# *TERRESTRIAL MANUAL* REVISION: ADVICE FOR MEMBER COUNTRY COMMENTS

Chapter Number and Title: Chapter 3.10.2. Cryptosporidiosis

Country making the comments:

Date:

It would be appreciated if the following guidance is followed when making a reply:

1. Comments may be general or specific, but specific comments are more valuable. General comments should be such that some conclusion and action can be taken in response to them. For example, instead of stating “This test is no longer used in our laboratory”, indicate the reasons the test is no longer used and what test is used instead.

2. Specific comments should be identified by indicating the line number in the text, to facilitate the editorial process.

3. Highlighting typing or technical errors is welcome, but the correct word or figure should be indicated in its place. For example, instead of indicating simply “0.8 M is too high”, the preferred value should also be indicated.

4. Bear in mind that the introductory chapters (Part 1 of the *Terrestrial Manual*) set general standards for the management of veterinary diagnostic laboratories and vaccine facilities and are not intended to be exhaustive, and indeed none of the chapters can give a completely comprehensive cover of the subject, otherwise the *Terrestrial Manual* would be too long. However, assistance in indicating priorities is always helpful.

5. The *Terrestrial Manual* is intended for world-wide use. The chapters need to reflect the development of new technology, while maintaining the established methods, usually requiring less sophisticated apparatus. New technology should not be described in detail until it has gained wide acceptance as a reliable method.

6. We recommend that if you have no specific comments, please respond to the OIE to that effect.

7. Any comments, proposed changes or revisions should be supported by clear evidence (the scientific rationale) such that some conclusion and action can be taken in response to them.

*Your participation in the OIE Standard-setting process is valued. Thank you for your engagement in the process!*

*General Comments*

*Specific Comments* (*add continuation sheets if required*)

*line:*

Chapter 3.10.2.

cryptosporidiosis

SUMMARY

**Description of the disease:** Cryptosporidiosis is the pathological condition caused by infection with the protozoan Cryptosporidium. Following infection, theCryptosporidium life cycle, comprising asexual and sexual phases, is completed in a single host, producing sporulated oocysts. There are at least ~~32~~ 43 ‘valid’ Cryptosporidium species, some of which cause disease in humans, livestock, poultry and game birds, and companion animals. Cryptosporidium parvum infects mainly the gastrointestinal tract and ~~causes~~ is an important cause of scour in young, unweaned livestock. Mortality is generally low but severe outbreaks may occur occasionally. Weaned and adult animals do not normally exhibit signs of disease, but can excrete oocysts that may contaminate the environment facilitating onward transmission. Cryptosporidium parvum is one of the major causes of zoonotic human cryptosporidiosis. Cryptosporidium andersoni infects the digestive glands of the abomasum of older calves and adult cattle and also bactrian camels. Some infected cows exhibit reduced milk yields and poor weight gain, but do not develop diarrhoea. Cryptosporidium baileyi affects primarily the upper respiratory tract, bursa of Fabricius and cloaca, kidneys and eyes of gallinaceous birds, and has caused outbreaks and mortalities in game and poultry units. Cryptosporidium meleagridis affects primarily the ileum of turkey poults and game birds, and can cause enteritis, diarrhoea and death, and C. galli infects the surface, ductal, and glandular epithelium of the proventriculus of adult hens and some wild birds.

**Detection of the agent:** Laboratory identification is required for diagnosis. Microscopic observation of stained oocysts~~, with acid-fast Ziehl–Neelsen, auramine phenol or immunofluorescent stains applied to faecal smears are~~ is used commonly. Enzyme immunoassays, immunochromatographic lateral flow assays, and molecular diagnostic tests are ~~also~~ widely ~~used but may have a low specificity. Molecular diagnostic tests are becoming more widely~~ available. The infecting species cannot be identified by oocyst morphology or antibody-based assays, but downstream analysis of DNA amplified by the polymerase chain reaction can be used to determine species. Most cases of cryptosporidiosis in young mammalian livestock are likely to be caused by C. parvum, which is also the most important zoonotic species. There is no standardised subtyping scheme, but sequencing the gp60 gene may be informative in outbreak investigations. Multilocus subtyping schemes are in development but need to be standardised. Oocysts can survive in moist environments for many months, and foodborne and waterborne transmission occurs. However, application of genotyping to the small numbers of oocysts likely to be present in food, water and environmental samples is challenging.

**Requirements for vaccines:** There is no commercially available vaccine for cryptosporidiosis.

A. INTRODUCTION

Cryptosporidiosis is the pathological condition caused by infection with the protozoan *Cryptosporidium.* Infection of the gastrointestinal tract is most common; the primary symptom is diarrhoea. Respiratory infection occurs in some hosts, especially birds. Other sites may also be infected, again most commonly in birds, and in immunocompromised patients.

1. ~~Causal pathogen~~ Nature and classification of *Cryptosporidium*

Cryptosporidiosis is caused by protozoa of the genus *Cryptosporidium,* classified traditionally asphylum Apicomplexa, class Sporozoasida, subclass Coccidiasina, order Eucoccidiorida, and family *Cryptosporidiidae*. A revised classification of the Eukaryotes ~~places~~ placed *Cryptosporidium* in the following descending hierarchical groups: Diaphoretickes; Sar (Stramenopiles, Alveolata and Radiolaria) supergroup; Alveolata; and ultimately Conoidasida wherein *Cryptosporidium* ~~is~~ was classified separately from the *Coccidia* and *Gregarinasina* (Adl *et al.,* 2012). Single-cell genomics and transcriptomics analyses also separated *Cryptosporidium* from the gregarines (Mathur *et al.,* 2019). At the time of writing (~~July 2015~~ November 2020), there are ~~26~~ 43 *Cryptosporidium* species with formally and adequately described biological and genetic characteristics (Table 1).

Laboratory detection of the genus is required for a diagnosis of cryptosporidiosis, but only molecular methods will differentiate *Cryptosporidium* species and subtypes as many of the oocyst sizes are similar (Table 1) and there are few species-distinguishing antigens. In the past, many ‘species’ were described on a generally false premise of host specificity (Fayer, 2010). In livestock, poultry and game birds, *C. parvum*, *C. andersoni*, *C. baileyi* and *C. meleagridis* have been reported to cause morbidity and outbreaks of disease. In humans, *C. parvum, C. hominis, C. meleagridis* and *C. cuniculus* are considered the main pathogenic species, causing sporadic cases and outbreaks (Table 1). Most cases of cryptosporidiosis in young mammalian livestock are likely to be caused by *C. parvum*, which is also the most significant zoonotic threat for humans. In addition, ~~over 40~~ tens of *Cryptosporidium* ‘genotypes’ have been identified in animals on the basis of DNA sequencing but lack sufficient biological data for species status (Fayer, 2010). The *Cryptosporidium* horse genotype, skunk genotype, ferret genotype, chipmunk genotype I, deer mouse genotype III and the *C. hominis* monkey genotype~~s~~ have also been described in humans.

