Update on the role of cryptosporidiosis in calf diarrhoea

The protozoan parasite *Cryptosporidium parvum* is increasingly being recognised as the primary cause of diarrhoea in young calves in the UK, with peak prevalence of clinical disease and intensity of oocyst shedding at one to 3 weeks of age. There are limited safe and effective therapeutic options that specifically treat, or prevent, cryptosporidiosis. Control on farms should be directed towards management strategies that minimise within- and between-farm transmission of the parasite. These include regular, appropriate disinfection and hygiene measures to reduce contamination of the environment and optimising calf health by a proactive approach to colostrum management and control of other enteropathogens.

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Diarrhoea continues to affect the cattle industry both in the UK and worldwide, with significant impact on the welfare and productivity of the animals as well as huge time and cost implications for farmers. While the true prevalence of diarrhoeic disease is difficult to quantify, it is estimated to affect over 30% of all calves and cause almost 50% of calf mortality (Defra, 2008). Whether or not a case is reported to a veterinary surgeon or submitted for passive surveillance will depend on a number of factors including the severity of clinical signs and on the farmer's perception of the problem.

Determining the primary aetiology of an outbreak is not straight forward, with many cases presenting as mixed infections. However, analysis of veterinary investigation surveillance report (VIDA) data over a 5 year period (2007–2011) indicated that *Cryptosporidium parvum* is the leading cause of enteric disease in calves under 1 month old; detection of *C. parvum* alone, in the absence of any other enteropathogen, accounted for 37% of all diagnosable submissions (25% for rotavirus; *Figure 1*) (Hall et al, 1980). *C. parvum* was also found in conjunction with the other major enteropathogens and these mixed infections have been shown to increase severity of clinical signs. Scotland's Rural College (SRUC) reports for 2011 found *C. parvum* to be the leading cause of calf diarrhoea outbreaks, comprising 36% of diagnosable submissions (rotavirus 27%). While there will be inherent bias associated with data from passive surveillance, they confirm that *C. parvum* is a genuine and widespread primary enteropathogen, capable of causing significant clinical disease. Historically cryptosporidiosis was seen as a mild self-limiting diarrhoeal disease, a less important contributor to the calf diarrhoea complex than rotavirus, coronavirus and *Escherichia coli* K99. However, this situation appears to have changed in recent years, perhaps due to the increased awareness and farmer uptake of vaccines against the other three pathogens. The remerging role of *C. parvum* is supported by an increasing wealth of anecdotal evidence that has become impossible to ignore.

Cryptosporidium is a protozoan parasite. The infective stage is the microscopic oocyst which is thick-walled, rendering it highly resistant in the environment and to many disinfectants. Infectious dose can be as low as 10 oocysts and these are massively amplified in the intestine, resulting in potentially billions of oocysts being shed into the environment by an acutely infected calf. Transmission occurs either directly between shedding individuals via faeco-oral contact, or indirectly for example via fomites, watercourses and wildlife vectors.

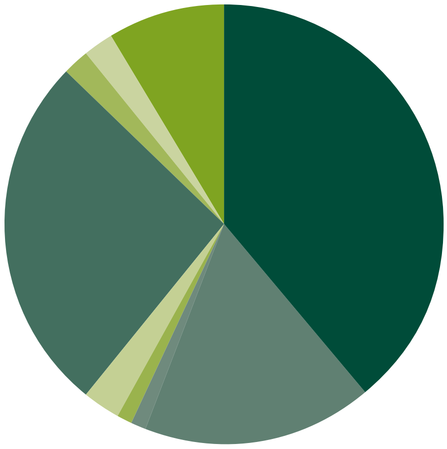

Figure 1 Pathogens causing diarrhoea in calves less than one month of age, as a percentage of diagnosable submissions to veterinary surveillance centres (VIDA, 2007-2011).

