Cryptosporidium parvum is a coccidian parasite that infects the microvilli of intestinal epithelial cells. Strains that infect horses and cattle can also infect man, and therefore cryptosporidiosis is a zoonotic disease. In horses, cryptosporidiosis is most commonly seen in foals (most frequently 1–4 weeks of age) and is associated with diarrhoea and weight loss. Immunocompromised foals (including foals with severe combined immunodeficiency syndrome) are particularly at risk.

Introduction
Cryptosporidium is increasingly gaining attention as a human and an animal pathogen mainly due to its dominant involvement in worldwide waterborne outbreaks (Grinberg et al. 2003; Karanis et al. 2007; Smith et al. 2007). Diarrhoea in horses, primarily foals, caused by Cryptosporidium parvum is also being increasingly recognised (Xiao et al. 2004; Sunnotel et al. 2006). C. hominis (formerly known as the C. parvum human genotype or genotype I) almost exclusively infects man, while C. parvum (formerly known as the C. parvum bovine genotype or genotype II) infects man, ruminants and other animal species (Morgan-Ryan et al. 2002). It is classically known to be responsible for the majority of the zoonotic cryptosporidial infections. More recently, other species including C. meleagridis, C. suis, C. felis and C. canis have been detected in man, which emphasises the risk posed due to the zoonotic transmission of the parasite (Xiao et al. 2001, 2004; Cama et al. 2007; Llorente et al. 2007).

Although classically described as ‘unusual’ or ‘unique’ coccidia, Cryptosporidium species are probably better considered as a distantly related lineage of apicomplexan parasites that are not in fact coccidia but that do occupy many of the same ecological niches (Barta and Thompson 2006).

Epidemiology
The prevalence of cryptosporidiosis in horses in different geographical locations is poorly documented. Prevalence varies with the method of detection used and the population studied. The prevalence of faecal shedding of oocysts by horses is low (Reinemeyer et al. 1984; Coleman et al. 1989; Olson et al. 1997; Cole et al. 1998; Atwill et al. 2000; Majewska et al. 2004; Chalmers et al. 2005; Bakheit et al. 2008). In contrast, a serological survey in the UK indicated that 20 of 22 horses (91%) were seropositive, suggesting that sub-

**Keywords:** horse; coccidia; Cryptosporidium parvum; cryptosporidiosis; zoonosis; diarrhoea

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**Summary**
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clinical infection may be common (Tzipori and Campbell 1981). In another study, the prevalence of faecal shedding of *C. parvum* oocysts in 152 horses in Texas was found to be 8.5% (Cole *et al.* 1998). Specific risk factors for faecal shedding in this study included residence on specific breeding farms, age <6 months and a history of diarrhoea within the preceding 30 days. Prevalence may be 100% among diarrhoeic foals at farms during an outbreak.

Horses become infected by ingesting infective *C. parvum* oocysts. Transmission occurs either via the faecal-oral route or by ingestion of contaminated food or water. In people, contaminated water supplies are an important source of infection (Smith *et al.* 2006), and a similar route may be important in horses. Three features of *Cryptosporidia* ensure a high level of environmental contamination and enhance the likelihood of waterborne transmission. Firstly, they are responsible for disease in a broad range of hosts including man, and have a low infectious dose enhancing the possibility of zoonotic transmission. Secondly, their transmissive stages are small in size and environmentally robust. Thirdly, they are insensitive to the disinfectants commonly used in the water industry (Smith and Nichols 2006). The oocyst stage can remain infective under cool, moist conditions for many months, especially where water temperatures in rivers, lakes, and ponds remain low but above freezing (Frayer 2004). Exposure of foals to cattle and adult horses was not found to be a risk factor for cryptosporidiosis (Cole *et al.* 1998). Likewise, there is little evidence to suggest that mares are an important source of infection of their foals, although infection of calves from their dams at birth has been reported (Pearson and Logan 1978). Inapparently-infected foals may represent a source of infection for other foals.

**Pathogenesis**

Unlike typical coccidia, *Cryptosporidium* oocysts are sporulated and infectious from the time they are excreted into the faeces (Huang and White 2006). The oocysts exsheath and sporozoites are released during passage through the gastrointestinal tract, allowing infection of enterocytes. Cryptosporidia develop at the apical surfaces of gastrointestinal epithelial cells, beneath the cell membrane, but separate from the host cell cytoplasm (intracellular but extracytoplasmic) (Marcial and Madara 1986) (Fig 1). They are closely associated with the
microvillous border of the epithelial cells (Levine 1973; Pearson and Logan 1978, 1983). They damage the intestinal microvilli, resulting in malabsorption, maldigestion and diarrhoea. Amplification occurs through asexual and sexual multiplication. Oocysts are formed that are capable of autoinfection prior to excretion (thin-walled oocysts) or that are immediately infectious when shed in faeces (thick-walled oocysts) (Moon and Woodmansee 1986). In foals with severe combined immunodeficiency, sites other than the small intestine may be infected, including the stomach, common bile duct, colon and pancreatic ducts (Field 2002).