~~A new genus, designated~~ *~~Piscicryptosporidium~~*~~, has been proposed for~~ *~~C. molnari~~* ~~and~~ *~~Cryptosporidium~~*~~-like other species and genotypes infecting fish but further genetic and biological characterisation is required to determine whether or not~~ *~~Piscicryptosporidium~~* ~~is a valid genus.~~

***Table 1.*** *Some differences among species within the genus* Cryptosporidium

| ***Cryptosporidium* species** | **Mean oocyst dimensions (µm)a** | **Major host(s)** | **Usual site of infection** | **Infections reported in humans** |
| --- | --- | --- | --- | --- |
| *C. alticolis* | 5.4 × 4.9 | Voles | Small intestine | No |
| *C. andersoni* | 7.4 × 5.5 | Cattle | Stomach | Yes, but only rarely |
| *C. apodemi* | 4.2 × 4.0 | Mice | Intestine | No |
| *C. avium* (syn. avian genotype 5) | 6.3 × 4.9 | Birds | Ileum, ceacum, kidney, ureter, and cloaca | No |
| *C. baileyi* | 6.2 × 4.6 | Poultry | Upper respiratory tract | No |
| *C. bollandi* (previously piscine gt 2) | 3.1 × 2.8 | Fish | Gastric mucosa | No |
| *C. bovis* (previously bovine B genotype) | 4.9 × 4.6 | Cattle | Small intestine | Yes, but only rarely |
| *C. canis* (previously dog genotype) | 5.0 × 4.7 | Dog | Small intestine | Yes, occasionally |
| *~~C. cichlidis~~* ~~(previously piscine genotype 1 or~~ *~~C. molnari-~~*~~like)~~ | ~~4.6 × 4.4~~ | ~~Tilapia~~ | ~~Stomach~~ | ~~No~~ |
| *C. cuniculus* (previously rabbit genotype) | 5.6 × 5.4 | Rabbit, humans | Small intestine | Yes, occasionally. One waterborne outbreak |
| *C. ditrichi* (syn. UK E6) | 4.7 × 4.2 | Mice | Small Intestine | Yes, but only rarely |
| *C. ducismarci* |  | Tortoises | Intestine | No |
| *C. erinacei* | 4.9 × 4.4 | Hedgehog | Small intestine | Yes, but only rarely |
| *C. fayeri* (previously marsupial genotype I) | 4.9 × 4.3 | Marsupials | Intestine | Yes, but only rarely |
| *C. felis* | 4.6 × 4.0 | Cat | Small intestine | Yes, occasionally |
| *C. fragile* | 6.2 × 5.5 | Black spined toad | Stomach | No |
| *C. galli* | 8.3 × 6.3 | Chicken | Proventriculus | No |
| *C. homai* | Not reported | Guinea-pig | Intestine | No |
| *C. hominis* (previously referred to as *C. parvum* human genotype, genotype 1, and genotype H) | 4.9 × 5.2 | Humans | Small intestine | Yes, commonly. Outbreaks are reported frequently |
| *C. huwi* | 4.6 × 4.4 | Guppy | Stomach | No |
| *C. macropodum* (previously marsupial genotype II) | 5.4 × 4.9 | Eastern grey kangaroo | Intestine | No |
| *C. meleagridis* | 5.2 × 4.6 | Birds, mammals | Intestine | Yes, frequency depends on setting. One farm-related and one school-related outbreak |
| *C. microti* | 4.3 × 4.1 | Voles | Large intestine | No |
| *C. molnari* | 4.7 × 4.5 | Sea bream | Intestine | No |
| *C. muris* | 7.0 × 5.0 | Rodents | Stomach | Yes, but only rarely |
| *C. occultus* | 5.2 × 4.9 | Rodents | Not reported | Yes, but only rarely |
| *C. ornithophilus* (previously avian gt 2) | 6.1 x 5.2 | Birds | Caecum, colon and bursa of Farbricius | No |
| *C. parvum* (also sometimes previously called bovine genotype, genotype II, and genotype B) | 5.0 × 4.5 | Humans, pre-weaned mammalian livestock, wild mammals | Small intestine | Yes, commonly and outbreaks are reported frequently |
| *C. proliferans* | 7.7 × 5.3 | Rodents | Stomach | No |
| *C. proventriculi* | 7.4 × 5.7 | Birds | Ventricuus and proventriculus | No |
| *C. ratti* (previously Rat gt 1) | 4.9 × 4.6 | Rats | Jejunum and ileum | No |
| *C. rubeyi* | 4.7 × 4.3 | Squirrels | Not reported | No |
| *C. ryanae* (previously deer-like genotype) | 3.7 × 3.2 | Cattle |  | No |
| *~~C. reichenbachklinkei~~* ~~(previously piscine genotype 2)~~ | ~~3.4 × 3.4~~ | ~~Gourami~~ | ~~Stomach~~ | ~~No~~ |
| *C. scophthalmi* | 4.4 × 3.9 | Turbot | Intestine | No |
| *C. scrofarum* (previously pig genotype II) | 5.2 × 4.8 | Pig | Small intestine | Yes, but only rarely |
| *C. serpentis* | 6.2 × 5.3 | Reptiles | Stomach | No |
| *C. suis* (previously pig genotype I) | 4.6 × 4.2 | Pig | Small intestine | Yes, but only rarely |
| *C. testudinis* | 6.4 × 5.9 | Tortoise | Not reported | No |
| *C. tyzzeri* (previously mouse genotype I) | 4.6 × 4.2 | Mice | Small intestine | Yes, but only rarely |
| *C. ubiquitum* (previously cervine genotype) | 5.0 × 4.7 | Various mammals | Small intestine | Yes, occasionally |
| *C. varanii* (syn. *C. saurophilum*) | 4.8 × 4.7 | Reptiles | Intestine | No |
| *C. viatorum* | 5.4 × 4.7 | Humans |  | Yes, occasionally |
| *C. wrairi* | 5.4 × 4.6 | Guinea pig | Small intestine | No |
| *C. xiaoi* (previously *C. bovis*-like genotype or *C. bovis* from sheep or *C. agni*) | 3.9 × 3.4 | Sheep, goat |  | No |

aFrom the original papers describing the species.

2. Description and impact of the disease in animals

Clinical and subclinical infections in animals have been reviewed by Santin (2013).

Occurrence of *Cryptosporidium* spp. in ungulate livestock has been reviewed systematically and subjected to meta-analysis by Hatam-Nahavandi *et al.* (2019). Most of the 245 studies included cattle (*n* = 163) and sheep (*n* = 46), and the overall sample positivity was 19% but with considerable variation. Occurrence was especially high in ruminant livestock, particularly in intensive animal production systems.

*Cryptosporidium parvum* is an important cause of ~~scour~~ diarrhoea in young, unweaned farmed livestock including calves, lambs, goat kids, alpaca and foals. In addition to welfare issues, ~~there are adverse consequences for productivity~~ production losses include death, diagnostic and treatment costs, additional feed and husbandry costs to achieve market weight and condition. Healthy and adult animals can also shed oocysts, often in large numbers, providing additional potential reservoirs of infection, ~~and~~ environmental contamination (including water supplies), and risk of zoonotic transmission.