Modern molecular methods are used to study the genetic profile of this parasite and the result is that new species and genotypes are being identified; currently over 28 species have been assigned, and the parasite can infect a huge range of animals (Chalmers and Katzer, 2013). *C. parvum* is the species most commonly associated with diarrhoea in young calves; it is also an important zoonotic pathogen. Other species are also identified in cattle and are not considered to be clinically significant. These include *Cryptosporidium bovis* and *Cryptosporidium ryanae*, typically thought to occur in older, weaned calves, and *Cryptosporidium andersoni*, which is associated with adult cattle and may cause mild milk drop (Brook et al, 2009; Santin et al, 2004).

At the Moredun Research Institute there is an active research group studying *Cryptosporidium* spp. working closely with farmers to tackle cryptosporidiosis, focusing on the following areas of pathogenesis and epidemiology.

Prevalence and distribution of species/genotypes

The prevalence of shedding of *C. parvum* oocysts in calves in the UK has been demonstrated by cross-sectional surveys to be extremely high, particularly where calves under 1 month old were targeted. In a recent study, carried out in northeast Scotland by the Moredun Research Institute in collaboration with the University of Glasgow, 48% of beef calves sampled were positive for *C. parvum* and this concurs with many other similar studies (Brook et al, 2008; Smith et al, 2014). Furthermore, cohort studies suggest that 100% of calves on *C. parvum*-positive farms may shed oocysts at some point in the first few weeks of life (S Thomson, unpublished observations). However, this does not necessarily infer prevalence of clinical disease attributable to cryptosporidiosis, as apparently healthy calves can shed oocysts.

Historically the prevalence of *Cryptosporidium* in older animals has been cited as being extremely low. Where oocysts have been detected, they have often been identified as *C. andersoni*. Ongoing research at MRI focusing on the parasite in older animals has confirmed that concentration of oocysts in the stools of these animals is often very low. However, by optimising processing it has been possible to detect a much higher prevalence of oocysts in faeces compared with literature and, significantly, molecular typing has identified the presence of *C. parvum* (Wells et al, 2015). While there is no evidence of clinical disease in older animals, this impacts on potential transmission within farms, especially considering the very low infectious dose.

Routes of transmission and source of infection for calves

Within farm transmission

The source of infection for young calves is important to determine in order to break the transmission cycle. Considering adults do shed *C. parvum*, transmission could occur directly via dams around calving/suckling or indirectly via a contaminated calving area. There is evidence of calves beginning to shed oocysts at only a few days of age which suggests they were infected around the time of birth, given that the pre-patent period is around 3 days.

Calf-to-calf transmission, either directly or via contaminated calf accommodation is likely to be an extremely important transmission route, given the high levels of oocysts shed by acutely infected calves (peak prevalence and intensity of shedding occurs at 1–3 weeks old) and also considering the low infectious dose and the resistant nature of the oocyst in the environment. Suckler herds that calve indoors often report an increase in disease as the calving season progresses due to the accumulation of contamination. For individually or grouped dairy calves, one study found that farms that regularly moved temporary, gate-style calf pens had a much lower prevalence of positive animals compared with farms with a limited number of fixed, concrete pens (Hotchkiss, personal communication).

In theory, calves could also be infected by contaminated water sources and other animals such as adult sheep and deer. The authors' recent research did detect *C. parvum* in these sources using sensitive processing methods (Wells et al, 2015), although semi-quantitative assessment of oocyst load suggested the risk was low, compared with the high intensity of shedding by young ruminants. Lambs had a high prevalence of *C. parvum* and could act as a reservoir of infection and contribute to environmental contamination.

Between farm transmission

Molecular studies can determine if farms in a local area share the same 'strains' or genotypes. In theory transmission between farms

Figure 2. Using temporary gates can allow calf pens to be moved regularly to allow proper disinfection and reduce environmental build-up of contamination (photo Kevin McCollum).

Figure 3. Research has shown that adult cattle can shed C. parvum *and are therefore may be potential sources of infection for neonates (photo Stephen Maley).*

can occur via on-farm movements or purchasing of shedding animals (potentially over long distances) or locally via watercourses, contiguous or shared grazing and free-roaming wildlife.

