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Introduction

Calf scours remains one of the most widespread diseases affecting young calves, and will affect virtually every dairy or beef calf rearing unit at some time. Severe outbreaks can kill up to a third of diseased calves. The impact of a scours outbreak on a farm is considerable – requiring a rapid response from veterinary professionals, and long hours from all on the farm.

Preventative veterinary advice is far preferable to an emergency response to a calf scour outbreak. Research shows farmers look to vets for advice on preventing scours.

80% of New Zealand farms have experienced calf scours.

Less than 20% of New Zealand farms have a preventative vaccination programme.

Calves that recover from scours may not perform as well as non-affected animals.

Calf scours results in:

- Mortality.
- Morbidity.
- Decreased productivity, including; reduced live weight gain, delayed onset of puberty, lowered first lactation yields, delayed finishing in beef cattle.

<table>
<thead>
<tr>
<th>Scour pathogens</th>
<th>Prevalence (single or mixed infection)</th>
<th>Age calves Affected</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus</td>
<td>50 – 60%</td>
<td>5 – 14 days</td>
<td>Destroys the intestinal villi, reducing digestive and absorptive capacity of the small intestine, resulting in diarrhoea.</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>7%</td>
<td>5 – 20 days</td>
<td>Causes damage, often permanent, to both the small and large intestine. Disease is usually more severe with higher mortality than Rotavirus.</td>
</tr>
<tr>
<td>E. coli K99</td>
<td>7%</td>
<td>2 – 3 days</td>
<td>Produces toxins that cause the calf to pump excessive water and electrolytes into the bowel. Rapid dehydration and death can follow.</td>
</tr>
<tr>
<td>Cryptosporidia</td>
<td>20 – 30%</td>
<td>7 – 14 days</td>
<td>Disease usually manifests as green, water intermittent diarrhoea. Morbidity is high, although few will die.</td>
</tr>
</tbody>
</table>

Aetiology

**Rotavirus**

Rotaviruses (RV) are a primary cause of neonatal diarrhoea in many species. RV are classified in seven groups (A - G), according to the antigenic similarity of the major structural protein of the virus VP6.

Group-A cause most clinical cases of diarrhoea in calves. Other serogroups (B - G) are a rare cause. Group-A RV are further classified into G and P types based on the genetic and antigenic variation of the two outer capsid proteins VP7 (glycoprotein) and VP4 (protease sensitive protein), respectively. At least 15 G serotypes and 26 P genotypes are recognized to date; G6P[5] is the most important epidemiologically. The only other serotypes isolated from cattle are G10 and G8 combined with P[5], P[11], and P[1] 3. No serotyping survey has been conducted in New Zealand.
Coronavirus

Bovine Corona Virus (BCV) is a primary cause of neonatal diarrhoea and mild respiratory disease in calves, and associated with winter dysentery in older cattle.

Only 1 serotype of BCV is thought to exist2.

Entertoxigenic Escherichia coli

Entertoxigenic Escherichia coli (ETEC) are a primary cause of neonatal diarrhoea in many species. ETEC is typical of the family Enterobacteriaceae and possess capsule (K antigens), flagella (H antigens) and a typical gram-negative cell wall composed of lipopolysaccharides (O antigens) and proteins. These three antigens are used to serotype isolates. ETEC must produce both adhesins (on their fimbria or pili) and an enterotoxin to be pathogenic. These adhesins allow ETEC to adhere to enterocytes and show some host specificity, with only F5 (formerly K99) of major importance in calves. Note as F5 is encoded on plasmid DNA, traditional serotyping is not useful in predicting the presence of F53.

Cryptosporidium parvum

Cryptosporidium parvum is a devastating parasite that, once present on a farm, maintains itself through several different pathways. Adult animals excrete oocysts of the parasite in low quantities in their faeces without showing clinical signs (carrier animals). These oocysts infect newborn calves during and directly after birth. Once a calf is infected, Cryptosporidium parvum rapidly multiplies and causes severe damage to the intestinal wall. Within 4 days of initial infection, calves start excreting oocysts in large quantities into their environment. This results in a source of infection for other calves and in rapid re-infection of the infected calf itself.