*Cryptosporidium parvum* infections of cattle are considered endemic globally. Prevalence and severity of disease peak in the second week of life. Endogenous stages infect enterocytes of the distal small intestine, caecum and colon. Villous atrophy, shortening of microvilli and sloughing of enterocytes are the major pathological changes. Affected animals usually recover within 2 weeks of showing signs of illness. Clinical signs can range from a mild to inapparent infection in older animals to severe scouring in young animals, and can cause varying degrees of dehydration, dullness, anorexia, fever and loss of condition. Mortality is generally low unless occurring as a mixed infection with other enteric pathogens such as *Escherichia* *coli* or rotavirus, although severe outbreaks of cryptosporidiosis are sometimes reported. Infection has been correlated with low live weight gain and poor production performance.

*Cryptosporidium parvum* infections of small ruminants (sheep, goats) commonly cause neonatal diarrhoea sometimes associated with high morbidity and mortality especially with concurrent infections or deficiencies in nutrition and husbandry. In ewes, a periparturient rise in oocyst shedding has been observed. Low carcase weights have been reported following acute cryptosporidiosis in lambs.

~~Although~~ *~~C. parvum~~* ~~appears to be pathogenic to piglets, causing inappetence, depression, vomiting or diarrhoea~~*~~, C. suis~~* ~~and~~ *~~C. scropharum~~* ~~are the most frequently reported species. However, natural infection does not appear to lead to disease, and clinical signs of cryptosporidiosis in pigs may be related to infection with different~~ *~~Cryptosporidium~~* ~~species or genotypes or simultaneous infection with other enteropathogens.~~

~~Treatment options for cryptosporidiosis are limited and centre on rehydration therapy. Halofuginone lactate and paromomycin are licenced in the UK for the prevention and treatment of bovine cryptosporidiosis, but only reduce not eliminate signs and oocyst shedding.~~

Other *Cryptosporidium* species also infect livestock and companion animals.

*Cryptosporidium bovis* and *C. ryanae* are generally more common than *C. parvum* in post-weaned calves. ~~, but~~ Infections with these host-adapted cattle species have not yet been widely confirmed as associated with illness although there is a report in diarrhoeic calves in Sweden. ~~and~~ There are no histological or pathological reports.

*Cryptosporidium* *andersoni* colonises the digestive glands of the abomasum of older calves and adult cattle. Infected cattle do not develop diarrhoea, but can excrete oocysts for several months. Some infected beef cattle exhibit reduced weight gain compared with uninfected controls, and one study found that infection may interfere with milk production in dairy cows.

*Cryptosporidium ubiquitum* and *C. xiaoi* infect lambs and kids. *Cryptosporidium ubiquitum* is prevalent in post-weaned lambs, ~~with one report of involvement with~~ *~~C. parvum~~* ~~in outbreaks of diarrhoea, and lambs with~~ *~~C. ubiquitum~~* ~~showed higher faecal consistency scores in one study~~ but is not commonly linked to diarrhoea. *Cryptosporidium xiaoi* infection has been associated with outbreaks of neonatal diarrhoea in goats.

*Cryptosporidium* *canis* is the most frequently reported species in dogs and although usually asymptomatic, infection has been linked to severe diarrhoea, malabsorption and weight loss ~~especially~~ in younger animals.

*Cryptosporidium* *felis* is the most frequently reported species in cats, often in the absence of clinical signs, although ~~infection has been~~ occasionally linked to persistent diarrhoea ~~in some cases~~. Cats with other enteric parasites, or with feline leukaemia virus infection, are more likely to develop cryptosporidiosis, which should be included in the differential diagnosis of chronic feline diarrhoea.

*Cryptosporidium* is a major protozoan parasite of birds and a primary pathogen in poultry ~~chickens, turkeys and quail~~, causing respiratory and/or intestinal disease, leading to morbidity and mortality, reviewed by Nakamura & Meireles, 2015. Three species ~~infect poultry~~ cause disease in birds: *C. baileyi,* *C. meleagridis* and *C. galli,* ~~reviewed by Current (1997) and Ryan (2010)~~.

*Cryptosporidium baileyi* is the most frequently detected species among Galliformes (e.g. chickens, turkeys, quail). It most commonly infects the upper respiratory tract, although other sites include the renal tract, bursa of Fabricius and cloaca, while the trachea and the conjunctiva are lesser sites of infection. Intestinal infection does not normally result in gross lesions or overt signs of disease, but respiratory cryptosporidiosis ~~of chickens and game birds such as red grouse~~ can result in severe morbidity and, on occasion, mortality. Initially, severe disease is accompanied by sneezing and coughing, followed by head extension to facilitate breathing. Epithelial cell deciliation and hyperplasia, mucosal thickening and discharge of mucocellular exudate into the airways are major pathological changes associated with disease in young broilers. Severe signs of respiratory disease can last up to 4 weeks post-infection. ~~Cryptosporidiosis in turkeys caused by~~ *~~Cryptosporidium baileyi~~* ~~is similar to that observed in chicken. Chicken isolates of~~ *~~C. baileyi~~* ~~cause infection in other birds. Respiratory and intestinal cryptosporidiosis has been reported in commercially grown quail caused by~~ *~~C. baileyi~~*~~, with pathological changes similar to those described in chickens. Oral infection of chickens with 100~~*~~C. baileyi~~* ~~oocysts can result in intestinal cryptosporidiosis (Current, 1997).~~ Possible immunosuppression has been reported due to infection with *C. baileyi*.

*Cryptosporidium* *meleagridis* is also mainly reported among Galliformes, ~~infects~~ especially turkeys~~, other poults and~~. Infections in chickens are infrequent. It is the only bird-adapted species also reported in mammals, including humans. Clinical infection is mainly of the small intestine with villous atrophy, crypt hyperplasia and shortening of microvilli reported as ~~are~~ major pathological changes causing diarrhoea and weight loss.

*Cryptosporidium* *galli* is most frequently reported amongPasseriformes and Psittaciformes, with~~produces a disease in adult hens and some wild and exotic birds. Unlike the life cycle stages of either~~ *~~C. meleagridis~~* ~~or~~ *~~C. baileyi~~*~~,~~ infection ~~with~~ *~~C. galli~~* ~~is~~ limited to the epithelial cells of the proventriculus. Clinical signs include puffed plumage with head held under the wing, unresponsiveness to external stimuli, and failure to thrive. Histopathology of haematoxylin and eosin stained sections from finches demonstrated necrosis and hyperplasia of proventricular glandular epithelial cells, and a mixed inflammatory cell infiltration into the lamina propria of the proventriculus associated with large numbers of oocysts attached to the surface of glandular epithelial cells.

Outbreaks of disease in game birds (e.g. pheasant, partridge and grouse) suggest cryptosporidiosis should be included among respiratory and enteric diseases routinely tested for in these birds. Both *C. meleagridis* and *C. baileyi* are involved.

Other *Cryptosporidium* species have been reported in bird faeces, including *C. parvum*, for which birds may be transport vectors rather than highly susceptible to infection.

~~The significance of~~ *~~Cryptosporidium~~* ~~infections for aquaculture and fish welfare is uncertain, although morbidity and mortality have been reported in hatcheries with high prevalence of infection (Gabor~~ *~~et al.,~~* ~~2011).~~

*Cryptosporidium* in terrestrial wildlife has been reviewed by Zahedi *et al*. (2016), indicating the widespread occurrence of this parasite including *C. parvum* and other zoonotic species.

