

Student Final Report No. 61110031

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Cryptosporidiosis in Calves



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1. Abstract

Cryptosporidiosis is a disease caused by the parasite *Cryptosporidium*. This parasite is zoonotic, meaning it can pass between animals and humans. Cryptosporidiosis in neonatal calves is commonly caused by *C. parvum* and the symptoms include watery diarrhoea, lethargy, reduced appetite and dehydration. This dehydration can be so severe that death can occur. Very little has been published about the transmission of this parasite to young calves and it is important to know this in order to reduce this transmission and therefore the impact that cryptosporidiosis has on farms. The potential routes examined in this thesis include transmission from the mother of the calf or other adult cattle on the farm, transmission from wild rabbits and transmission from pheasants. All of this has been done using very sensitive and the most up-to-date molecular techniques to diagnose the species and genotype of *Cryptosporidium* which is present. This work showed that adult dairy cattle, on the studied farm, are unlikely to play a major role in the transmission of *C. parvum* to their calves. Most of the adult cattle on the dairy farm were predominantly shedding *C. parvum* however calves on the same farm presented with a different genotype. On the beef farm, however, many of the adult cattle did share the same genotype of *C. parvum* as their calves, and so are more likely to pose a risk for *C. parvum* transmission to their calves.

Rabbits collected from 18 farms from across Scotland revealed *C. parvum* to be the most prevalent species; an unusual discovery as it was previously believed that the species *C. cuniculus* was the most prevalent. The parasite load in the faeces of rabbits in this work appeared to be small although PCR inhibition could account for this. The pheasants also had *C. parvum* as the most prevalent species, although very few shared the same type that was present in the calves at the pheasant sample's location. On the other hand, very few oocysts (the thick walled, infectious stage of the parasite which is found in the environment) are required to cause cryptosporidiosis in a calf, so even if co-located wildlife do not appear to be shedding high numbers of oocysts, there could still be a risk of transmission of *C. parvum* to calves.

It was unknown whether infection of a calf with *Cryptosporidium* in the first few weeks of life will have a detrimental effect on the growth of the calf over time. This would affect both the production capability of the calf and the profitability of the farm. This work shows that infection in the first few weeks of a calf's life significantly reduces the weight gained over six months.

As this parasite is both costly to the farming community and a risk to animal and public health, it is essential that the right disinfectants are used to combat it. *Cryptosporidium* is resistant to many commonly used disinfectants that are used on farm and this thesis shows that Hydrogen peroxide based disinfectants are the most effective at inactivating the parasite oocysts and KENOTMCOX was the best at maintaining efficacy seven days post preparation. These disinfectants however must be made up fresh and the area cleaned before use for them to be at their most effective.

2. Introduction

Cryptosporidium parvum is a protozoan parasite with zoonotic potential and a wide host range affecting in particular neonatal calves with a disease known as cryptosporidiosis. The clinical symptoms of this disease are watery diarrhoea, abdominal pain and loss of appetite although the disease is most often self-limiting. Occasionally if dehydration is severe, death can occur.

The main source of infection to neonatal calves is currently unknown due to both the lack of research and lack of sensitive diagnostic measures. Sensitive diagnostics and higher discrimination in genotype designation are required to study the epidemiology and transmission of this parasite to calves. This disease is self-limiting; however, it was unknown whether or not suffering from cryptosporidiosis as a neonatal calf will have a long-term effect on the growth of the calf. It has been shown that growth rates are reduced in human children and lambs and so it could be that there is a longer-term impact caused by this parasite on cattle farms.

A few disinfectants are available to help control *Cryptosporidium* oocysts, however, for some of them, their efficacy in a farm setting or even in the laboratory has yet to be proven. There are currently no published studies to compare commercial farm disinfectants on their ability to inactivate *Cryptosporidium* oocysts so it is quite difficult to be able to advise farmers on which would be the best to use to help manage *Cryptosporidium* contamination in the farm environment

2.1. Aims of the PhD

Investigate the transmission routes for *Cryptosporidium* to beef and dairy calves.

Previous work has found that the genotypes of *C. parvum* differ between calves and adult cattle (Thomson, 2015). This may suggest that calves do not acquire *C. parvum* infections from their mothers and it may be acquired from another source. However, this work has only been completed on one farm and so further farms need to be studied before a conclusion can be drawn. The main aim of this work will be to investigate the different potential sources of infection for neonatal calves within a dairy and a beef farm.

Determine the effect of cryptosporidiosis on the long-term health and weight gain of beef calves.

It has been shown that *Cryptosporidium* impairs the growth of children, mice and lambs although there has been no work looking at the long-term effect that infection with this parasite has on the growth of calves. Therefore, the primary aim of this work will be to examine the impact of this parasite on the long-term growth of beef calves.

This will be achieved by selecting a farm which has a range of clinical cryptosporidiosis which can be clinically scored on the farm. Calves will be scored on the farm as regards their clinical presentation of cryptosporidiosis. The highest scoring animals will be compared to the lowest scoring animals with regard to the animals' weight change over a time period spanning birth to 6 months. Faecal samples will be taken to confirm that these animals are infected with *C. parvum* and whether or not they are suffering from concurrent enteric infections.

Compare different disinfectants on their ability to inactivate Cryptosporidium oocysts

Cryptosporidium oocysts are very hardy and are resistant to many of the commonly used disinfectants on the farm (King & Monis, 2007). Some disinfectants such as Neopredisan 135 - 1 and KENOTMCOX have been shown to have a good efficacy against the oocysts (Joachim et al., 2003; Naciri et al., 2011) although some newer disinfectants lack data on their effectiveness. Therefore, the aim of this work is to determine the efficacy of new disinfectants Cyclex, Progiene Coxicur and Steriplex SD+ by comparing them to proven disinfectants Neopredisan, Kenocox and Hydrogen Peroxide and also to a commonly used farm disinfectant FAM-30.

3. Materials and methods

3.1. Genotyping *Cryptosporidium* from faecal samples

3.1.1. Acid flocculation and Salt flotation

The protocol used for processing adult cattle samples for *Cryptosporidium* oocyst concentration was performed as previously described (Wells et al., 2016). This protocol involved weighing 50 g of the faecal sample before adding it to a 1-litre glass cylinder, along with 700 ml H₂O and 7ml of 2% H₂SO₄ in dH₂O. The sample was mixed on a magnetic stirrer for 5 minutes before being left to settle for 20-30 minutes until two to three distinct layers, the sediment at the bottom, the supernatant in the middle and sometimes a fatty deposit layer at the top, could be seen. Once settled, the middle layer which contained the suspended oocysts was taken with a glass pipette and this was centrifuged at 1100 x g for 20 minutes to create a pellet. Following this, a wash step to transfer the oocysts from a 250 ml container to a 15 ml tube involved washing the container 3 times with 3 ml H₂O and moving this to the 15 ml tube. A centrifugation step of 3000 x g for 5 minutes created a pellet containing the oocysts and the supernatant was discarded.