The severity of clinical signs may be related to agent factors (inoculum size, virulence), host factors (age, immunocompetence), and environmental factors (water source, housing practices).

Clinical signs
In horses, clinical disease due to cryptosporidiosis is most commonly reported in foals, particularly from 1–4 weeks of age, although cryptosporidial diarrhoea has occasionally been diagnosed in younger foals (at age 2 days) and in weanlings and yearlings (Netherwood et al. 1996). The relatively naïve or immature immune system of newborn foals is likely a predisposing factor for cryptosporidiosis. Cryptosporidial diarrhoea is rare in mature horses; however, subclinical infection is probably common in both healthy foals and adult horses (Cohen and Snowden 1996). The incubation period of cryptosporidiosis is 3–7 days. The clinical features include persistent diarrhoea, with associated dehydration, weakness and death in some cases if untreated (Grinberg et al. 2003). Clinical signs usually persist for 5 to 14 days (Mayhew and Greiner 1986), but in older foals (i.e. 3–6 months) the diarrhoea may be more chronic and persist until the foals are aged 9–12 months. Reports of Cryptosporidium oocyst shedding in adult horses with diarrhoea are rare (McKenzie and Diffay 2000). Cryptosporidiosis has been recognised in foals that are hospitalised for other problems, suggesting that the stress of hospitalisation and other disease can predispose them to develop clinical disease. Cryptosporidiosis is also common in foals with severe combined immunodeficiency syndrome (Snyder et al. 1978; Gibson et al. 1983; Mair et al. 1990). Although the disease will generally be more severe in immunocompromised foals, severe or fatal diarrhoea can also occur in immunocompetent foals. Concurrent infection with other enteropathogens (including Salmonella spp, rotavirus, coronavirus, adenovirus) can occur, particularly in immunocompromised animals (Mair et al. 1990).

Some farms experience epidemics of cryptosporidial diarrhoea, although recurrence during subsequent years is rare. A high density of foals, a municipal water source, foaling in stalls (vs. pasture), and poor hygiene may be risk factors for infection and disease (Cohen 2002).

Diagnosis
Diagnosis is based on the detection of oocytes in the faeces. However, C. parvum oocysts are small (4–5 µm in diameter) and are difficult to identify by light microscopy using routine faecal parasitological examinations. Faecal samples should be submitted as fresh material or in recommended preservative (10% formalin or sodium acetate-acetic acid-formalin). Oocysts can be detected using either concentration or staining techniques. Concentration of oocysts may be accomplished by flotation or sedimentation. Regardless of technique, distinguishing oocysts from yeast is an important diagnostic issue.

A number of different diagnostic techniques are available, and include:

• Flotation of oocysts
• Acid-fast staining of oocysts (Cole et al. 1999)
• Detection of oocysts using an immunofluorescence assay (IFA) (Cole et al. 1999)
• Flow cytometry (Cole et al. 1999)
• ELISA (Werner et al. 2004)
• PCR and loop-mediated isothermal amplification of DNA (LAMP) (Bakheit et al. 2008).

Sedimentation techniques are rarely used. Of the flotation techniques used, flotation in Sheather’s sugar solution is most common. Prompt processing is important because oocysts collapse and lose their spherical shape when left in Sheather’s sugar solution.

Acid-fast staining of faecal specimens is widely used for detection of C. parvum. The organisms appear as red spheres (4–6 mm in diameter) against a dark, counter-stained background, while yeasts
generally do not appear red. The technique has relatively poor specificity making it a poor choice as a screening test. However, it is useful clinically as a diagnostic test because of its good sensitivity, availability and low cost.

The IFA test has relatively low sensitivity but excellent specificity. A commercial immunofluorescence assay is available\(^1\) that simultaneously detects cryptosporidial and giardial organisms. The high cost relative to staining techniques and specialised microscopic equipment needed are limitations of the IFA. To date, reliable enzyme-linked immunosorbent assays have not been developed and validated for detecting *C. parvum* in samples from horses. Flow cytometric methods are more sensitive than IFA or acid-fast staining, but are not widely available.

The pattern of oocyst shedding by foals is variable in duration (from days to many weeks) and can be intermittent. Shedding may be antecedent, concurrent or subsequent to the onset of diarrhoea. In view of the variable duration and the intermittent pattern of shedding, multiple samples (at least 3) should be submitted for detecting *C. parvum* in faeces from foals. It may be easier to detect oocysts in unformed than formed faeces.

**Treatment**

Although numerous different treatments have been tested in a variety of animals, to date no specific chemotherapy or immunotherapy has been proven to be convincingly effective for treating *C. parvum* in people and other mammals, and none has been evaluated in a controlled clinical trial among foals (Cohen 2002). In man, the most commonly used drugs for the treatment of cryptosporidiosis include paromomycin, nitazoxanide and azithromycin (Farthing 2006; Gargala 2008). A recent study of the prophylactic and therapeutic use of nitazoxanide in calves did not show the expected positive effect on the course of the *Cryptosporidium* infection, neither on reducing the clinical severity, nor on oocyst excretion (Schnyder *et al.* 2008). Those treatments that may have greatest potential for use in foals include paromomycin and bovine colostrum.