3. Human health risk and zoonotic potential

Human cryptosporidiosis has been reviewed by Chalmers & Davies (2010). Cryptosporidiosis is usually an acute, self-limiting gastrointestinal disease, characterised by watery diarrhoea, abdominal cramps, vomiting, low-grade fever, and loss of appetite. Symptoms can last for up to 1 month during which time apparent recovery and relapse occurs in about one third of cases. It can be a serious disease in the young, malnourished, and immunocompromised, and is a major cause of moderate-to-severe diarrhoea in young children in sub-Sarharan Africa and South-East Asia where it carries a significant risk of death (Kotloff *et al.,* 2013).

Long-term sequelae have been linked to infection with *Cryptosporidium* including myalgia, arthralgia, fatigue, continued gastrointestinal upset, irritable bowel syndrome, and an association with bowel cancer that ~~but~~ requires further investigation.

Patients with severe immunodeficiency may suffer from chronic, severe and intractable cryptosporidiosis with significant mortality.In malnourished young children, infection causes substantial morbidity and mortality (Kotloff *et al.*, 2013), and longer term consequences including growth faltering and cognitive defects. There is evidence for respiratory involvement in some populations.

*Cryptosporidium parvum* is themain zoonotic species (Table 1), but there are also human-adapted subtypes that seem to be transmitted without animal involvement. These occur globally but are most prevalent in Africa where anthroponotic transmission predominates in a landscape of generally extensive and pastoral husbandry (Robertson *et al.,* 2020).

I~~n addition to zoonotic~~ *~~Cryptosporidium~~* ~~species (Table 1),~~ *~~C.~~ Cryptosporidium hominis* is an important cause of gastrointestinal disease in humans. Humans are the major host, and although there are a small number of reports of *C. hominis* infections in ~~cattle and sheep~~ livestock, there is no evidence for maintenance of infection in, or transmission between, herds or flocks, ~~or of~~ and few reports of clinical signs in animals.

4. Transmission, biosafety and biosecurity requirements

Transmission is by the faecal-oral route and may involve a vehicle such as contaminated food or drinking water. Food- and especially water-borne outbreaks carry significant economic, health and social impacts. *Cryptosporidium* *parvum* is highly infectious for young livestock and humans; older livestock can remain infected and excrete oocysts that can be transmitted to other susceptible hosts. The nature of animal production systems and husbandry (e.g. whether intensive or extensive, pastoral or otherwise) is an influence on transmission to humans. A review of One Health approaches to tackle zoonotic cryptosporidiosis has been produced by Innes *et al.* (2020).

Transmission of *C. hominis* is considered to be anthroponotic; relatively rare findings in animals may be influenced by human activity.

Isolates vary in infectivity, and susceptibility is influenced by host-related factors (Borad & Ward 2010; Flores & Okhuysen, 2009; Yang *et al.,* 2010). Dose–response models indicate that there is a high likelihood of human and pre-weaned livestock infection with single numbers of *C. parvum* oocysts. ~~, and also that~~ There is a positive relationship between pre-existing antibodies and protection from infection, whether with the same or a different *Cryptosporidium* species. However, neither the level of immunity nor the extent of cross-protection has been determined. Ingestion of a sufficient quantity of good quality colostrum soon after birth is important in controlling cryptosporidiosis in livestock.

Oocysts can survive for long periods (>6 months) in cool, moist environments, and on fomites such as farm gates, buildings and utensils. Oocysts can be transmitted following direct contact with faeces from an infected individual, or contact with contaminated fomites, or by ingestion of contaminated food or water. Agricultural practices likely to enhance the spread of cryptosporidiosis include indoor calving and lambing and the communal feeding and husbandry of neonates, where young susceptible animals are in close contact with infected animals. Transmission from clinically normal dams to suckling neonates may occur, ~~but little is known about carriage~~ and improved sample preparation from large faecal mass and sensitive detection methods such as polymerase chain reaction (PCR) need to be applied to detect carriage in adult, asymptomatic animals. The disposal of faeces, farmyard manure or other contaminated waste in land-based dumps, and the spreading of slurry, when followed by periods of heavy rainfall or melting snow can lead to oocyst contamination of water courses and transmission through drinking water supplies. Manure and slurry should be well composed or fermented before spreading.

Farm management practices directed at controlling environmental contamination include removal of dung and contaminated bedding from animal housing, steam-cleaning and disinfection, although *Cryptosporidium* is resistant to many commonly used disinfectants. Thorough cleaning of surfaces and utensils with hot, soapy water > 60°C followed by drying can be effective.

Wild mammals may act as hosts to *Cryptosporidium* spp. (Fayer, 2010; Xiao *et al*., 2004) and provide a reservoir, but little is known of the scale of importance of their involvement in transmitting infection to, or maintaining infection in, livestock in agricultural environments (Sturdee *et al*., 1999). Animals such as birds or fish normally infected with host-adapted *Cryptosporidium* species may act as transport vectors of other species including *C. parvum.* Contamination of drinking water supplies by wildlife is a possible transmission route.

5. Differential diagnosis

The differential diagnosis for *Cryptosporidium* includes other entero-pathogens involved in diarrhoea. Multiple pathogens can be present in faeces including other parasites, viruses such as rotavirus or coronavirus, pathogenic strains of *E. coli,* and *Salmonella* spp. *Cryptosporidium* is a significant pathogen and cryptosporidiosis in livestock is confirmed by finding significant numbers of oocysts in diarrhoeic faeces in the absence of other pathogens. Although it has been widely speculated that co-infection may lead to more severe cryptosporidiosis (Lorenz *et al.,* 2011) experimental data to support this are lacking. Gastro-intestinal upset may also have non-infectious causes, for example inflammatory bowel disease in humans.

B. DIAGNOSTIC TECHNIQUES

Table 2. Test methods available for diagnosis of cryptosporidiosis and their purpose

| Method | **Purpose** | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| Population freedom from infection | Individual animal freedom from infection prior to movement | Contribute to eradication policies | Confirmation of clinical cases | Prevalence of infection – surveillance | Immune status in individual animals or populations post-vaccination |
| **Detection of the agent[[1]](#footnote-2)** | | | | | | |
| **Conventional, stained microscopy** | – | – | – | +++ | ++ | – |
| **FAT** | – | – | – | +++ | ++ | – |
| **Antigen detection by IC** | – | – | – | + | + | – |
| **Antigen detection by ELISA** | – | – | – | +++ | +++ | – |
| **PCR** | – | – | – | +++ | +++ | – |
| **Detection of immune response** | | | | | | |
| **Antibody detection by ELISA** | – | – | – | – | ++ | – |

Key: +++ = recommended for this purpose; ++ recommended but has limitations;   
+ = suitable in very limited circumstances; – = not appropriate for this purpose.   
FAT = fluorescent antibody test; IC = immunochromatography;   
PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay.

1. Introduction to tests available

Confirmation of the infection is most commonly by microscopic detection of *Cryptosporidium* oocysts in faeces (Casemore, 1991). Organisms can also be detected in intestinal fluid, tissue samples, or biopsy specimens; antigens in faeces or intestinal fluid; or nucleic acid by PCR-based detection in faeces, intestinal fluid, tissue samples, or biopsy specimens. Haematoxylin and eosin stain can be used for histological diagnosis in biopsy material or confirmation of the diagnosis post-mortem.