The pellet obtained from the acid flocculation was used in a salt flotation protocol as previously described (Chalmers et al., 2009a). This involved suspending the pellet in 8 ml of saturated salt followed by trickling 2 ml of H₂O on top. The samples were then centrifuged at 1000 x g for 8 minutes and the oocysts were removed by creating a vortex with a pastette and adding these oocysts to 6 ml H₂O in a 15 ml tube. Samples were centrifuged at 1000 x g for 5 minutes. The pellet, containing the purified oocysts, was re-suspended in 1ml TE buffer ready for DNA extraction. Samples were then processed directly for DNA extraction.

3.1.2. DNA extraction

Before DNA was extracted from both the adult cattle and calf samples, they underwent 10 freeze-thaw cycles in liquid nitrogen in order to break the hard shell of the oocyst and release the parasite DNA. DNA was then extracted according to the manufacturer's protocol for DNA extraction using the NucleoSpin Tissue DNA, RNA and protein purification kit (Macherey-Nagel, NZ740952250).

3.1.3. 18S PCR

Cryptosporidium in cattle was determined using a nested species-specific PCR which amplified the 18S region (Thomson et al., 2016) and allowed the identification of the common cattle species *Cryptosporidium parvum*, *Cryptosporidium bovis*, *Cryptosporidium ryanae* and *Cryptosporidium andersoni*. Samples were run in triplicate with a negative control, a DNA extraction control and positive controls for all species in the PCR. Samples from wildlife were analysed using the 18S nested PCR as described previously (Xiao, Alderisio, Limor, Royer & Lal., 2000).

3.1.4. Genotyping

For all *C. parvum* positive samples, the GP60 gene was amplified by PCR and the positive products were submitted for sequencing. This was done by using a nested PCR (Brook et al., 2009). The PCR products were then run on a 1.5 % agarose gel, along with the 100bp Promega ladder (Cat No.G210A), using gel electrophoresis and stained with GelRed™ (Biotium, UK). The gel was then examined under UV light using an Alphamager 2000 before positive products were submitted for sequencing.

Microsatellite analysis was carried out on DNA samples which were positive for *C. parvum* by PCR. Four microsatellite markers which were previously described (Brennan, 2009; Mallon, MacLeod, Wastling, Smith & Tait., 2003; Morrison et al., 2008) were used to identify alleles and assign multilocus genotypes to each sample. PCR products underwent fragment analysis (Applied Biosystems; University of Dundee) using Genescan ROX500 as a size standard and the results analysed using STRand (<https://www.vgl.ucdavis.edu/informatics/strand.php>).

The maximum peak was recorded as the primary fragment size and secondary peaks recorded if there were over one third of the size of the primary peak (Hotchkiss et al., 2015). Only primary peaks are used for the visual representation of the multilocus genotype which was done using Phyloviz (<http://www.phyloviz.net>).

3.2. Scoring for Cryptosporidiosis

Limousin x Belgian Blue calves were scored for severity of cryptosporidiosis every second day from their birth until they reached 16 days of age. It has been reported that diarrhoea due to cryptosporidiosis is typically seen in calves in the first 15 days of life (Glombowsky

et al., 2017). Preliminary visits and an interview with the farmer confirmed that calves often showed signs of cryptosporidiosis between 6-10 days old.

Determining the severity of cryptosporidiosis in calves infected with *C. parvum* on a beef farm was done by developing a clinical scoring system, incorporating scores for both the faecal consistency and the demeanour of the animal.

Calves were weighed at 3, 4 and 6 months of age in the first year using an aluminium cattle platform (Allied Weighing. <http://www.alliedweighing.co.uk>). The birth weights of calves in the first year were estimated by the farmer due to the lack of weighing facilities. As the lack of weighing facilities at calf birth in 2016 resulted in the use of estimated birth weights, the study was repeated for the second year where animals were weighed at birth and then again at 4, 5 and 6 months of age. Calves on the farm were weighed at birth using a small scale designed for sheep (IAE Ltd) and then afterwards using the cattle platform incorporated into a cattle race.

3.3. Excystation of *Cryptosporidium* oocysts

Disinfectants were sourced through donations from participating companies. Each disinfectant was made up fresh according to the manufacturer's guidelines on the same day it was to be used for the experiment. Approximately 1×10^6 *Cryptosporidium* oocysts were placed into 8 separate 0.25 ml microcentrifuge tubes, one for each of the 7 disinfectants and 1 control tube with no disinfectant added. Each disinfectant was added at manufacturers recommended concentration 1:2 with oocysts. After the recommended contact time had elapsed, excystation were done to determine the oocyst viability.

Degradation of disinfectants over a 7-day period was examined by making up each disinfectant according to the manufacturer's instructions and left for 7 days before conducting the experiment using the 'Manufacturers guidelines' was repeated and was also repeated on 5 separate occasions so that each disinfectant had 5 replicates. All disinfectants were kept at 4 °C in the dark during the 7-day period.

Samples containing 1×10^6 *Cryptosporidium* oocysts in 0.25 ml tubes were centrifuged at 12,500 x g for 30 seconds and the supernatant discarded. If disinfectant was present, 50 µl phosphate buffered saline (PBS) was added and mixed before another spin at 12,500 x g for 30 seconds. This was repeated until 3 PBS washes had taken place and supernatant

discarded. Afterwards 40 µl of 1 x Hanks Buffered Salt Solution (HBSS) was added and the pellet resuspended. Fifty microliters of 1 x Trypsin (prepared in HBSS at pH 3 (add 7.5 µl 2% HCL)) was added and mixed. The tubes were then placed into a water bath at 37°C for 1 hour. Once the hour had elapsed the eppendorfs were centrifuged at 12,500 x g for 30 seconds and the supernatant discarded.

The addition of 90 µl of 1 x HBSS, 10 µl 2.2% Sodium Bicarbonate and 10 µl 1% Sodium Deoxycholate at this stage changes the pH to mimic intestinal conditions. The contents in the tubes were mixed and placed in a water bath at 37°C for 40 minutes. After this time the tubes were centrifuged at 12,500 x g for 30 seconds and the supernatant discarded.