Oocysts are resistant to nearly all antimicrobial and anticoccidial agents, as well as most disinfectants, and, due to their thick wall, can survive in the environment for long periods.

Epidemiology

Reservoir, Transmission, and Excretion

The primary reservoir for all these pathogens is the intestinal tract of cattle. Clinically normal sero-positive adult cattle shed rotavirus, coronavirus, and E. coli2, 4, 5 and therefore are probably responsible for the maintenance of infection in seasonal calving beef or dairy herds. Once infected, neonates multiply these infections leading to heavy environmental contamination. Viral excretion in the faeces of both clinically normal and abnormal calves lasts for 3 - 14 days6.

Transmission of all four pathogens is primarily by the ingestion of faeces and oral contact with faecally contaminated fomites. Coronavirus is however also excreted via the respiratory route, so aerosol may be another means of transmission2.
Occurrence

Distribution is worldwide and almost all herds have serological evidence of exposure to RV and BCV. Rotaviruses are the most common virus isolated from clinical cases of neonatal calf diarrhoea; whereas BCV is isolated less often. In a 2002 NZ unpublished survey of the aetiology of neonatal clinical diarrhoea on dairy farms, the farm (n = 57) prevalence (95% C.I.) of single or mixed infections was 75% (62 to 88%) for RV, 9% (3 to 19%) for BCV and 7% (3 to 17%) for ETEC.

Pathogenesis

Rotavirus replicates in villous epithelial cells of the small (and rarely large) intestine. BCV replicates in villous and crypt epithelial cells of the small and large intestine. Viral invasion results in cell necrosis, loss of enterocytes, atrophy of villi, malabsorption, and diarrhoea. Note BCV induced diarrhoea is often more severe and of longer duration than RV due to damage of crypt epithelial cells prolonging villous regeneration, and reduced fluid reabsorption in the colon.

Bovine coronavirus can also replicate in nasal, tracheal and lung epithelium. This may lead to mild respiratory disease in older cattle (5 – 13 months). However respiratory disease is yet to be reported in New Zealand.

ETEC (F5) adheres to receptors on the surface of proximal small intestinal epithelial cells. They multiply and produce enterotoxins that cause secretion of fluids and electrolytes. Note the disappearance of these cell receptors at about a week of age, explains why ETEC diarrhoea is only seen in very young neonates.

After orally infecting a calf, Cryptosporidium parvum follows a complex life cycle within the enterocytes of the small intestine. Going through a number of stages, the parasite rapidly multiplies in the calf. This results in the excretion of large quantities of oocysts in faeces. Oocysts are infectious immediately after excretion.

The loss of fluids and electrolytes causes severe dehydration, electrolyte imbalances, hypoglycaemia, acidosis and hyperkalaemia. These can lead to cardiac abnormalities, circulatory failure, shock and death.

Clinical signs

Infection of most (especially older) cattle is subclinical. Clinical disease usually involves mixed or sequential infections. Morbidity and mortality vary with pathogen virulence, environmental conditions, nutrition and colostrum / immune status; hence there is no typical clinical picture.

ETEC outbreaks are usual only in calves under 3 days of age. RV and BCV associated scours occur mostly between 1 – 4 weeks of age; but cases are seen up to several months of age.

Cryptosporidium parvum typically causes diarrhoea in calves of 1 – 3 weeks of age. The first clinical sign is a reduction in appetite and this is followed by diarrhoea, characterised by loose to watery faeces, and dehydration. It is practically impossible to differentiate cryptosporidiosis from other causes of neonatal calf diarrhoea on clinical grounds alone.

Autopsy findings;

Non-specific gross lesions are similar for all pathogens and include dehydration, fluid filled intestines, and abdominal distension.
Control

Because RV, BCV and ETEC are ubiquitous and they do not generate sterilising immunity, preventing exposure is impossible.

Control relies on reducing challenge and enhancing innate immunity by attention to hygiene, housing, calf density, stress, and effective case management; together with enhancing specific passive immunity by vaccination of the dam. Together with an effective vaccine, specific immunity relies on correct colostrum management and feeding. In addition to these measures, Halocur can be used to both treat and prevent Cryptosporidiosis.