Paromomycin is an aminoglycoside antibiotic that is poorly absorbed from the gastrointestinal tract. Paromomycin reduced the duration and severity of diarrhoea and eliminated oocyst shedding in neonatal calves experimentally infected with *C. parvum*. Doses used in calves have ranged from 50–100 mg/kg bwt administered orally once or twice daily. No data exist for the use of this drug in foals. Adverse effects of paromomycin in man include diarrhoea, nausea and abdominal cramps. As for all other agents used to treat cryptosporidial infection, experimental and clinical evidence also exists indicating a lack of effectiveness of paromomycin. No antibiotic approved for use in horses has been demonstrated to be effective in the treatment of cryptosporidial diarrhoea.

Hyperimmune bovine colostrum has been used with varying success as a means of prophylaxis and therapy of cryptosporidiosis in animals and patients with AIDS. A factor limiting the use of hyperimmune bovine colostrum is its availability. Pooled bovine colostrum, however, is more readily available. Pooled bovine colostrum from nonimmunised animals also may be protective in controlling cryptosporidiosis; non-immunoglobulin factors in the colostrum may provide protection. Use of hyperimmune or pooled bovine colostrum has not been uniformly successful (Cohen 2002). The benefits of administration of colostrum or hyperimmune colostrum to foals, regardless of their age, with cryptosporidiosis are unknown.

Treatment of foals with severe combined immunodeficiency is likely to be unsuccessful. In immunocompetent foals, infection is often subclinical or mild and self-limiting; in these foals no treatment or supportive care is needed. In more severely affected foals further treatment may be necessary.

**Control and prevention**

The prevention and control of cryptosporidiosis can be difficult. Currently, immunisation effective at preventing cryptosporidiosis in horses and foals is lacking. Although some chemotherapeutic agents have shown preventive potential, the cost-effectiveness of such prophylaxis is often a limiting factor. Oocysts shed in feces are infective, extremely resistant to environmental factors, and can survive for months if not exposed to extremes of temperature or desiccation. Oocysts are highly resistant to most chemical disinfectants (Tzipori 1983). Moist heat (pasteurisation to >55°C or live steam), freezing, or thorough drying
may be the most effective means of killing oocysts (Anderson 1985). Exposure to 5% ammonia solution or 10% formalin for 18 h will also kill oocysts (Tzipori 1983). Good sanitation may help by decreasing the oocyst burden in the foals’ environment. Specific sanitation strategies would include providing uncontaminated water, rigorous cleaning (preferably with steam) and disinfecting foaling stalls, removing all the bedding, and isolating diarrhoeic foals.

Zoonotic considerations

Ingestion of oocysts can cause gastrointestinal disease in immunocompetent and immunosuppressed human patients. Those working with animals, including farmers and veterinarians, are considered to be at increased risk (Moon and Woodmasnee 1986; Konkle et al. 1997; Mahdi and Ali 2002). Cryptosporidiosis has occurred in veterinary students exposed to infected calves and foals (Anderson et al. 1982; Pohjola et al. 1986; Levine et al. 1988; Gait et al. 2008). Cryptosporidium hominis is spread only among humans, but the major reservoir for C. parvum is domestic livestock, predominantly cattle, and direct contact with infected cattle is a major transmission pathway along with indirect transmission through drinking water (Hunter and Thompson 2005). Efforts to minimise transmission in people handling infected foals should include instruction regarding, and rigorous attention to, hygiene, protective clothing (possibly to include face mask, gloves, gown or coveralls, and boots) and efforts to disinfect contaminated areas. Those with primary or acquired immunodeficiency should not be exposed to foals with contaminated areas. Those with primary or acquired immunodeficiency should not be exposed to foals with diarrhoea in which a diagnosis of cryptosporidiosis is possible. In view of the low prevalence of infection, mature horses do not appear to be an important source of environmental contamination (Johnson et al. 1997; Atwill et al. 2000).

Over the past decade molecular methods have enabled the characterisation and identification of species and genotypes within the Cryptosporidium genus. The taxonomy is under continual review, but so far 20 valid species and numerous genotypes have been described. Recently, a long-term genotyping study in the United Kingdom identified 3 unusual Cryptosporidium genotypes (skunk, horse and rabbit) in human patients with diarrhoea (Robinson et al. 2008). The horse genotype was found in a 30-year-old immunocompetent woman from a rural area of southwest England, who reported swimming and foreign travel (destination unknown) but no contact with animals during the incubation period. A genetic study of 9 C. parvum isolates from diarrheic foals in New Zealand were genetically diverse, markedly similar to human and bovine isolates, and carried GP60 I1aA18G3R1 alleles, indicating a zoonotic potential (Grinberg et al. 2008).

Manufacturer's address

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References


