An Irish perspective on Cryptosporidium*

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Cryptosporidiosis, a protozoal disease which causes significant morbidity in humans, is one of the chief causes of diarrhoea in neonatal ruminants. Although the parasite poses a significant threat to public health and animal health in Ireland, its epidemiology on the island is only poorly understood. Environmental studies have shown the waterborne parasite to be widespread in some untreated waterbodies around Ireland. The island’s hydrogeological situation, combined with high stocking rates of livestock and the absence of filtration from regular water treatment, render it vulnerable to large-scale outbreaks. This review discusses the parasite in the Irish context and underlines the need for a reference facility to provide active surveillance on the island.

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Pathology and prevalence of cryptosporidiosis in domestic animals

Cryptosporidiosis in ruminants is said to be on the increase (Castro-Hermida et al., 2002) and it is now considered a major disease and one of the main causes of morbidity and mortality in newborn livestock (Casey, 1991; Munoz et al., 1996; de Graaf et al., 1999). C. parvum is the predominant parasite in cattle, sheep and goats, while C. andersoni and C. bovis also occur in cattle (one C. bovis infection has also been reported from a two-week-old lamb: Fayer et al., 2005). The latter two are not infective to humans and, apparently, are less pathogenic to cattle than C. parvum (Santin et al., 2004; Xiao et al., 2004). Cryptosporidiosis in calves, lambs and goat kids is characterised by diarrhoea, anorexia, abdominal pain, apathy and depression. It has been suggested that destruction of the intestinal epithelia by the parasite may also increase susceptibility to other enteric pathogens (Casey, 1991; de Graaf et al., 1999; Lefay et al., 2001) but, so far, synergistic effects have not been reported (Browning et al., 1991; Munoz et al., 1996; Netherwood et al., 1996). Severe cases are characterised by excessive loss of fluid and electrolytes with subsequent metabolic acidosis, which may prove fatal in some circumstances (de Graaf et al., 1999; Constable, 2002). Generally, the disease lasts between three and 12 days with protracted oocyst shedding well beyond recovery (Casemore et al., 1997). There are no reports of clinical cryptosporidiosis in adult cattle, sheep or goats, although they may shed oocysts asymptomatically (Lorenzo-Lorenzo et al., 1993; Casemore et al., 1997; de Graaf et al., 1999; Causapé et al., 2002; Sturdee et al., 2003).

The parasite is common in ruminants throughout Europe but reported prevalence rates vary widely. Among calves up to 100% of a herd may be affected, especially if the animals are housed communally (Casemore et al., 1997; de Graaf et al., 1999; Joachim et al., 2003; Sturdee et al., 2003). In Ireland, two studies have been carried out in commercial beef abattoirs: they reported prevalences between 5.5% (adult cattle only: de Waele et al., 2003) and 7.3% (including both adults and calves: Moriarty et al., 2005) with a marked spring peak. In both studies, most infections were due to the non-zoonotic species C. andersoni. Among lambs and goat kids, particularly high prevalences of 40% to 70% have been reported from Spain (Munoz et al., 1996; Casemore et al., 1997; Castro-Hermida et al., 2002; Causapé et al., 2002). In the UK, infection rates appear to be somewhat lower: Sturdee et al. (2003) reported an average incidence of 23%. Information is not available about the prevalence of cryptosporidiosis in sheep or goats in Northern Ireland or in the Republic of Ireland. A study of the prevalence of cryptosporidiosis in a commercial deer herd reported widespread asymptomatic infections in both calves and...
adult deer hinds associated with low levels of oocyst shedding all year around (Skerrett and Holland, 2001). Among all ruminants, incidence rates usually peak during calving and lambing seasons. This is thought to be due to the presence of large groups of highly susceptible neonates in close proximity to each other and to dams, which may experience a periparturient rise in oocyst shedding (Casemore et al., 1997; Skerrett and Holland, 2001; Moriarty et al., 2005).