Species identification is usually by a reference laboratory test, for which the benchmark is sequencing the small subunit (SSU) rRNA gene (Roellig & Xiao, 2020). Subtyping tools ~~are~~ targeting the gp60 gene may be used in epidemiological investigations of *C. parvum, C. hominis, C. meleagridis,* ~~and~~ *C. ubiquitum* and *C. felis* infections~~. The usual genetic target is the gp60 gene~~ (Roellig & Xiao, 2020). There is no standardised multilocus subtyping scheme, and although sequencing or fragment size analysis of mini- and microsatellite markers has been described (Xiao, 2010), marker selection, analysis and relationship algorithms need to be harmonised (Widmer & Caccio, 2015). High throughput sequencing technologies are not yet routinely applied to *Cryptosporidium* in diagnostic laboratories. There are no reproducible *in-vitro* culture techniques available routinely to amplify parasite numbers prior to identification.

Serological tests are not appropriate for diagnosis but can be used for seroepidemiological surveys of exposure.

2. Detection of *Cryptosporidium*

2.1. Safety and quality

*Cryptosporidium* is a ~~laboratory~~ risk to laboratory workers and all laboratory procedures that can give rise to infectious aerosols must be conducted in a biosafety cabinet. Specimens may contain other pathogenic organisms and should be processed accordingly. To safeguard the health of laboratory workers, all laboratory manipulations must be performed at an appropriate biosafety and containment level determined by biorisk analysis (see Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*).

2.2. Collection and submission of samples

Specimens for primary diagnosis should be collected during acute infection. If *Cryptosporidium* only is sought, short-term storage of faeces at 4°C is appropriate as oocyst morphology and antigen structure will be retained. Longer term, –20°C can be used. Alternatively, an equal volume of 5% K2Cr2O7 can be added to facilitate storage at ambient temperature. However, if other parasites and especially trophozoites are sought in differential diagnosis, faeces need to be examined promptly. The deterioration of the morphology of other parasite stages, and overgrowth by other microorganisms particularly yeasts, can be reduced by the addition of preservatives including 10% (v/v) aqueous formalin, merthiolate–iodine–formaldehyde (MIF), sodium acetate–acetic acid–formalin (SAF) and polyvinyl alcohol (PVA). Downstream tests must be considered for preservative compatibility; for example formalin and SAF are generally compatible with enzyme-linked immunosorbent assay (ELISA) and immunochromatographic (IC) kits, but refer to manufacturers’ instructions. Preservatives may interfere with PCR-based tests; faecal samples can be preserved in 90% ethyl alcohol for later PCR testing. Faeces in preservative may require concentration by a recognised method before microscopy, but this is not appropriate if ELISA and IC kits are used because soluble antigens may be lost in the process. Some faeces may need additional processing, for example very liquid faeces may be concentrated, high fat faeces may require defatting, mucoid samples may need treatment with KOH or dithiothreitol, high-fibre faeces may need sieving to remove fibres.

Procedures for packaging and shipping of specimens must be as outlined in the International Air Transport Association’s Dangerous Goods Regulations (IATA, ~~2003~~ 2021). These regulations are summarised in Chapter 1.1.2 *Collection, submission and storage of diagnostic specimens* and Chapter 1.1.3 *Transport of biological materials*.

2.3. Microscopy – sample preparation and staining

2.3.1. Preparation of faecal (or appropriate body fluid) smears

Most unpreserved samples can be smeared directly on to microscope slides prior to staining. Include a positive control slide each time this procedure is performed.

2.3.1.1. Test procedure

i) Wear protective clothing and disposable gloves. Score the reference number of the specimen on a microscope slide with a diamond marker[[2]](#footnote-3), and use separate microscope slides for each specimen. For formed faeces, place 1 drop of saline (about 50 µl) in the centre of the slide.

ii) For liquid faeces (or other appropriate body fluid) dispense one drop (about 20 µl) directly on to the slide. For formed faeces, use the tip of a clean applicator stick to remove about 2 mg sample[[3]](#footnote-4) and emulsify in the saline by thorough mixing.

iii) Prepare a medium to thick smear with areas of varying thickness. Ensure that the smear is of the correct transparency[[4]](#footnote-5).

iv) Air dry the smear at room temperature.

v) Fix the smear[[5]](#footnote-6) in methanol for 3 minutes.

vi) Stain using modified Ziehl–Neelsen or auramine phenol stains as described below.

2.3.2. Concentration of oocysts from preserved or liquid samples by flotation

2.3.2.1. Preparation of flotation solution

Sucrose, zinc sulphate or sodium chloride solutions may be used to separate oocysts from faecal debris. Selection may depend on downstream applications.

2.3.2.1.1. Preparation of sucrose or zinc sulphate solution

Either prepare sucrose solution (specific gravity 1.18) in a glass beaker by adding 256 g of sucrose to 300 ml of deionised water or prepare zinc sulphate solution (specific gravity 1.18) in a glass beaker by adding 100 g of zinc sulphate to 300 ml of deionised water. Gently heat the solution (<60°C) and stir continuously on a hot plate stirrer until the sucrose or zinc sulphate has dissolved completely. Place the solution on ice or in a refrigerator until it cools to 4°C. Pour the cold solution to a 500 ml measuring cylinder and adjust the specific gravity to 1.18 by adding sufficient cold, deionised water (4°C). Pour the solution into a screw-cap glass bottle, labelled, dated, initialled and stored at 4°C until used.

2.3.2.1.2. Preparation of saturated salt solution

Prepare saturated salt solution (specific gravity 1.2) by adding approximately 200 g of sodium chloride to 200 ml of deionised water. Gently heat the solution (<60°C) and stir continuously on a hot plate stirrer. Add further, small amounts of sodium chloride (approximately 10 g) at 10-minute intervals until the solution becomes saturated. Pour the saturated salt solution into a clean glass bottle and either place on ice or in a refrigerator until it has cooled to 4°C. Pour the solution into a 500 ml measuring cylinder and adjust its specific gravity to 1.2 by adding cold, deionised water (4°C). Pour the saturated salt solution into a screw-cap glass bottle, labelled, dated, initialled and stored at 4°C until used.

Alternatively, a cold method may be used; approximately 1.5 kg of sodium chloride is required to saturate 4 litres of deionised water by adding the sodium chloride in small quantities, taking care not to stop the magnetic stirrer and keeping the solution stirring briskly. Continue to add sodium chloride until specific gravity 1.2 is reached. Pour the saturated salt solution into screw-cap glass bottles, labelled, dated, initialled and stored at 4°C until used.

Before use, ensure that the salt solution is mixed by inversion and allowed to settle for 5 minutes.