Considering biosecurity and limiting purchasing of animals, particularly youngstock, is vitally important. Farms that purchase animals are more likely to harbour multiple strains and the genotypic profile of these strains is less stable compared with closed herds (Brook et al, 2009).

Basis of variation in clinical manifestation

It is known that there is a spectrum of clinical outcome with *C. parvum* infection (ranging from asymptomatic infection to death), but the factors driving this variation are largely unknown. Research into this pathogen is technically challenging as the life cycle cannot be completed in vitro; isolates must be propagated and maintained in vivo, in animal models. This means that many fundamental questions regarding how the disease progresses remain unanswered. In particular, the biological mechanisms that determine disease severity are unknown and the multifactorial nature of the syndrome means pathogenesis is complex, with clinical outcome potentially depending on host factors (age, immune status, genetics), environmental factors (concurrent enteropathogens, other stressors) and pathogen factors (number or strain of pathogen). Identifying the relative importance of these factors is very difficult on farms; controlled conditions are required. If virulence genes or markers could be identified, then this may inform the design of vaccines in the future.

Control

Depending on the individual farm, the aim of a control strategy might be to reduce shedding of oocysts (and therefore environmental contamination), or to reduce the clinical signs of infection.

Therapeutics

Understandably, many farmers are frustrated at the apparent lack of progress towards a 'silver bullet' that will treat the signs of cryptosporidiosis. Many agents have shown promising results in vitro (Gargala, 2008), but few have given consistent efficacy against clinical signs or shedding in affected ruminant species (Shahiduzzaman and Daugschies, 2012). Currently there is one licensed product available, halofuginone lactate (Halocur: MSD Animal Health), which can be used prophylactically on problem farms and has been shown to reduce or delay oocyst shedding (and therefore environmental contamination) in some studies. Conflicting reports of the effectiveness of halofuginone lactate in reducing clinical disease are to be found in published studies and anecdotally on farms.

For prophylaxis, one study found treated calves were less likely to shed oocysts and mortality was lower, although there was no effect on the incidence of diarrhoea (Trotz-Williams et al, 2011). No effect was seen on diarrhoea in other studies (Lallemand et al, 2006) and a small delay in disease onset (of 3 days) was observed on another (Jarvie et al, 2005). Conversely other studies have reported a decrease in severity of disease (Joachim et al, 2003) and mortality (Naciri et al, 1993).

The disadvantage of this product is that is must be given orally for 7 days from birth and this is not always practical, especially on beef suckler units. In addition, it is vitally important that farmers are aware of the potential effects of overdosing as toxic effects (including diarrhoea) have been shown at only twice the recommended dose. The product is also licensed for treatment of diarrhoea due to cryptosporidiosis however it must not be given to dehydrated or inappetant animals.

When the authors have surveyed farmers, many claim to be effectively treating *Cryptosporidium* spp. with antimicrobials and products such as decoquinate (Deccox; Zoetis). While these products may improve clinical signs by treating concurrent or other enteropathogens they are not licensed to treat cryptosporidiosis; the weight of evidence suggests decoquinate is ineffective against *Cryptosporidium* spp. (Lallemand et al, 2006; Moore et al, 2003). On problem farms vaccination against rotavirus, coronavirus and *E. coli* K99 may also help the overall health of neonatal calves.

Other control strategies

While there remains to be limited licensed products for treating cryptosporidiosis, there are practical measures that farmers can implement to reduce the impact of the infection in their animals and to reduce the amount of environmental contamination.