The importance of cryptosporidiosis in horses is unclear. Until recently, clinical disease was thought to be restricted to immunodeficient foals, in which the infection can spread throughout the intestine and bile duct and become life-threatening (Bjorneby, 1991). Later studies demonstrated cryptosporidiosis in immunocompetent foals and adult horses. In foals, the disease may be asymptomatic (Browning et al., 1991) or it may cause mild to severe diarrhoea, in which case it may become fatal (Grinberg et al., 2003; Chalmers et al., 2005; Chalmers and Grinberg, 2005:). There is a single report in the literature of Cryptosporidium-associated diarrhoea in a mature horse (McKenzie and Diffay, 2000). Generally, infected mature horses shed small numbers of oocysts without developing clinical signs (Majewska et al., 2004). With the exception of a novel horse genotype identified in a single Przewalski’s wild horse (Ryan et al., 2003a), the only Cryptosporidium species identified in horses is C. parvum. In the UK, reported prevalence rates in foals and horses range between 3% and 20% (Netherwood et al., 1996; Sturdee et al., 2003; Chalmers et al., 2005a). A study of 21 normal and 285 diarrhoeic foals from British and Irish stud farms reported prevalences of 27% and 29%, respectively (Browning et al., 1991).

Three species infect pigs: C. suis (formerly pig genotype I), pig genotype II and, occasionally, C. parvum (Ryan et al., 2003b; Ryan et al., 2004). Experimental Cryptosporidium infections in pigs and cats (caused by Cryptosporidium cesticola and Cryptosporidium felis, respectively) has only been reported from animals that are immunosuppressed by concurrent viral infections such as canine distemper, parvoviral gastroenteritis, feline leukaemia or feline immunodeficiency (Morgan et al., 1998; Denholm et al., 2001; Fayer et al., 2001). Furthermore, it has been reported that dogs and cats only rarely act as asymptomatic carriers (Casemore et al., 1997; Fayer et al., 2001; Cirak and Bauer, 2004), while PCR screening has shown that many are chronically infected at levels that are too low to be detected microscopically (Professor Una M. Ryan, personal communication, March 2006).

In chickens and other domestic fowl, extra-intestinal infections are much more common than in mammals. The parasite infects the microvillous regions of the digestive tract, the respiratory tract, the bursa of Fabricius and the urinary tract. Mortality rates may be high (de Graaf et al., 1999). It has been suggested that respiratory cryptosporidiosis is due to inhalation of oocysts associated with aerosolised faecal dust. This may be followed by intestinal infections as oocysts are coughed up and swallowed (Trampel et al., 2000). Respiratory infections are characterised by coughing, sneezing, dyspnea and rales (Fayer et al., 1997). Chickens with intestinal infections suffer diarrhoea and severe enteritis. Renal cryptosporidiosis is associated with pale and enlarged kidneys, while the ureters may become partially obstructed, resulting in vesicular gout (Trampel et al., 2000). Most infections occur in chickens under 11 weeks of age (Goodwin and Brown, 1990; Fayer et al., 1997). The chief avian species are Cryptosporidium baileyi, Cryptosporidium galli and Cryptosporidium meleagridis.

**Diagnosis and detection**

Most microbiological departments of larger hospitals now test for Cryptosporidium if requested by the clinician or if cryptosporidiosis is indicated by the clinical presentation or the age of the patient. In Northern Ireland, faecal samples from suspected animal cases are tested on request at the laboratories of the Department of Agriculture and Rural Development (Veterinary Sciences Division). In the Republic of Ireland, that service is available at the Regional Veterinary Laboratories, at the Veterinary Hospital, University College Dublin and at the Central Veterinary Research Laboratory, Abbotstown, Dublin. The majority of these laboratories use either modified Ziehl-Neelsen stain or phenol-auramine stain (OIE, 2004: UK National Reference Method 002: www.defra.gov.uk/animals/diseases/vetsurveillance/pdf/nrm-002crypto.pdf), or a combination of both, to detect oocysts (safefood website: www.safefoodonline.com/safefood/login_extranet.asp; Lowery et al., 2001).