Passive Protective Immunity

Protection against neonatal clinical disease caused by ETEC, BCV and RV depends on specific neutralising antibody in the lumen of the intestine binding to epitopes on the surface receptors of these pathogens. The bound antibodies inhibit attachment to intestinal cell receptors and promote removal by peristalsis. These antibodies are only available passively via colostrum and milk.

In ruminants IgG1 is the only isotype secreted from serum into the colostrum and milk in significant amounts; the aim of vaccination is therefore to maximise the amount and duration of IgG1 specific neutralising antibody in dam serum.

In the calf, IgG1 derived from on-going colostrum/milk feeding (i.e. lactogenic immunity) and IgG1 originally absorbed into the blood as a neonate and then actively re-secreted (a process coined reverse transudation) back into the lumen are significant sources of passive antibodies in the lumen of the intestine. Re-secretion alone (i.e. no feeding of stored colostrum) has been demonstrated to reduce the severity of diarrhoea and increase weight gain, although not statistically significantly from 302gm/day in a control group to 366gm/day in the treatment group. Therefore vaccination of the dams and feeding of colostrum to calves sold at a young age for rearing may have a practical benefit. This type of trial has not, to date, been repeated in NZ.

However, given the level of challenge on most farms, circulating antibodies re-secreted into the lumen are not sufficiently protective and must be augmented with lactogenically derived antibodies.

Exposure to ETEC, BCV and RV takes 2 – 3 weeks to generate mucosal immunity, via secretory IgA. This time lag in generating a primary response is why colostrum deprived calves can develop severe disease.

Colostrum Management and Feeding

Colostrum is essential to the neonatal calf providing an important source of nutrients, non-specific immune factors, and specific immunoglobulin protection against a variety of pathogens. Protection depends on the quantity, quality, and timing of ingested colostrum.

Newborn calves ideally require 150g of IgG1. As first colostrum varies markedly in Ig concentration (20 – 150g/L), and the ability to absorb Ig declines rapidly (i.e 50% within 12 hours), to ensure adequate intake, calves require 10 – 15% of their body weight in colostrum within the first few hours of birth. For example a 40kg calf needs 2L at birth and a further 2L 6 – 12 hours later.

Note as colostral Ig decreases markedly (i.e. a 50% reduction after the 1st milking) colostrum fed to neonates should be from the first milking.

To maintain lactogenic immunity, stored colostrum must be fed (2 – 3Ls daily) as part of the diet. The length of colostral supplementation depends on disease risk, a minimum of 3 weeks is recommended.
Rotavec Corona – The Vaccine

Rotavec Corona is a white liquid water-in-oil emulsion vaccine specifically formulated to raise protective neutralising IgG₁ antibodies against Bovine rotavirus, Bovine coronavirus and E. coli F5 (K99).

It is made by emulsifying the inactivated aqueous antigens that are absorbed on aluminium hydroxide gel in a light surfactant/mineral oil mixture. Sterility and shelf-life is enhanced by the addition of the preservative thiomersal.

As the vaccine pathogens are ubiquitous, the majority of cows will have pre-existing antibodies prior to vaccination. To overcome this “natural” immunity, to ensure an initial very high concentration of neutralising antibodies for transfer to colostrum, and to prolong antibody production for transfer into the milk, the vaccine must stimulate a hyper-immune response. Furthermore, the response must stimulate production of a specific immunoglobulin (Ig) subtype, namely IgG₁, as this is the only Ig that is concentrated from serum into the udder.¹⁶

Antigens

The vaccine strain (G6P5) of rotavirus is representative of serogroup-A, the most common (>99%) serogroup isolated from cases of rotaviral calf scours. The epitopes targeted are on 2 outer capsid proteins (VP4 and VP7). These proteins induce protective neutralising antibodies. There appears to be limited (especially VP4) diversity of these proteins.

All Bovine coronaviruses are antigenically similar. The vaccine strain, isolated from a calf with clinical disease, induces neutralising IgG₁ and in addition haemaglutinating IgG₁. Furthermore, the vaccine contains a “large” dose of antigen. This is vital for inducing an appropriate antibody titre and duration of response¹⁷.