Colostrum

The importance of colostrum cannot be understated and a proactive approach to colostrum management can have a huge impact on calf disease. Simple and practical measures, such as recording when a calf has been seen to suck or is administered colostrum via a bottle or tube have been shown to be very successful. The three Qs of colostrum management should be adhered to, namely quality, quantity and quickly. The recommendations are that 3 litres (or 10% of bodyweight) are given within the first 2 hours of life, with a second feed given within 12 hours. Few farmers check colostrum quality but this can be done fairly simply. Colostrum with less than 20 g/l IgG should not be used. There are some excellent online tutorials focusing on colostrum management (http://dairy.ahdb.org.uk/technical-information/youngstock/3-qs-of-colostrum/).

Disinfection

It is not widely understood by farmers or veterinarians that most commonly used disinfectants (such as FAM, Sorgene and lime) will not kill *Cryptosporidium* oocysts at recommended concentrations. Steam cleaning is one effective and safe option if carried out correctly. Otherwise effective agents are based on ammonia and hydrogen peroxide, although they need to be used at the correct concentration and given the appropriate contact time. Care must be taken when preparing and using many of these agents; caustic fumes preclude the use of ammonia in occupied housing. Current available products are: 2–3% KenoTMCox (Naciri et al. 2011), 2–4% Neopredisan (Keidel and Daugschies 2013), 10% Ox-Virin (Quilez et al. 2005) and 3% hydrogen peroxide.

Farm management practices

There are many practical measures that farmers can put in place to reduce within- and between-farm transmission of the parasite including individual housing for dairy calves, low stocking density in group pens and outdoor calving (or early turnout), where possible. Grouping of calves according to age may also be beneficial — in particular farmers should avoid mixing calves of 1-3 weeks of age (i.e. during peak shedding) with neonates. Strict hygiene must be adhered to in calving pens and calf accommodation, with regular and appropriate disinfection. In addition, rapid isolation of scouring individuals is crucial in preventing an outbreak of cryptosporidiosis.

KEY POINTS

- *Cryptosporidium parvum* is extremely common in UK calves.
- Most commonly used farm disinfectants do not inactivate *Cryptosporidium* oocysts at recommended concentrations.
- Older animals, as well as youngstock, can act as reservoirs of *C. parvum* infection.
- There is a real risk to public health from *C. parvum*, particularly to those not regularly exposed to the pathogen.
- Halocur may help on problem farms, but farmers should be aware of the toxic effects of overdosing.

Public health

Farmers and veterinarians should consider the real potential risks to public health from *C. parvum*. On farms, good hygiene practices should be adhered to, particularly with students seeing practice who may not have been previously exposed to the parasite (Gait et al, 2008). Immune-compromised individuals should avoid contact with young calves. Farms that regularly open to the public should also be advised that alcohol-based gels will not suffice to protect visitors and are no substitute for thorough hand washing with soap and water (Gormley et al, 2011; Hoek et al, 2008). Slurry management should be carried out according to legislation before application to fields, as contamination of drinking and recreational water is an extremely common route of large outbreaks in humans with agricultural runoff often implicated as the source of infection (Lake et al, 2007). One important aspect of ongoing work at Moredun Research Institute is working with stakeholders at water catchment level (including the water industry, farmer, landowners, gamekeepers and environmental agencies) to safeguard public water supplies (Wells et al, 2015).

Future

Funding has recently been secured to study more closely the host–parasite interaction with the neonatal gut, in order to better understand early response to infection. In addition, the authors are interested in farmer behaviour, especially in terms of risk perception. From the authors' own research and from personal experience as a veterinarian, it is clear that different farmers will accept very varying degrees of morbidity and mortality in young calves. Schemes that allow benchmarking of calf health performance can be very powerful in changing attitudes and this is a potential area that can be driven by the veterinary practice.

Conclusion

While there is unlikely to be a novel licensed therapeutic product or vaccine in the near future, there is much that can be done to control cryptosporidiosis on farms. Veterinary advice should be targeted at minimising the build-up of environmental contamination and reducing the clinical impact by optimising the neonatal environment and controlling other enteropathogens. Veterinarians concerned about potential *Cryptosporidium* problems are welcome to contact the research team at the Moredun Research Institute. LS

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