Oocysts, shells and sporozoites were then suspended in 50 µl of 3% glutaraldehyde in PBS to fix and spot onto a microscope slide. A coverslip was placed over the spotted liquid and the edges of the cover slip were sealed using mineral oil. The slides were then left to settle for a few minutes.

Slides were examined under a microscope using x 40 phase contrast magnification. The oocysts, shells and sporozoites were then counted to a combined total count of 250. The sporozoite per shell ratio was calculated as follows:-

$$(\text{Sporozoite Count})/(\text{Shell Count}) = \text{Sporozoites per Shell}$$

The excystation percentage was calculated as follows:-

$$(\text{Shell Count})/(\text{Oocyst Count} + \text{Shell Count}) \times 100 = \text{Percentage Excystation}$$

4. Results

4.1. Species of *Cryptosporidium* in dairy calves over a 6 week period

Calves on the dairy farm were tested for *Cryptosporidium* species from birth until they reached 6 weeks of age. In week 1, 25/38 calves were positive for *C. parvum*. In week 2, 23/35 calves were positive for *C. parvum* with one calf showing a *C. parvum* and *C. ryanae* mixed infection. In week 3, 23/37 calves were positive for *C. parvum* with one animal showing a *C. parvum* and *C. bovis* mixed infection and two showing a showing a *C. parvum* and *C. ryanae* mixed infection. In week 4 10/35 calves were positive for *C. parvum*. Two calves were positive for *C. bovis*, 3 calves for *C. ryanae*. One calf was positive for *C. ryanae* and *C. bovis* as a mixed infection, three calves had a *C. parvum* and *C. bovis* mixed infection and one a *C. parvum* and *C. ryanae* mixed infection. Calves at 5 weeks had 16/38 positive for *C. parvum*, one for *C. bovis* and two calves for *C. ryanae*. Four calves had a *C. parvum* and *C. bovis* mixed infection and two calves a *C. parvum* and *C. ryanae* mixed infection. In week 6, 7/38 calves were positive for *C. parvum*, two for *C. bovis*, and four for *C. ryanae*. There was also one *C. parvum* and *C. bovis* mixed infection.

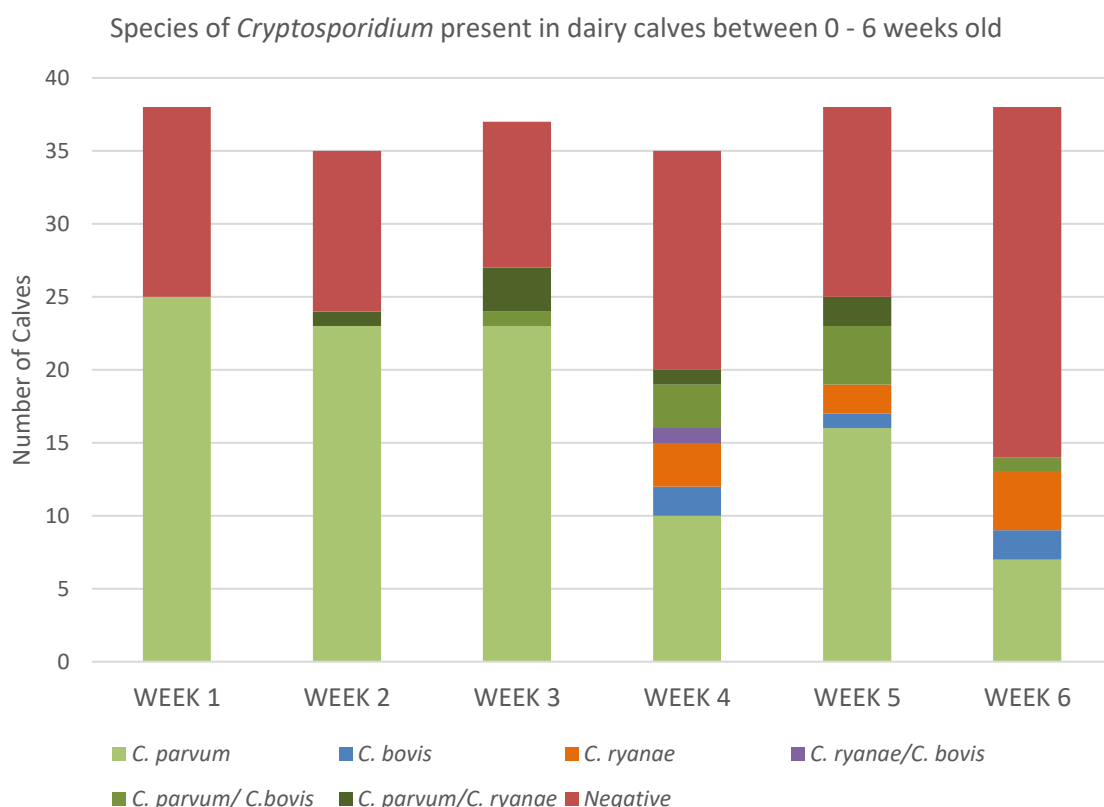


Figure 1 - Bar chart showing the species of *Cryptosporidium* found in 38 dairy calves from 0 - 6 weeks old, based on sampling 3 times per week for the first three weeks and twice per week for the following three weeks.

4.2. Adult cattle as a source of *C. parvum* to their calves

On the dairy farm, only two adults shared the same multilocus genotype which was predominant in the calves, which can be seen in Figure 2. This suggests that the adult cattle on this farm are very unlikely to contribute to the transmission of *C. parvum* to their calves. This has been indicated in previous work which genotyped using GP60 where only one adult cow shared the same genotype with the calves (Thomson, 2015).