In the UK, the legal limit for the presence of Cryptosporidium is one oocyst/10 litres drinking water (UK Drinking Water Regulations 1999, S.I. No 1527). However, numerous outbreaks have been reported at oocyst concentrations well below this threshold (Report of Waterborne Cryptosporidiosis Subcommittee of the Scientific Advisory Committee, 2004). Current EU legislation does not set limits for Cryptosporidium oocysts in drinking water. Similarly, there are no guidelines for the presence of oocysts in food, even though the number of reported foodborne outbreaks is on the increase. To our knowledge there are currently three laboratories on the island that test environmental samples for the presence of Cryptosporidium oocysts: in Northern Ireland, the Water Service Northern Ireland, Altnagelvin, Derry and, in the Republic of Ireland, the Central Laboratory, Dublin City Council, Dublin 8, and City Analysts Ltd., Ringsend, Dublin 4. Detection of oocysts in water samples and viability testing involves immunomagnetic separation (IMS), immuno-fluorescence (IFA) and vital dye staining as recommended by the Drinking Water Inspectorate (Regulations 1999, SI No.1524, June 1999) and the EPA (USEPA Methods 1622 and 1623).

Genotyping to species level helped to identify the sources of contamination responsible for the three outbreaks in Northern Ireland during 2000 and 2001 (Glaberman et al., 2002) and the 2002 outbreak in Westmeath (Jennings and Rhatigan, 2002). However, in the absence of a National Cryptosporidium Reference Laboratory, genotyping is not routinely carried out, although this information would be vital for a better understanding of the chief transmission routes of the parasite on this island.

**Treatment**

In most immunocompetent patients and young animals, the disease is self-limiting and resolves without chemical intervention. For the treatment of more severe cases, over 100 agents have been tested with varying degrees of success (Hommer et al., 2003). Currently,
the only drug approved by the US Food and Drug Administration for treatment of cryptosporidiosis in children is the anti-protozoal agent nitazoxanide (Alinia) (Carey et al., 2004). Although some studies reported obvious benefits to both immunocompetent and immunocompromised people (Carey et al., 2004; Smith and Corcoran, 2004), other workers have questioned its usefulness to HIV-infected patients (Zardi et al., 2005). Another drug paromomycin, an aminocyclitol antibiotic isolated from Streptomyces, was previously reported to alleviate symptoms and reduce oocyst excretion in human infections. More recently, inconsistent results have raised questions concerning its efficacy against human cryptosporidiosis (Harp, 2003; Siripanth et al., 2004; Zardi et al., 2005).

To date, no agent has been approved for the prevention or therapy of cryptosporidiosis in calves (Jarvie et al., 2005). Though of questionable efficacy in humans, paromomycin was successfully used as a prophylaxis in dairy calves, where it reduced the duration and severity of diarrhea (Fayer and Ellis, 1993). Other studies have shown that the efficiency of paromomycin can be improved by combined use with protease inhibitors (Homer et al., 2003) or recombinant IL-12 (Gamra and el-Hosseiny, 2003). The antiprotozoal drug halofuginone lactate (Halocur), a synthetic quinazolinone, can delay the establishment of infections in calves and reduce the severity of cryptosporidiosis. During treatment, oocyst excretion is reduced, contributing to decreased contamination of the environment (Jarvie et al., 2005). However, low levels of oocyst shedding may recur following withdrawal of the drug (Lefay et al., 2001; Joachim et al., 2003). Unfortunately, both drugs have disadvantages that preclude their widespread use in food animals: both are relatively toxic and require exact doses. Paramomycin is very expensive. Decoquinate, which is highly effective against some other apicomplexans, was found by some to be beneficial in both infected calves and goat kids (Redman and Fox, 1994; Mancassola et al., 1997). Others reported little activity against Cryptosporidium in vitro, or in vivo in calves or suckling mice (Lindsay et al., 2000; Moore et al., 2003). The starch-derived excipient beta-cyclodextrin was shown to be highly effective as a prophylactic and therapeutic agent in neonatal lambs (Castro-Hermida et al., 2002). Promising results have also been achieved with a number of other drugs, such as lasalocid, nigericin (two ionophorous antibiotics) and alpha-cyclodextrin in vivo and in the murine model (Castro-Hermida et al., 2000; Giacometti et al., 2000; Castro-Hermida and Ares-Mazos, 2003), but so far they have not been tried in ruminants.