2.3.2.2. Recovery of *Cryptosporidium* oocysts by centrifugal flotation

2.3.2.2.1. Test procedure

i) Wear protective clothing and disposable gloves. Transfer approximately 1 to 2 g of faeces[[6]](#footnote-7) with an applicator stick, or pipette 1 to 2 ml liquid faeces, into 10 ml of flotation solution in a 15 ml centrifuge tube and mix thoroughly.

ii) Place the centrifuge tube in a bench top centrifuge with swing out buckets, add a balance tube, if necessary, and centrifuge at 1100 ***g*** for 5 minutes[[7]](#footnote-8).

iii) Remove the top 2 ml of fluid (containing the oocysts) from the meniscus, wash 3× in deionised water and finally resuspend in a minimum volume of deionised water.

iv) Transfer the re-suspended contents on to a microscope slide with a disposable pipette, and air dry.

2.3.3. Concentration of oocysts in preserved or liquid samples by sedimentation

All steps that can generate aerosols (excluding centrifugation) should be performed in an operator protection safety cabinet.

2.3.3.1. Test procedure

i) Wear protective clothing and disposable gloves. Sample approximately 500 mg to 1 g faeces[[8]](#footnote-9) with an applicator stick[[9]](#footnote-10) and place in a clean 12–15 ml centrifuge tube containing 7 ml of 10% formalin. If the stool is liquid, dispense about 750 µl into the centrifuge tube.

ii) Break up the sample thoroughly and emulsify using the applicator stick.

iii) Filter the resulting suspension through a sieve[[10]](#footnote-11) into a beaker, then pour the filtrate back into the same centrifuge tube.

iv) Add 3 ml of ethyl acetate[[11]](#footnote-12) to the formalinised solution, seal the neck of the tube with a rubber bung and shake the mixture vigorously for 30 seconds. Invert the tube a few times during this procedure and release the pressure developed gently by removing the rubber bung slowly.

v) Centrifuge the tube at 1100 ***g*** for 2 minutes[[12]](#footnote-13).

vi) Loosen the fatty plug with a wooden stick by passing the stick between the inner wall of the tube and the plug. Discard the plug and the fluid both above and below it by inverting the tube, allowing only the last one or two drops to fall back into the tube. Discard this fluid, containing ethyl acetate and formalin, into a marked re-sealable liquid waste container.

vii) Re-suspend the pellet[[13]](#footnote-14) by agitation. Transfer the re-suspended contents on to a microscope slide with a disposable pipette, and air dry.

Commercial devices for concentrating helminth ova, larvae and protozoan cysts and oocysts using the formalin-ether method are available.

2.3.4. Staining methods

2.3.4.1. Modified Ziehl–Neelsen (mZN)

i) Strong carbol fuchsin

Dissolve 20 g basic fuchsin in 200 ml absolute methanol and mix on a magnetic stirrer until dissolved. Add 125 ml liquid phenol (general purpose reagent [GPR; 80% w/w in distilled water]) carefully until well mixed, and make up to the final volume with 1675 ml deionised water. Mix thoroughly. Filter before use through Whatman No.1 filter paper to remove debris and store in a stock reagent bottle. Label, date and initial. Store the stock reagent in a dark cupboard at room temperature. Commercial supplies are also available. The concentration of basic fuchsin can vary within the acceptable range of 1 to 3%.

ii) 1% acid methanol

Carefully add 20 ml concentrated hydrochloric acid to 1980 ml of absolute methanol and mix. Transfer to a stock reagent bottle, and label, date and initial. Commercial supplies are also available.

iii) 0.4% malachite green

Add 2 g malachite green to 480 ml deionised water and mix on a magnetic stirrer. Filter through Whatman No.1 filter paper into a stock reagent bottle, label, date and initial. Commercial supplies are also available.

2.3.4.1.1. Test procedure

Include a positive control slide each time this procedure is performed.

i) Wear protective clothing and disposable gloves. Fix the air-dried smear[[14]](#footnote-15) in methanol for 3 minutes.

ii) Immerse or flood the slide in cold strong carbol-fuchsin and stain for 15 minutes.

iii) Rinse the slide thoroughly in tap water.

iv) Decolourise in 1% acid methanol for 10–15 seconds[[15]](#footnote-16).

v) Rinse the slide in tap water.

vi) Counterstain with 0.4% malachite green for 30 seconds.

vii) Rinse the slide in tap water.

viii) Air-dry the slide.

ix) Examine for the presence of oocysts by scanning the slide systematically using the ×40 objective lens of a bright-field microscope. Confirm the presence of oocysts under the oil immersion objective lens[[16]](#footnote-17).

x) Measure the size and shape of the red-stained bodies using a calibrated eyepiece graticule.

*Cryptosporidium* spp. oocysts stain red on a pale green background. The degree and proportion of staining varies with individual oocysts. In addition, the internal structures take up the stain to varying degrees. Some may appear amorphous while others may contain the characteristic crescentic forms of the sporozoites. *Cryptosporidium parvum* oocysts appear as discs, 4–6 µm in diameter. Yeasts and faecal debris stain a dull red. Some bacterial spores may also stain red, but these are too small to cause confusion.

2.3.4.2. Auramine-phenol

i) Auramine phenol (AP)

Dissolve 3 g phenol in 100 ml deionised water and slowly add 0.3 g Auramine O. Filter through Whatman No. 1 filter paper into a stock reagent bottle. Label, date and initial the stock reagent. Store at room temperature in a light-proof glass bottle with an airtight stopper. Commercially available stains, such as Lempert’s reagent, are also acceptable. Commercial supplies are also available.

ii) 3% Acid methanol

Carefully add 60 ml concentrated hydrochloric acid to 1940 ml absolute methanol and mix. Transfer to a stock reagent bottle, and label, date and initial. Commercial supplies are also available.

iii) 0.1% potassium permanganate

Add 0.5 g potassium permanganate to the 499.5 ml deionised water and mix using a magnetic stirrer. Filter through Whatman No. 1 filter paper into a stock reagent bottle, and label, date and initial. Commercial supplies are also available.

2.3.4.2.1. Test procedure

Include a positive control slide each time you perform this procedure.

i) Wear protective clothing and disposable gloves. Fix air-dried smears or concentrates[[17]](#footnote-18) in absolute methanol for 3 minutes.

ii) Immerse the slides in AP stain for 10 minutes.

iii) Rinse in tap water to remove excess stain.

iv) Decolourise with 3% acid alcohol for 5 minutes.

v) Counterstain in 0.1% potassium permanganate for 30 seconds.

vi) Air dry slide at room temperature[[18]](#footnote-19).

vii) Examine for the presence of oocysts, using an epifluorescence microscope equipped with fluorescein isothiocyanate (FITC) or UV filters, by scanning the slide systematically under the ×20 objective lens. Confirm the presence of oocysts under the ×40 objective lens.

viii) Measure the size and shape of the fluorescent bodies using a calibrated eyepiece graticule (see below)[[19]](#footnote-20).

*Cryptosporidium* spp. oocysts appear ring or ovoid shaped and exhibit a characteristically bright apple-green fluorescence against a dark background. *Cryptosporidium parvum* oocysts are ring or doughnut shaped, measuring 4–6 µm in diameter. If available, view the preparation under a UV filter (excitation 355 nm, emission 450 nm), as sporozoites are more readily seen under the UV rather than the FITC filter set. Under the UV filter, oocysts appear light green and sporozoites appear yellow green.