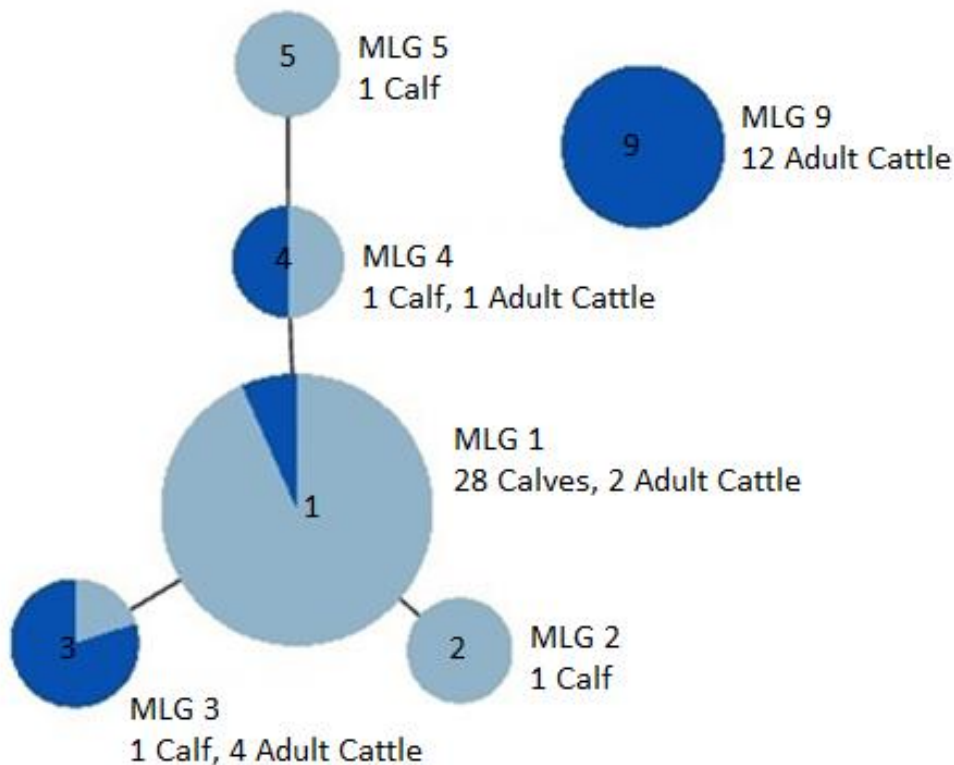


Figure 2 - Multilocus genotypes of *C. parvum* for paired adult cattle and their female calves on a dairy farm. Each node represents a single multilocus genotype (MLG) and these are joined by a line if they share 4 out of the 5 alleles. The size of the node represents the number of animals which have that MLG (adult cattle are indicated by dark blue and calves by light blue).

On the other hand, 7/13 of the adult beef cattle shared the predominant genotype which was found in the calves MLG 10 which can be seen in Figure 3. This could indicate that adults on the beef farm are more likely to be a source of infection to their calves, however the adults still showed a much higher variety of genotypes and were housed together with their calves, which only presented with a single multilocus genotype, and so, it could be hypothesised that transmission of *C. parvum* is more likely to occur the other way around; the calves are actually a source of *C. parvum* transmission to the adults. Some *C. parvum* genotypes have been found to be more virulent than others (Bouzid et al., 2013; Cama et

al., 2007) and so would be more efficient at causing infection in very young calves with an immune system which is still developing. Calves could be amplifying this more virulent genotype and causing mass environmental contamination, infecting subsequent calves which are then born. Based on the results from the dairy farm, separating cow and calf may not resolve any issues with *Cryptosporidium* on the beef farm, which would not be a practical approach for the beef industry.

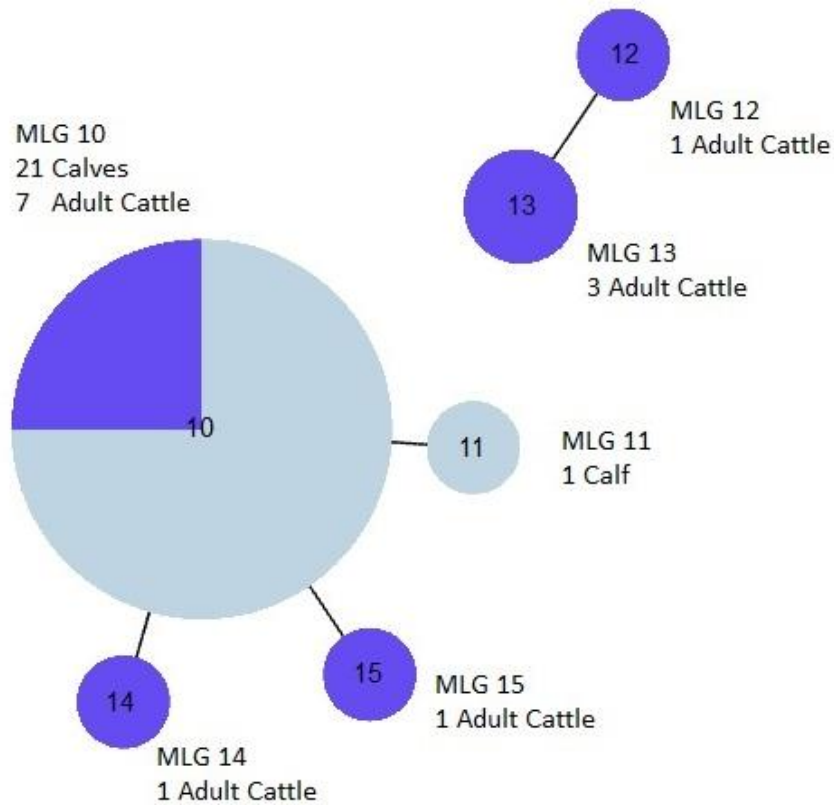


Figure 3 - Multilocus genotypes for *C. parvum* in paired adult cattle and their calves on a beef farm. Each node in Figure represents a single multilocus genotype (MLG) and these are joined by a line if they share 4 out of the 5 alleles. The size of the node represents the number of animals which have that MLG split into adult cattle (dark blue) and calves (light blue).

4.3. Wildlife transmission

Faecal samples from 359 wild rabbits located on 18 different farms in Scotland were tested for *Cryptosporidium*. The overall prevalence of *Cryptosporidium* species in rabbits was 37.33%. The most common species identified was *C. parvum*, which was present in the faeces of 78 rabbits (21.73 %), one of which was a mixed infection with *C. cuniculus*. The majority of the tested rabbits (225/359 – 62.67 %) were negative for *Cryptosporidium* based on the 18S PCR.

Samples from 30 individual pheasants in 2016 and 20 individual pheasants in 2017 gave a 40 % (11/30) and a 65 % (13/20) prevalence of *Cryptosporidium* in 2016 and 2017 respectively. The total prevalence of *Cryptosporidium* found in faeces of pheasants over the two years was 48 % (24/50).

The species identified in the pheasants was primarily *C. parvum*. In 2016, 8/11 *Cryptosporidium* positive pheasants were shedding *C. parvum* and 3/11 were positive for both *C. bovis* and *C. parvum*. In 2017, 12/13 *Cryptosporidium* positive pheasants were shedding *C. parvum* and the other pheasant *Cryptosporidium* positive PCR product failed sequencing. Therefore pheasants do harbour *C. parvum* and so could pose a risk to calves, although further work is required to confirm this as further genotyping showed pheasants to shed multiple genotypes. It would be an important area of further research to determine whether these birds are infected, or if they are acting as mechanical vectors.