The vaccine contains purified cell-free F5 (K99) pili antigen. This raises neutralising antigen protective against enterotoxigenic E. coli, the most common E. coli associated with neonatal calf diarrhoea.

Adjuvant System

Along with the appropriate quantity of antigen, the adjuvant (a blend of a different light mineral oil and a different emulsifier/surfactant to that in Rotavec K99) selected for Rotavec Corona drives a hyper-immune type of response. The performance of this new adjuvant is similar to that of Rotavec K99 for antibody production; however is less reactogenic, resulting in less local inflammatory responses. Furthermore Rotavec Corona is completely free of ingredients of animal origin; a bonus given global TSE risks.

This adjuvant enhances the immune response in a number of ways, including:

• Acting as a local and regional lymph node antigen depot.
• Helping to deliver the antigen to the spleen and lymph nodes. The microdroplets of oil containing antigen formed by this oil-in-water adjuvant emulsion, are readily ingested by local antigen presenting cells (e.g. macrophage and dendritic cells). These antigen-loaded cells rapidly emigrate, via lymphatics, to the draining lymph nodes or spleen where the necessary cell to cell interactions take place to generate antibody secreting plasma.
• Activation of various cells involved in the immune response.
Rotavec Corona – Usage

Dose

2mL, by deep intramuscular injection into the anterior third of the neck of pregnant cows or heifers. Alternative sites are not acceptable. The bottle must be well shaken before any vaccine is withdrawn.

Injection site reactions

Occasional injection site reactions (ISR) are expected with oil emulsion adjuvanted vaccines; this includes Rotavec Corona.

In the first year (2007) of widespread Rotavec Corona use in New Zealand, occasional ISR typical of those experienced with Rotavec K99 were reported; i.e. round raised abscess-like swellings that in some cases persisted for several months as a small granulomatous lump. However, a distinct type of ISR was also reported following Rotavec Corona use. These ISR were diffuse, raised plate-like swellings 2 – 20cm in diameter that varied in consistency from soft to hard. These cellulitis-like ISR usually regressed completely within 4 – 6 weeks, with a minority resolving after 12 weeks.

The reported within-herd incidence was usually 20 – 50% and ranged from <5% up to 100% in 2 small herds.

In a number of these cases, vaccination was in areas other than the recommended vaccination site, including varying areas of the ‘rump’, or the back of the thigh, and subcutaneous or dermal injection were also reported.

Depression and/or anorexia 2 – 5 days post-vaccination was also reported in 2 herds.

The adjuvant in Rotavec Corona has inherently less tissue reactivity than that used in Rotavec K99. However, notwithstanding the type of reaction and site of vaccination, the possibility of site reactions remain. To minimise risk, ensure hygienic deep intramuscular injection using sharp needles into the anterior neck.

Vaccination Timing

The label claim states vaccination can be between 12 – 3 weeks before calving. This refers to the individual cow; therefore to maximise herd coverage, vaccination should be done 3 weeks prior to the planned start of calving. This will cover cows calving in the first nine weeks and in the average NZ herd should represent 90 – 93% of cows. For herds with a wider calving spread, split herd vaccination is recommended.

Whole herd vaccination

All cows, including 1st calving heifers should be vaccinated. This ensures that every neonatal calf has the opportunity to get antibody rich colostrum via natural suckling, the best route for maximal absorption. Furthermore, it ensures the maximum amount of specific antibody rich colostrum is available; for calves reared off the mother this allows greater volumes to be fed for longer. This is particularly important given the varied response in individual cows within a herd.

Aim

The vaccine should:

• prevent clinical symptoms,
• reduce shedding by infected calves and hence spread to other calves, and
• increase the threshold for infection of susceptible calves therefore
• contribute to the interruption of the infection chain.

This protection relies on correct; vaccination timing, neonatal calf management, colostrum storage and feeding, and calf housing and environmental hygiene.
Cross-protection with Rotavec Corona

Rotaviral Serotyping

The capsid (outer protein shell) of Bovine Rotavirus (BRV) has three layers. The middle layer contains a virus protein (VP) called VP6. This protein is used to classify BRV into one of 7 serogroups (A to G). Group A is considered the only significant pathogen of cattle. Within serogroup A, isolates are serotyped based on differences between two proteins (G and P) in the outer layer. Important serotypes in cattle include G6 and G10 and P1, P5 and P11. The isolate used in Rotavec Corona, G6P5, is the most important cause of bovine diarrhoea.