Since there is no drug that achieves the complete removal of Cryptosporidium from an infected host, and no compound is known to eradicate the infection, prophylactic and supportive treatment are the only options. The most effective method of prophylaxis is to use drugs, such as tetracycline, which have been shown to delay the establishment of infections in calves and reduce the severity of cryptosporidiosis in humans. A number of studies have shown that the efficiency of tetracycline can be improved by combined use with protease inhibitors (Homer et al., 2003) or recombinant IL-12 (Gamra and el-Hosseiny, 2003). The antiprotozoal drug halofuginone lactate (Halocur), a synthetic quinazolinone, can delay the establishment of infections in calves and reduce the severity of cryptosporidiosis. During treatment, oocyst excretion is reduced, contributing to decreased contamination of the environment (Jarvie et al., 2005). However, low levels of oocyst shedding may recur following withdrawal of the drug (Lefay et al., 2001; Joachim et al., 2003). Unfortunately, both drugs have disadvantages that preclude their widespread use in food animals: both are relatively toxic and require exact doses. Paramomycin is very expensive. Decoquinate, which is highly effective against some other apicomplexans, was found by some to be beneficial in both infected calves and goat kids (Redman and Fox, 1994; Mancassola et al., 1997). Others reported little activity against Cryptosporidium in vitro, or in vivo in calves or suckling mice (Lindsay et al., 2000; Moore et al., 2003). The starch-derived excipient beta-cyclodextrin was shown to be highly effective as a prophylactic and therapeutic agent in neonatal lambs (Castro-Hermida et al., 2002). Promising results have also been achieved with a number of other drugs, such as lasalocid, nigericin (two ionophorous antibiotics) and alpha-cyclodextrin in vivo and in the murine model (Castro-Hermida et al., 2000; Giacometti et al., 2000; Castro-Hermida and Ares-Mazos, 2003), but so far they have not been tried in ruminants.
Ireland, the body called for a National Cryptosporidium Reference Laboratory responsible for typing of isolates and provision of expert advice (Report of Waterborne Cryptosporidiosis Subcommittee of the Scientific Advisory Committee, 2004). In 2004, a cross-border Cryptosporidium Research Network was set up to promote and facilitate interdisciplinary research and the transfer of information about cryptosporidiosis on the island of Ireland. In addition, facilities for genotyping Cryptosporidium to species and strain level has been set up in the Central Veterinary Laboratory and at University College Dublin. More such measures and collaborations between medical, veterinary and environmental scientists and health care professionals will be necessary to enhance our knowledge of cryptosporidiosis on the island and to avert or minimise future outbreaks.

**Cryptosporidium in Ireland: recent initiatives**

In 2004, Professor Grace Mulcahy, School of Agriculture, Food Science and Veterinary Medicine, University College Dublin (UCD) set up the cross-border Cryptosporidium Research Network. Funded by ‘safefood’, the Food Safety Promotion Board, the network set out to promote and facilitate interdisciplinary research and the transfer of information about cryptosporidiosis on the island of Ireland. Genotyping of cattle isolates has been carried out under Dr Tom Murphy at the Central Veterinary Laboratory since 2002. Professors Grace Mulcahy and Seamus Fanning have set up a facility for genotyping Cryptosporidium to species and strain level at the UCD School of Agriculture, Food Science and Veterinary Medicine. Dr Nicholas Holden, School Of Agriculture, Food Science and Veterinary Medicine, UCD and Dr Tom Murphy, Central Veterinary Laboratory, are currently setting up a project which will use a microbial risk assessment model to identify catchments where potable water is at high risk of being contaminated.

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Clarification

Part one of this scientific paper appeared in the previous edition of the Irish Veterinary Journal [59 (8) pp 442-447]. It has been noted that, within that article, the content of Figure 4 was not made immediately clear. Thus, for purposes of clarification, Figure 4 is repeated here, with highlighting (smaller box) of the relevant area..

Figure 4: Identification of C. hominis (lanes 2, 3 and 5, 6) and C. parvum (lanes 4 and 7) by PCR-RFLP. Digestion with the endonuclease Sspl results in products of approx 500 and 250bp in both spp. Digestion with the endonuclease Vspl renders products of approx 590 (C. hominis) and 610 bp (C. parvum). Lanes 1 and 8 are molecular weight markers.