4.4. Cryptosporidiosis and the growth of beef calves

In year 1, results indicate that animals with severe cryptosporidiosis have reduced growth over a 6-month period when compared to animals with no clinical signs of the disease using estimated birth weights. A boxplot for the weight gain over a 6-month period in the three groups of animals can be seen in Figure 4. Preliminary results suggest that the likely weight difference would be around 29.95 kg between severely infected and calves with no clinical signs of disease.

In year 1 there was no significant association between the gender of the calf and the severity of cryptosporidiosis which occurred ($p=0.386$).

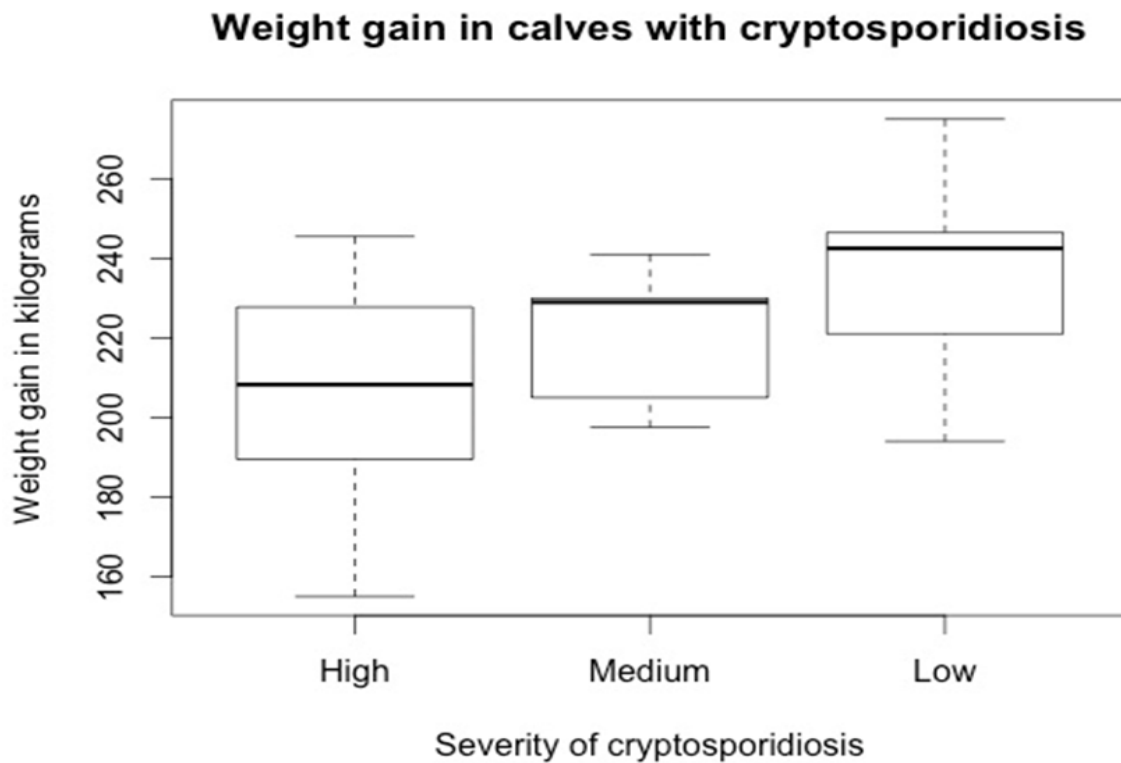


Figure 4 Comparison of weight gain from birth to 6 months in calves with different severity of cryptosporidiosis for year 1 (2016) 1. Severe clinical disease 2. Mid-range disease 3. No clinical disease. Note: Birth weights are an estimation in this year.

In the second year, calves in the severely infected group were 39.7 kg lighter on average than calves with no clinical signs of disease. Figure 5 shows a boxplot displaying these results.

Animals with severe cryptosporidiosis had a significantly reduced growth over a 6-month period ($p=0.008$) when compared to those animals with no clinical disease.