2.3.4.3. Reporting results of microscopic examination

Negative specimens should be reported as ‘*Cryptosporidium* oocysts NOT seen’.

Positive specimens should be reported as ‘*Cryptosporidium* oocysts seen’.

A scoring system for positive samples can be used, based on the number of oocysts observed under the ×40 objective lens. However, microscopic examination cannot be considered as a quantitative determination as oocyst numbers vary considerably during the course of infection.

+ = less than 1 per field of view

++ = 1 to 10 oocysts per field of view

+++ = 11 or more oocysts per field of view

2.3.5. Immunological methods

Three approaches to the immunological detection of *Cryptosporidium* oocyst antigens have proven useful, immunofluorescence microscopy (IFM), ELISA and IC, and a variety of commercial kits are available. IFM kits are more specific for, and can be more sensitive at, detecting *Cryptosporidium* oocysts in faecal smears than conventional stains (Chalmers & Katzer, 2013). Detection limits of ELISA and IC have been reported in the region of 3 × 105 to 106 oocysts per ml (Anusz *et al.,* 1990; Smith, 2008), which is no more sensitive than conventional microscopical methods, and less sensitive than IFM. However, ELISA in 96-well plate format offers the advantage of streamlined testing of large numbers of samples while IC can be applied outside of the laboratory with less qualified staff. Positive reactions should be confirmed by another method.

2.3.5.1. Direct immunofluorescence microscopy (dIFM)

In dIFM, a FITC-conjugated anti-*Cryptosporidium* MAb (FITC-C-MAbs) that recognises surface-exposed epitopes of oocysts is used. It does not distinguish different species of *Cryptosporidium*. Epifluorescence using a FITC filter system causes the labelled oocysts to exhibit a bright apple-green fluorescence. Materials provided with commercial kits vary but *C. parvum* oocyst positive and negative controls, FITC-labelled anti-*Cryptosporidium* MAb (provided at the working dilution; it is false economy to dilute), and glycerol-based mounting medium containing a photo-bleaching inhibitor may be included.

Include a positive and negative control slide (usually supplied with the kit) each time this procedure is performed. *Cryptosporidium parvum* oocysts can be purchased from commercial suppliers, diagnostic veterinary laboratories or research facilities.

After staining and mounting slides according to manufacturers’ instructions, scan the preparation for oocysts under the ×20 and confirm under the ×40 objective of an epifluorescence microscope equipped with an FITC filter set (maximum excitation wavelength 490 nm, mean emission wavelength 530 nm). Measure oocysts under the ×100 objective18. If necessary, slides can be stored at room temperature, in the dark, until read.

*Cryptosporidium* spp. oocysts are round or slightly ovoid objects that exhibit a bright apple-green fluorescence under the FITC filter set. Their measurements (measured length × breadth) are presented in Table 1. Often the fluorescence has an increased intensity around the entire circumference of the oocyst, with no visible breaks in oocyst wall staining. If Evans’ blue, which reduces nonspecific fluorescence, is included in the kit, the background fluorescence will be red. If DAPI is included as counterstain, the nuclei will show blue fluorescence. Nonspecific fluorescence is usually yellow. Always refer to the positive control to ensure that the size, shape and colour of the putative oocyst is consistent with those of the positive control.

Results should be reported as for conventional microscopical methods (above). Numbers can be recorded as identified previously.

2.3.5.2. Detection of *Cryptosporidium* antigens by enzyme linked immunosorbent assay (ELISA)

In the ELISA, the presence of soluble *Cryptosporidium* antigens in faeces (coproantigen) is sought. Depending on the commercial kit, *Cryptosporidium* coproantigens are captured and detected using a mixture of monoclonal and polyclonal antibodies.

Commercially available sandwich ELISA antigen detection kits contain anti-*Cryptosporidium*-coated well strips for capturing *Cryptosporidium* coproantigens, anti-*Cryptosporidium* antibodies for developing the reaction that is conjugated to an enzyme (frequently horseradish peroxidase), substrate, chromogen/substrate development system and stopping solution (which inhibits further enzyme catalysis when added to the reaction mixture). The test may also be provided as a rapid membrane enzyme immunoassay in a single-test cartridge system.

These tests have been developed to detect *C. parvum* antigens in stool samples, but they are also capable of detecting common epitopes from infections with other *Cryptosporidium* species. Known negative and positive samples are included in commercial kits. Commercial kits normally contain all the necessary reagents to perform the analysis and the manufacturers’ instructions must be followed. It is false economy to dilute kit reagents to increase testing capacity. A comprehensive method and a formula for calculating the cut-off value and assigning positive or negative status to samples are usually included. Kit reagents are normally stored at 4°C when not in use. All reagents should reach room temperature before being used. The diagnostician should always determine whether any contraindications apply to the use of a commercial test and any stool/sample fixative used. Because of the variation in the methods described for different commercial kits, no method for ELISA or IC coproantigen detection is included in this chapter.

Negative reactions should be reported as ‘‘*Cryptosporidium* antigen NOT detected’.

Positive reactions should be reported as ‘*Cryptosporidium* antigen detected’.

It is good practice to confirm positive ELISA reactions using a test of equal or better sensitivity and specificity, such as dIFM or PCR with the result included in the report.

2.3.5.3. Detection of *Cryptosporidium* antigens by immunochromatography

Rather than relying on molecular diffusion to dictate the rate of antigen binding by the capture antibody as in the ELISA format, which normally takes about an hour per reaction, in lateral flow IC, the speed of antigen binding to the solid phase-bound capture antibody is increased by a wicking action. This draws all fluids rapidly through a membrane enclosed in the immunochromatography cassette and reduces the time required for analysis from hours to minutes or seconds. Soluble *Cryptosporidium* antigens in the test sample are drawn through the membrane and come into contact with, and bind to, immobilised antibodies raised against *Cryptosporidium* antigens, which dramatically increases the speed of antigen–antibody interaction. Positive reactions are qualitative and are seen as a band of colour at a specific location on the membrane, normally identified by a line on the cassette. The assay format can vary between commercial kits. The diagnostician should always determine whether any contraindications apply to the use of a commercial test and any fixative used.

IC is a convenient and rapid method for detecting *Cryptosporidium* antigen in stool samples, although false-positive reactions have been reported and positive reactions must be confirmed by a different test. Sensitivity is less than reported for ELISA, dIFM and PCR.

Negative reactions should be reported as ‘*Cryptosporidium* antigen NOT detected’

Positive reactions should be reported as ‘*Cryptosporidium* antigen detected’

It is good practice to confirm positive IC reactions ~~and confirmed~~ using a test of equal or better sensitivity and specificity, such as dIFM or PCR, with the result included in the report.

2.3.6. Nucleic acid recognition methods

PCR offers improved diagnostic sensitivity compared with microscopy and immunological assays for detecting *Cryptosporidium* in faeces (de Waele *et al.,* 2011). The target is the sporozoite DNA within the oocysts. The reported sensitivity of published PCR methods can range between 1 and 106 oocysts, depending on the copy number of the gene target, the oocyst disruption, DNA extraction, amplification and detection reagents, procedures and platforms.

