patterns determined for *Cryptosporidium parvum* in bovine examined during a visit to a farm (northeastern Spain). *Veterinary Parasitology*, 81(3), pp. 185-193.
- Atwal H.k, Z. E., 2022. A comparison of assays for the detection of *Cryptosporidium parvum* in the feces of scouring calves. *J Vet Diagn Invest.* , Volume 34, pp. 284-287.
- Beth Wells, H. S. E. H., 2015. Prevalence, species identification and genotyping of *Cryptosporidium* from livestock and deer in a catchment in the Caringorms with a history of a contaminated public water supply. *Parasites and Vectors*, p. 66.
- Chalmers R.M, S. R. H. S., n.d. Zoonotic linkage and variation in *Cryptosporidium parvum* from patients in the United Kingdom. *Parasitol Res*, Volume 108, pp. 1321-1325.
- DuPont HL, C. C., 1995. The infectivity of *Cryptosporidium parvum* in healthy volunteers. *The New England Journal of Medicine*, Volume 332, pp. 855-859.
- Enbom T, S. K. L. S., 2023. *Cryptosporidium parvum*: an emerging occupational zoonosis in Finland. *Acta Veterinaria Scandinavica*, 65(25).
- Fatma Mahmoud, T. E.-a., 2016. Conventional and molecular diagnosis of cryptosporidiosis in calves. *Assiut Veterinary Medical journal*, 62(151), pp. 12-21.
- Faubert GM, L. Y., 2000. Natural transmission of *Cryptosporidium parvum* between dams and calves on a dairy farm. *Journal of Parasitology*, Volume 86, pp. 495-500.
- Fayer, R., 2004. *Vet Parasitol.*
- Haas CN, R. J., 1994. Reconciliation of microbial risk models and outbreak epidemiology: The case of the Milwaukee outbreak. *Water Quality*, pp. 517-523.
- Lichtmannsperger K, H. B. J. A., 2019. Molecular Characterization of *Giardia intestinalis* and *Cryptosporidium parvum* from calves with diarrhoea in Austria and evaluation of point of care tests. *Comp Immunol Microbiol infect Dis*, Volume 16.
- Lichtmannsperger, K. H. J., 2020. *Crptosporidium parvum*, *Cryptosporidium ryanae* and *Cryprosporidium bovis* in samples from calves in Austria. *Parasitology research*, Volume 119, pp. 4291-4925.
- Murray P.R, B. E., 2003. Manual of clinical microbiology 8th Edition. *Diagn Microbiol Infect Dis*, 47(4), pp. 625-626.
- O'Connor, A., 2017. *The curse of cryptosporidia*. [Online]  
Available at: <http://www.Teagasc.ie>  
[Accessed 30 October 2023].
- Quinn PJ, e. a., 1994. *Clinical Veterinary Microbiology*. 1 ed. s.l.:Mosby.
- Razakandrainibe R, D. E. H., 2018. Common occurrence of *Cryptosporidium ovinis* in asymptomatic and symptomatic calves in France. *PLoS Negl Trop Dis*, 12(3).
- Smith RP, C.-H. F. C. T., 2014. Prevalence and molecular typing of *Cryptosporidium* in dairy cattle in England and Wales and examination of potential on-farm transmission routes. *Veterinary Parasitology*, Volume 204, pp. 111-119.

Trotz-Williams Lisa, W. M. S. M. D., 2005. Multiattribute evaluation of two single sets for the detection of *Cryptosporidium parvum* in cattle faeces. *Veterinary Parasitology*, pp. 15-23.

U Ryan, F. R. X. L., 2014. *Cryptosporidium* species in human and animals: current understanding and research needs. *Parasitology*, pp. 1667-1685.

Xaio Peng, A. F. J. A., 1997. Genetic Polymorphism among *Cryptosporidium parvum* isolates: evidence of two distinct human transmission cycles. *Emerging Infectious Diseases*, Volume 3, pp. 567-573.

Zambriski, J. N. D., 2013. *Cryptosporidium parvum*: Determination of ID50 and the dose response relationship in experimentally challenged dairy calves. *Veterinary Parasitology*, Volume 197, pp. 104-112.

Zintl A, M. G., 2006. An Irish perspective on *Cryptosporidium*. Part 1. *Irish Veterinary Journal*, 59(442).

## Acknowledgements

The author recognises the support of Dr. Jurkovich as the supervisor for this literature review and the School of Veterinary Medicine.