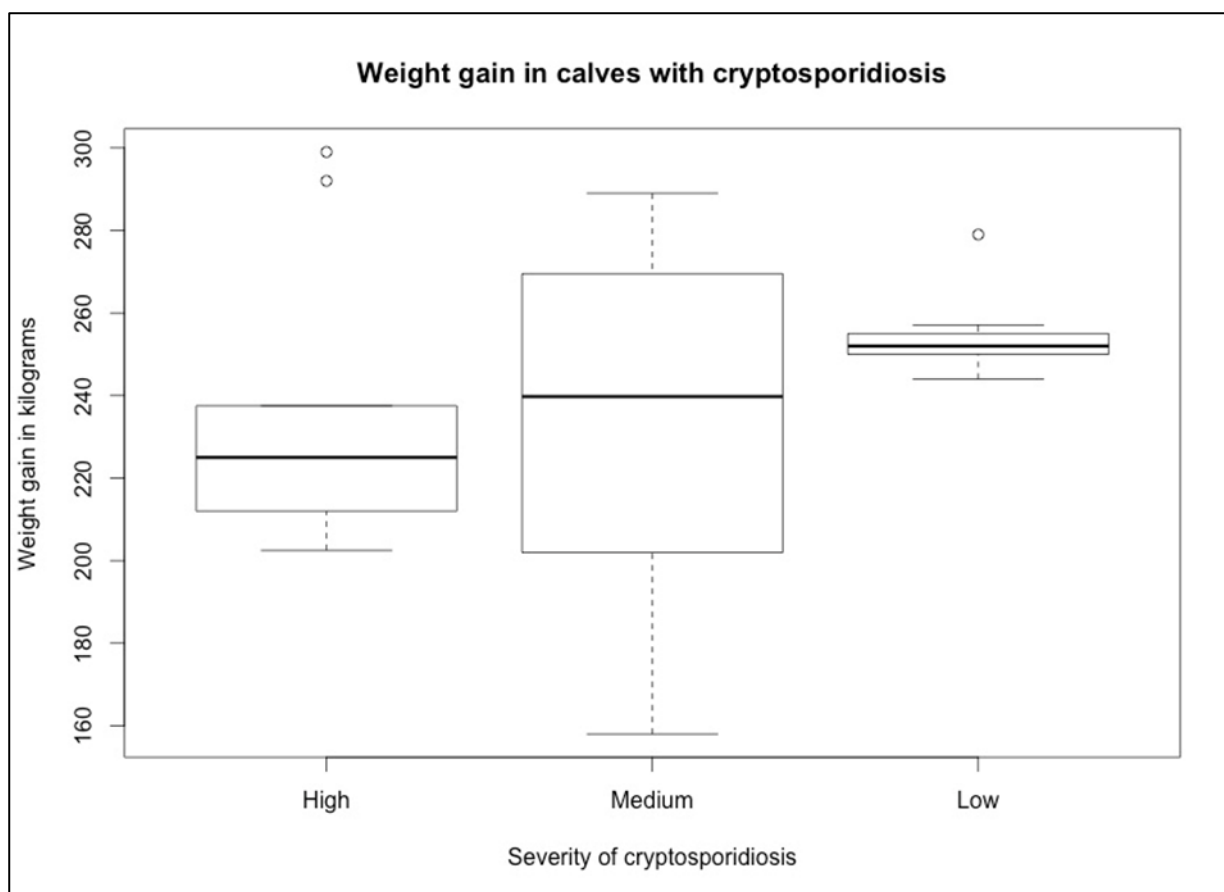


Figure 14 Comparison of weight gain from birth to 6 months in calves with different severity of cryptosporidiosis for year 2 (2017) 1. Severe clinical disease 2. Mid-range disease 3. No clinical disease.

4.5. Disinfectant studies

Disinfectants were tested for their efficacy against *Cryptosporidium* oocysts based on the manufacturer's guidelines for use. The best performing disinfectant with regard to excystation rate alone was Hydrogen peroxide which after 5 repeats gave a mean excystation rate of 0.40 % with a standard deviation (STDEV) of 0.40 %. Steriplex SD+, a hydrogen peroxide-based disinfectant, also performed very well with a mean oocyst excystation rate of 4.97 % after exposure, with a standard deviation of 3.55 %.

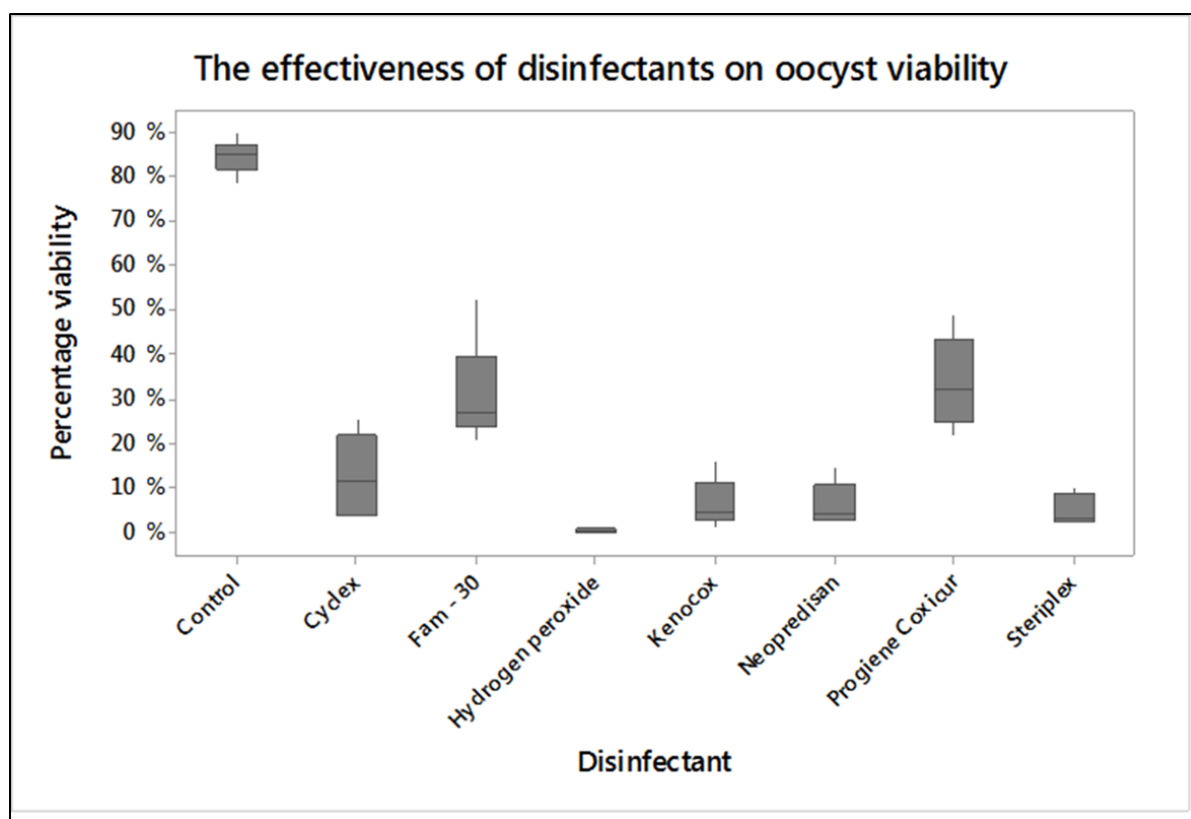


Figure 23 Excystation rate for *Cryptosporidium* oocysts undergoing excystation following exposure to various disinfectants used according to the manufacturers guidelines.

With regard to sporozoite to shell ratio, all disinfectants except FAM-30 performed very well. Steriplex SD+ had a mean sporozoite to shell ratio of 0 with a standard deviation of 0, meaning no sporozoites were seen in any of the five replicates. Cydex and KENOCOX both had a mean sporozoite to shell ratio of 0.1, Neopredisan 135-1 and Progiene Coxicur had a mean sporozoite to shell ratio of 0.2 and Hydrogen Peroxide had a mean ratio of 0.3.

The effect of time following preparation of disinfectants on the efficacy of the various commercial disinfectants was analysed to see how the excystation rate and sporozoite to shell ratio is altered 7 days after the disinfectant had been prepared. Overall, every disinfectant had a worse performance when the 7-day old disinfectant was used with a higher excystation rate being observed. The disinfectant with the smallest excystation rate using disinfectant made up 7 days prior to analysis was Hydrogen peroxide.