There is little cross-protection between serotypes. For example Woode et al (1983)\textsuperscript{18} noted that “cross-protection studies in gnotobiotic calves showed that cross-protection only occurred between rotaviruses of the same serotype, and even a minor serotype difference was sufficient for the calves to show a lack of cross-protection”. This has meant vaccination of calves to prevent BRV has not been successful.

Lactogenic Immunity and Cross-protection

When we vaccinate the dam and use lactogenic immunity (i.e. antibodies in colostrum and milk) for protection, we cannot think about cross-protection in the classical way. Rather cows vaccinated with a single bovine rotavirus serotype produce increased antibody titres to all serotypes to which they have had prior exposure. Cows therefore respond heterotypically to monovalent vaccination.

This heterotypical response has been demonstrated by numerous researchers (Snodgrass et al 1984\textsuperscript{19}; Brussow et al 1988\textsuperscript{20}; Green et al 1990\textsuperscript{21}) and is also observed with other viruses that have marked antigenic variability such as influenza.

Importantly, challenge data in the registration dossier for Rotavec Corona included studies conducted using both the G6 and G10 serotypes. The serological data associated with these studies clearly indicates vaccination with Rotavec Corona boosts levels of both G6 and G10 specific antibodies (Figure 1).

Farm Specific Response

One of the key advantages of using heterotypical lactogenic immunity is that the protection provided by passive antibodies is determined by the serotype or serotypes on a particular farm. Given the numerous possible BRV serotypes, heterotypic cross-protective antibodies have a distinct advantage over the limited number of serotypes found in colostrum supplements and allows a single serotype vaccination to be efficacious. Veterinarians can therefore focus on the critically important aspects of colostrum collection, storage, and feeding when prescribing products designed to stimulate lactogenic immunity.
Rotavec Corona - A Summary of Recent Studies

1. A UK study in 25 dairy cows, 15 vaccinated with Rotavec Corona and 10 unvaccinated controls showed a significant increase in the mean specific antibody titre against all 3 antigens in the serum of vaccinated cows measured at intervals from vaccination 30 days before calving until 7 days after calving. There was a subsequent significant increase in colostrum and milk antibody levels measured at 7 day intervals from calving until 28 days post calving. Calf serum antibody levels measured at 7 day intervals from birth up to 28 days of age were higher than in calves from unvaccinated cows. (Fig 2, 3 and 4).

Fig 2. Mean *E. coli* F5 (K99) antibody levels in serum and milk from vaccinated and control cows, and in sera from their calves. Error bars show 95% confidence levels.
Higher levels (>4 fold) of specific rotavirus and coronavirus in the milk of vaccinated cows for at least 28 days after calving means that calves will have available the necessary levels of virus neutralising antibodies for a significant time after birth.

Vaccination of cows with Rotavec Corona provides increased rotavirus, coronavirus and *E.coli* F5 (K99) antibodies in colostrum and milk for at least 28 days following calving and will significantly improve the level of passive immunity in calves for at least 28 days.
2a. A French study in commercial beef cattle showed significantly increased colostrum antibody response to *E. coli* and coronavirus in Rotavec Corona vaccinated cows compared to a competitor vaccine and to unvaccinated controls measured at calving and 7 days after calving. Rotavirus antibody levels were higher, but the difference was not significant. (Figure 5)

Figure 5. Mean (SE) levels of maternal antibodies to rotavirus serotype G6, bovine coronavirus (BoCV) and *E. coli* fimbrial antigen F5 (K99) in a) colostrum at the time of calving and b) milk 7 days after calving; from Charolais beef cattle reared under commercial conditions in France vaccinated with Rotavec Corona (vaccine a) and a competitor vaccine (vaccine b), and from unvaccinated controls.
A Spanish study\textsuperscript{23} in 2 commercial dairy herds showed Rotavec Corona produced significantly higher antibody levels in colostrum to \textit{E. coli}, rotavirus and coronavirus when measured at calving and 4 days after calving when compared to a competitor vaccine and to unvaccinated controls. (Figure 6)