## Statements

### HuVetA

#### ELHELYEZÉSI MEGÁLLAPODÁS ÉS SZERZŐI JOGI NYILATKOZAT\*

**Név:** Shane Roger Ryan

**Elérhetőség (e-mail cím):** shaneryan526@gmail.com

**A feltöltendő mű címe:** Clinical and morphological investigation in cryptosporidium enteritis in calves

**A mű megjelenési adatai:** Not published

**Az átadott fájlok száma:** 01 Documents

---

Jelen megállapodás elfogadásával a szerző, illetve a szerzői jogok tulajdonosa nem kizárólagos jogot biztosít a HuVetA számára, hogy archiválja (a tartalom megváltoztatása nélkül, a megőrzés és a hozzáférhetőség biztosításának érdekében) és másolásvédett PDF formára konvertálja és szolgáltatssa a fenti dokumentumot (beleértve annak kivonatát is).

Beleegyeznek, hogy a HuVetA egynél több (csak a HuVetA adminisztrátorai számára hozzáférhető) másolatot tároljon az Ön által átadott dokumentumból kizárólag biztonsági, visszaállítási és megőrzési célból.

Kijelenti, hogy az átadott dokumentum az Ön műve, és/vagy jogosult biztosítani a megállapodásban foglalt rendelkezéseket arra vonatkozóan. Kijelenti továbbá, hogy a mű eredeti és legjobb tudomása szerint nem sérti vele senki más szerzői jogát. Amennyiben a mű tartalmaz olyan anyagot, melyre nézve nem Ön birtokolja a szerzői jogokat, fel kell tüntetnie, hogy korlátlan engedélyt kapott a szerzői jog tulajdonosától arra, hogy engedélyezhesse a jelen megállapodásban szereplő jogokat, és a harmadik személy által birtokolt anyagrész mellett egyértelműen fel van tüntetve az eredeti szerző neve a művön belül.

A szerzői jogok tulajdonosa a hozzáférés körét az alábbiakban határozza meg (**egyetlen, a megfelelő négyzetben elhelyezett x jellel**):

- engedélyezi, hogy a HuVetA-ban -ban tárolt művek korlátlanul hozzáférhetővé váljanak a világhálón,
- az Állatorvostudományi Egyetem belső hálózatára (IP címeire) korlátozza a feltöltött dokumentum(ok) elérését,
- a Könyvtárban található, dedikált elérést biztosító számítógépre korlátozza a feltöltött dokumentum(ok) elérését,
- csak a dokumentum bibliográfiai adatainak és tartalmi kivonatának feltöltéséhez járul hozzá (korlátlan hozzáféréssel),

Kérjük, nyilatkozzon a négyzetben elhelyezett jellel a helyben használatról is:

Engedélyezem a dokumentum(ok) nyomtatott változatának helyben olvasását a könyvtárban.

Amennyiben a feltöltés alapját olyan mű képezi, melyet valamely cég vagy szervezet támogatott illetve szponzorált, kijelenti, hogy jogosult egyetérteni jelen megállapodással a műre vonatkozóan.

A HuVetA üzemeltetői a szerző, illetve a jogokat gyakorló személyek és szervezetek irányában nem vállalnak semmilyen felelősséget annak jogi orvoslására, ha valamely felhasználó a HuVetA-ban engedéllyel elhelyezett anyaggal törvénytörtő módon visszaélne.

Budapest, 2023/11/03

*Shane Roger Ryan*

---

aláírás

szerző/a szerzői jog tulajdonosa

---

*A HuVetAMagyar Állatorvos-tudományi Archívum – Hungarian Veterinary Archive az Állatorvostudományi Egyetem Hutýra Ferenc Könyvtár, Levéltár és Múzeum által működtetett egyetemi és szakterületi online adattár, melynek célja, hogy a magyar állatorvos-tudomány és -történet dokumentumait, tudásvagyonát elektronikus formában összegyűjtse, rendszerezze, megőrizze, kereshetővé és hozzáférhetővé tegye, szolgáltatassa, a hatályos jogi szabályozások figyelembe vételével.*

*A HuVetA a korszerű informatikai lehetőségek felhasználásával biztosítja a könnyű, (internetes keresőgépekkel is működő) kereshetőséget és lehetőség szerint a teljes szöveg azonnali elérését. Célja ezek révén*

- a magyar állatorvos-tudomány hazai és nemzetközi ismertségének növelése;*

- *a magyar állatorvosok publikációira történő hivatkozások számának, és ezen keresztül a hazai állatorvosi folyóiratok impakt faktorának növelése;*
- *az Állatorvostudományi Egyetem és az együttműködő partnerek tudásvagyonának koncentrált megjelenítése révén az intézmények és a hazai állatorvos-tudomány tekintélyének és versenyképességének növelése;*
- *a szakmai kapcsolatok és együttműködés elősegítése,*
- *a nyílt hozzáférés támogatása.*