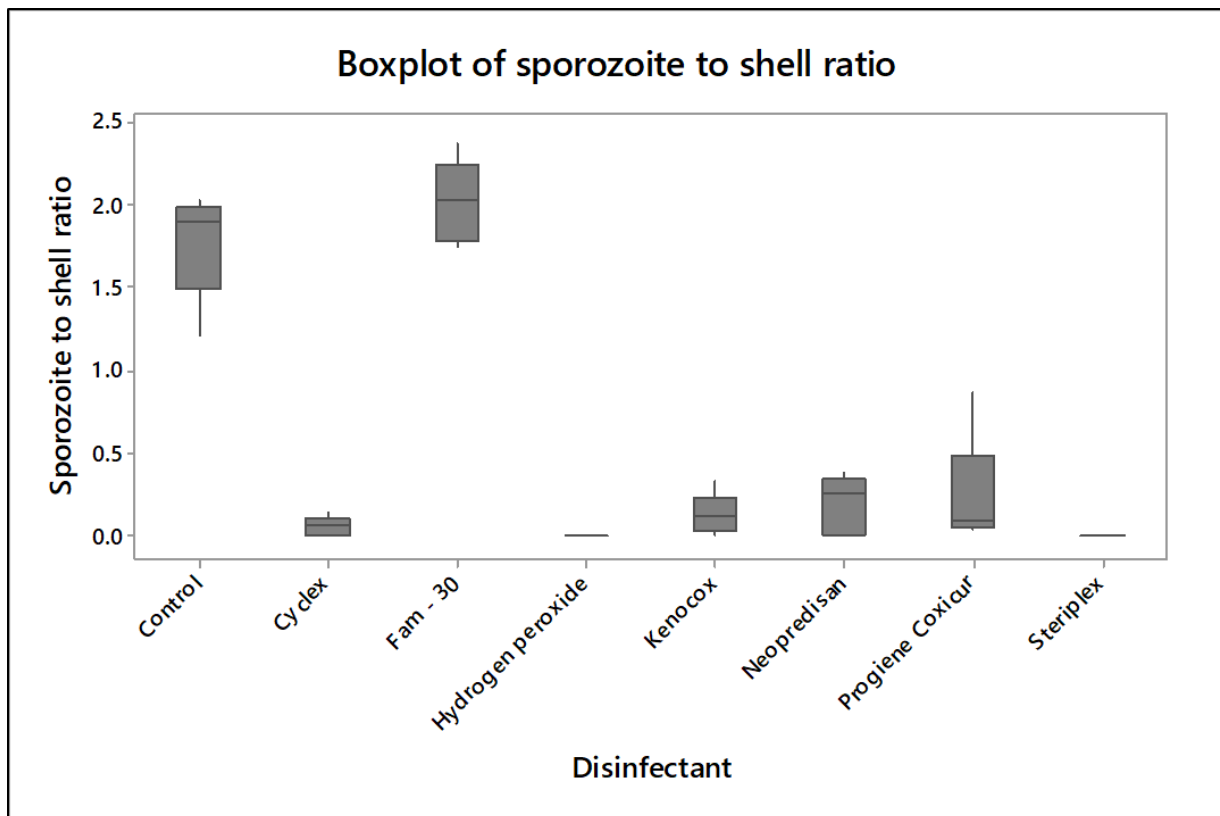


Figure 24 Shell to sporozoite ratio for *Cryptosporidium* oocysts undergoing excystation following exposure to various disinfectants used according to the manufacturers guidelines.

5. Discussion

Cryptosporidiosis, caused by the protozoan parasite *Cryptosporidium*, is a very important diarrhoeal disease as it is a major cause of animal mortality and economic loss on the farm (Ralston et al., 2010; Sweeny et al., 2011; Goater et al., 2014) and is widespread throughout the world. Prevalence in UK cattle herds varies from 28 – 80% (Brook et al., 2008; Wells et al., 2015) and it is thought that all calves in infected herds will shed *Cryptosporidium* oocysts at some point during the first few months of life (Santin et al., 2008). Not only is *Cryptosporidium* a problem for livestock on farms, but it is a risk to public health, being responsible for many human diarrhoeal outbreaks (Chalmers, 2012). Typically, neonatal livestock show clinical signs of cryptosporidiosis when they are infected with the species *C. parvum*, although other species *C. bovis*, *C. ryanae* and *C. andersoni* are also found in cattle (Thomson et al., 2016). This PhD found that neonatal and pre-weaned calves predominantly shed *C. parvum* with the occasional mixed infection with *C. bovis* and *C. ryanae*. The species *C. bovis* and *C. ryanae* did not occur as a single infection until the calves were at least one month of age. The adult cattle are shedding both *C. parvum* and *C. andersoni*. This provides further evidence to the conclusions drawn following a longitudinal study of the species of *Cryptosporidium* found in calves; that the predominant species present tends to follow an age-related distribution (Thomson, 2015).

Peak shedding of *C. parvum* occurred between weeks 2 and 3 of age which supports previous findings that young calves tend to show clinical signs of disease in the second week of life (Faubert & Litvinsky, 2000; Sanford & Josephson, 1982). A second peak of infection occurred when the calves were 5 weeks of age and this was with another genotype of *C. parvum*. The role that adult cattle could play in the transmission of *C. parvum* to calves has been addressed on both dairy and beef cattle farms. Using sensitive concentration techniques and highly discriminatory genotyping tools it appears that adult cattle do shed *C. parvum* which supports work done by Faubert and Litvinsky (2000) and also work looking at *Cryptosporidium* species in livestock in a Scottish water catchment (Wells et al., 2015) both of which found *C. parvum* in adult cattle. In this work 33 % of adult dairy cattle and 56 % of adult beef cattle were shedding *C. parvum*. Results showed that only 2/38 (5.26%) adult dairy cattle were shown to be shedding the same genotype as calves. This was following multi-locus genotyping on the dairy farm which showed the majority of the adults (12) were shedding a genotype which was different at more than two loci. Therefore, it is unlikely that adult dairy cattle on this farm play a major part in transmission of *C. parvum* to their calves. Further work needs to be undertaken to determine the role that adult beef cattle play in the

transmission of *Cryptosporidium* to their calves. The results for the beef cattle and calf transmission were inconclusive, however, as the rearing system means that adults and calves are kept together it is more likely that transmission will occur between them. Performing this work in a similar way to the dairy farm (sampling the adult cattle before the calves are born) would allow for a more confident conclusion as to the role of adult beef cattle in the transmission of *C. parvum* is occurring.

The parasite is known to persist within the calf population on farms and commonly recurs each calving season. As *Cryptosporidium* is a very hardy environmentally ubiquitous parasite (Goater et al., 2014), this persistence could potentially be the *Cryptosporidium* oocysts persisting in the calving area for subsequent years. Many commonly used disinfectants are ineffective at killing *Cryptosporidium* oocysts (Weir et al., 2002) which also increases the chances that the parasite will persist in the environment. It is likely that the environmental load of oocysts increases as the calving season progresses, as more calves are born, become infected and start to shed oocysts. The first calves would likely receive a low infectious dose and therefore suffer reduced clinical disease compared to those born later in the season which are met with a much higher infectious dose following amplification in the first-born calves.