Figure 6. Mean (SE) levels of maternal antibodies to rotavirus serotype G6, bovine coronavirus (BoCV) and \textit{E. coli} fimbrial antigen F5 (K99) in a) colostrum at the time of calving and b) milk 4 days after calving, from Holstein-Friesian dairy cattle reared under commercial conditions in Spain vaccinated with Rotavec Corona (vaccine A) and a competitor vaccine (vaccine B), and from unvaccinated controls.
Halocur - A unique product for cryptosporidiosis

Reduced oocyst excretion with Halocur

Halofuginone lactate, the active ingredient of Halocur, is a unique molecule with explicit cryptosporidiostatic activity, mainly active against the early free-living stages of the parasite.

The molecule delays the lifecycle of the parasite, thus markedly reducing the excretion of infectious oocysts into the environment (see Figure 7). In addition to this, the cryptosporidiostatic action of Halocur allows the concurrent development of a specific serum and local antibody response to the parasite; this prevents calves from having severe clinical signs if re-infection occurs following a completed treatment course.

Figure 7: Faecal excretion of Cryptosporidium oocysts is reduced and delayed following treatment with Halocur

Results of Halocur treatment:

• Oocyst excretion is markedly reduced
• No interference with development of active immunity (Peeters et al., 1993).

Reduced severity of diarrhoea with Halocur

In a multicentre, randomised, double-blind clinical trial the faecal character of 158 naturally infected newborn calves was scored (0 = no diarrhoea, 1 = semi-liquid diarrhoea, 2 = liquid diarrhoea).

Half of the animals were treated with Halocur (2 mL/10 kg bodyweight once daily on 7 consecutive days, orally), and the other half received a placebo treatment (see Figure 8).

The difference in faecal indices was greatest after 7 days, when the mean faecal index of the placebo group was twice the mean of the Halocur group. If considering calves with the highest faecal index, the risk of diarrhoea in the treated group was reduced by 65%.
The analysis shows there is a statistically significant beneficial effect of treatment (P=0.0001) on the development of faecal indices from day 0 to day 21 in favour of the Halocur group (Lefay et al, 2001).

Successful control of cryptosporidiosis

Step 1: Diagnosis
Demonstrate cryptosporidiosis is a problem on the farm using clinical signs, examination of faecal samples (AxCSS-4 Diagnostic Kit/laboratory) and/or pathology.

Step 2: Therapy
To ensure rapid control of clinical signs, all calves aged less than 3 weeks with diagnosed cryptosporidiosis should be treated with Halocur within 24 hours of onset of diarrhoea, in addition to symptomatic therapies.

Step 3: Prevention
Treat all newborn calves with Halocur
• within 24 – 48 hours of birth;
• with 2mL per 10kg of bodyweight, orally;
• once daily, for 7 consecutive days.

Step 4: Improve management practices
• improve hygiene around calving, paying attention to both the cow and the environment;
• review the housing of newborn calves, consider individual rather than group housing;
• prevent other causes of neonatal calf diarrhoea;
  » produce antibody-rich colostrum by vaccinating cows with Rotavec Corona.
  » calves need 10% of their body weight (e.g. 2 – 3.5 litres) of colostrum from the 1st milking within 6 – 12 hours of birth.
**Indication**

**Newborn calves**
- Prevention of diarrhoea caused by *Cryptosporidium parvum* on farms with a known history of this disease. Treatment should start within 24hrs of birth.
- Reduction of diarrhoea caused by *Cryptosporidium parvum* in clinical cases. Treatment should start within 24hrs after the onset of clinical signs.

**Composition**

0.50mg halofuginone-base (as lactate) per mL.

**Dose and administration**

For oral use in calves, directly after feeding:
2mL per 10kg bodyweight, once daily for 7 consecutive days.

**Warning**

Overdosing may cause adverse effects.
Follow manufacturers dosage recommendations strictly.

**Contra-indications**

- Do not administer to calves with an empty stomach.
- Do not administer to weakened calves or to calves that have been ill for more than 24 hours.
References


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