Faecal samples can contain many PCR inhibitors. In addition to bilirubin and bile salts, complex polysaccharides are also significant inhibitors. Boiling faecal samples in 10% polyvinylpolypyrrilidone (PVPP) before extraction can reduce inhibition, but may not be necessary if abrogation steps are taken during DNA extraction (for example, spin columns) and in the PCR (including bovine serum albumin or appropriate mastermix). Faeces or partially purified oocysts stored in an equal volume of 5% K2Cr2O7 and intended for PCR should be washed in deionised water to remove residual preservative prior to DNA extraction. For oocysts in suspension, a series of three washes each followed by centrifugation (1100 ***g*** for 10 minutes), removal of the supernatant and resuspension of the pellet in deionised water should minimise PCR inhibition.

No standard method for disrupting oocysts and extracting *Cryptosporidium* sporozoite DNA exists. *Cryptosporidium* DNA can be extracted either following partial purification of oocysts using one of the flotation/sedimentation techniques described above, or directly from oocysts in faeces. If concentration by formol–ethyl acetate sedimentation is the routine laboratory test, oocyst concentrates suitable for lysis and amplification by PCR can be made by washing the pellets by centrifugation in deionised water. Options for oocyst disruption include bead-beating, freeze–thaw cycles, heating or chemical/enzymatic treatments (Elwin *et al*., 2012). Options for subsequent DNA extraction include commercial spin columns, glassmilk, and chelex resin.

Care is necessary when choosing PCR primers, as some are genus-specific whereas others are species-specific. Validated hydrolysis probe-based real-time PCR assays have been used for detection and identification of *Cryptosporidium parvum* and other selected species in livestock (De Waele *et al.,* 2011) and human (Robinson *et al.,* 2020) samples.

Commercial single and multiplex PCR-based assay kits for the detection of comprehensive panels of gastrointestinal pathogens are becoming available (including for veterinary applications), utilising robotic platforms for DNA extraction, assay set-up and amplicon detection. Pathogen panels should ideally be tailored for the population under investigation. Loci incorporated in such assays include SSU rRNA and *Cryptosporidium* oocyst wall protein (COWP) genes.

2.3.7 Detection of oocysts in drinking water and food

There are standard methods for the detection of *Cryptosporidium* oocysts in drinking water (e.g. International Organization for Standardization [ISO, 2006], The Environment Agency [2010], Environmental Protection Agency [2012]) and fresh leafy green vegetables and berry fruits (ISO, 2016), based on high volume filtration, elution, concentration, immunomagnetic separation and IFM. ~~A standard method for the detection of~~ *~~Cryptosporidium~~* ~~in leafy green vegetables and soft berry fruits is in preparation by the International Standards Organisation.~~

2.3.8. Typing and subtyping for disease and source tracking

Molecular tools for inter- and intra-species discrimination are usually applied in specialist or reference laboratories for investigating transmission, identifying sources of infection and identifying specific risk factors.

DNA sequence analysis of the *Cryptosporidium* SSU rRNA gene using the “Xiao/Jiang primers” (Roellig & Xiao, 2020; Xiao & Ryan, 2008) is widely regarded as the benchmark for species identification, as not all *Cryptosporidium* species or genotypes can be identified by procedures such as restriction fragment length polymorphisms (RFLP); however, most of the species that are currently known to be commercially important for livestock can be identified by PCR-RFLP using *Vsp*I and *Ssp*I (Xiao & Ryan, 2008). For bovine samples, *Dde*I can be included for differentiation of *C. andersoni* and *C. muris*, and *Mbo*II for differentiation of *C. parvum* from *C. bovis* or *C. ryanae* (Feng *et al.,* 2007; Roellig & Xiao, 2020). Sequencing of the amplicon is needed for investigation of water or environmental samples where any species or genotype may be present.

Recent work has confirmed the utility of mini- and micro-satellite markers in the study of the population structure of *Cryptosporidium*, but there is a need to harmonise the entire method from marker selection to analytical algorithms (Widmer & Caccio, 2015).

3. Serological tests

Most assays for *Cryptosporidium* antibodies are ELISA based, using various aqueous extracts of native antigens (e.g. Hill *et al*., 1990) or recombinant proteins (e.g. Priest *et al.,* 2006) derived from *C. parvum* oocysts. They have limited application for epidemiological surveillance, and results should be interpreted with caution as the tests are not fully validated.

C. REQUIREMENTS FOR VACCINES

There is no commercial or rigorously tested vaccine available.

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**NB**: First adopted in 2004. Most recent updates adopted in 2016.

1. Positive reactions in ELISA or IC tests should be confirmed. [↑](#footnote-ref-2)
2. Alternatively, a pencil can be used to mark the etched (frosted) portion of a frosted glass microscope slide. [↑](#footnote-ref-3)
3. For formed stools, the sample should include portions from the surface and from within the stool. [↑](#footnote-ref-4)
4. Moderately thick smears are recommended for this procedure.If the smear is too thin or thick, oocysts will be missed. An acceptable thickness can be achieved when either the hands of your watch or the print on this page can just be read when viewed through the preparation. [↑](#footnote-ref-5)
5. Air-dried, methanol-fixed smears can be kept at room temperature for >6 months before staining. [↑](#footnote-ref-6)
6. For formed stools, the sample should include portions from the surface and from within the stool. [↑](#footnote-ref-7)
7. Centrifugation at speeds higher than 1100 ***g*** for longer (>5 minutes) periods of time is not advised as some parasites may deform or rupture and collapse. [↑](#footnote-ref-8)
8. This is the size of a pea. [↑](#footnote-ref-9)
9. The sample should include portions from the surface and from within a formed stool. [↑](#footnote-ref-10)
10. 425 µm aperture, 38 mm diameter is equivalent to 36 mesh British Standard (BS 410-86) or 40 mesh American Standard (ASTM E11-81). The skirt of the sieve should fit neatly into the rim of the beaker. Both the sieve and the beaker should be washed thoroughly in running tap water between each sample. [↑](#footnote-ref-11)
11. Ethyl acetate, although less flammable than diethyl ether, which was used previously, is nevertheless flammable, therefore the procedure should be performed in well ventilated areas, ensuring that they contain no naked flames. Avoid prolonged breathing or skin contact. [↑](#footnote-ref-12)
12. Centrifugation at speeds higher than 1100 ***g*** for longer (>5 minutes) periods of time is not advised as some other parasites may deform or rupture and collapse. [↑](#footnote-ref-13)
13. Too large a pellet is indicative of one or more of the following: centrifuging above the recommended speed or time, insufficient shaking (step iv), taking too large a faecal sample. [↑](#footnote-ref-14)
14. Moderately thick smears are recommended for this procedure. [↑](#footnote-ref-15)
15. Over-destaining must be avoided. [↑](#footnote-ref-16)
16. The smear can be examined with or without a cover-slip. [↑](#footnote-ref-17)
17. Moderately thick smears are recommended for this procedure. [↑](#footnote-ref-18)
18. Do not blot slides dry, as some blotting papers contain fluorescent fibres. [↑](#footnote-ref-19)
19. Putative oocysts are measured by slowly increasing the voltage (light intensity) of the bright-field light source so that both fluorescent and bright-field images can be seen concurrently. Objects can then be measured with the eye-piece graticule. [↑](#footnote-ref-20)