It was found that a high level of *C. parvum* oocysts were being shed by both wild rabbits and pheasants. This is a surprising find for the rabbits, as previously the most common species shed has been reported to be *C. cuniculus* (Robinson, & Chalmers. 2010). The high prevalence of *C. parvum* in the rabbit is likely due to the rabbits living in close proximity to farmland. These rabbits were sampled initially for the examination of paratuberculosis transmission to cattle (Fox et al., 2018), and so were selected based on their proximity to cattle. The pheasants too were also located very close to the calving shed on the farm they were sampled from. Despite this, the genotypes present in the pheasants were mixed and in the first sampling year of 2016, none of the pheasants were shedding the same genotype of *C. parvum* as the calves. It would be interesting to expand this work to determine if the pheasants were infected with *C. parvum* or if they are acting as a transport host.

Clinical cryptosporidiosis is thought to have a long-term effect on calf growth as it has already been proven to be detrimental to the growth of children (Ajjampur et al., 2010; Checkley et al., 1998), and the weight gain and carcass condition of lambs (Jacobson et al., 2016; Sweeny et al., 2011). This work has shown that calves with severe clinical disease

have a significantly reduced weight gain when compared to calves with no clinical disease at 6 months of age. Those animals suffering from a mid-range disease still suffered a reduction in weight gain and so any form of clinical cryptosporidiosis could have longer-term effects. This is supported by similar work which was done in children, which found that children which suffered a single episode of cryptosporidiosis had similar weight-for-age and height-for-age scores as children which suffered multiple infections, which was significantly lower than children with no infections (Ajjampur et al., 2010). The impact of these findings could be much larger than just having smaller cattle. A reduction in growth rate is likely to result in a poor body condition score, which is associated with poor reproductive efficiency (Kadivar et al., 2014) with cattle taking longer to ovulate after having a calf. A low body condition score can also predispose cattle to lameness (Randall et al., 2015) and other infectious diseases (Roche et al., 2013). Reduced growth rate would also impact dairy cattle as milk production is known to decrease with reduced body condition and body weight (Roche et al., 2013).

Further work should include following beef calves for a longer period of time until they reach slaughter age. This will allow for an analysis of cryptosporidiosis on the carcass quality and score. Those animals which are kept could have their reproductive performance and milk yield analysed in order to determine if the changes that cryptosporidiosis causes at a young age in calves could have a larger economic impact on the farm. Calves showed a range of clinical manifestations of the disease despite being kept under the same management in the same shed and so are likely to suffer similar exposures to *Cryptosporidium* oocysts. In fact, in this study it was shown that almost all calves tested positive for *C. parvum* following PCR. Therefore, genetic studies to determine why some calves are more affected by *C. parvum* than others would be a very useful area of research and may point towards selective breeding opportunities.

Cryptosporidium is very difficult to manage on farms due to the parasite's ability to survive many of the commonly used disinfectants and environmental conditions. The lack of control means that once a farm has a problem with cryptosporidiosis, it is currently almost impossible completely inactivate the parasite from the environment. This work has shown that some disinfectants do exist which are capable of inactivating the oocysts, however, none of them are 100 % effective. It is therefore essential for farmers to follow the guidelines provided by disinfectant manufacturers, abiding by usage, concentration, contact time and storage recommendations. The pilot study in chapter 5 on Steriplex SD+ shows that the

disinfectant is much less effective when used in dirty environments (oocysts in faeces). Therefore, it is essential to make sure pens are cleaned out before disinfectants are used for the product to be the most effective.

Overall, many factors should be considered when determining the best disinfectant to use on the farm. Ease of use and short contact times are most desirable on a working farm in order to reduce the time spent with empty pens and sheds. The cost and shelf life of the disinfectant is also very important as some farms work with only limited budgets. Risk to user and to the environment should also be considered as it could pollute the surrounding environment. Despite hydrogen peroxide performing the best according to the results, the prepared product has a relatively short shelf life, losing efficacy after only 7 days following preparation. Not only this but there are restrictions on what percentage concentration can be purchased, which is 12% (less than the 30% stock used for this study) which is likely to reduce the shelf life even further. Therefore, a better alternative would be KENO™COX which has a much longer shelf life of the prepared product. Unfortunately, this product has one of the longest required contact times (2 hours) although it is cheaper, working out at £0.42 pence per litre of working solution, compared to £0.84 pence for hydrogen peroxide. Neopredisan 135-1 is commonly used against *Cryptosporidium* oocysts and did perform well, however, this disinfectant works out to be the most expensive at £1.12 per litre of working solution. Both are used at 0.4 litres per m². Another consideration is safety, as Neopredisan 135-1 is considered safe to use in the presence of animals and humans, environmentally friendly and biologically degradable. KENO™COX, on the other hand, is corrosive, requires protective clothing for the user and is considered dangerous to the environment.

What the farming community really requires is an effective drug or vaccine to combat cryptosporidiosis. Research in this area has been lacking for some time owing to the difficulties with maintaining the parasite in laboratory conditions without the use of animals. This means that less parasite is available for the tests required for drug and vaccine development. Despite this, progress has been made and new drugs could be on the horizon. A newly developed bumped kinase inhibitor which targets the calcium-dependant protein kinases in *Cryptosporidium* has effectively cured cryptosporidiosis in 5 out of 6 mice with no side effects (Castellanos-Gonzalez et al., 2016). These bumped kinase inhibitors were also used in a calf model where treatment resulted in a reduction in diarrhoea severity, *Cryptosporidium* oocyst shedding, and overall health of the calves (Schaefer et al., 2016).

6. Industry messages

6.1. Transmission of *C. parvum*

- Calves are predominantly infected with the species *C. parvum*, which is also present in adult cattle.
- Adult dairy cattle, on the studied farm, are unlikely to play a major role in *C. parvum* transmission.
- Rabbits and pheasants can carry zoonotic genotypes of *C. parvum* including those which infect calves.

6.2. Production impacts of cryptosporidiosis

- Calves with severe cryptosporidiosis in the first few weeks of life are significantly smaller at 6 months of age than calves which had no clinical signs of disease.
- The difference in weight occurs in the first month of life and these calves failed to catch up over a 6 month period.
- Using a cost analysis specific to the study farm, a calf with severe clinical cryptosporidiosis could be worth £100 less on average than a calf with no clinical disease.
- Calves with mid range disease still suffered a reduction in growth.

6.3. Disinfection

- Hydrogen peroxide based disinfectants are the most effective at inactivating *Cryptosporidium* oocysts.
- This disinfectant must always be made up fresh and according to the manufacturers guidelines.
- A dirty environment reduces disinfectant efficacy and so the pen must be cleaned properly prior to disinfection.

7. References

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