Enteric Viral Vaccines for Calves

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Introduction

The development of successful vaccines against viral infections of the intestine of the young calf depends on an understanding of the aetiology of the syndrome, the epidemiology and pathogenesis of the individual enteropathogens and the host immune response to them. These aspects will be examined briefly in this paper.

Aetiology of Viral Enteritis in Calves

In spite of the major economic importance of the disease, surprisingly few comprehensive surveys of the aetiology of calf diarrhoea have been undertaken. However, studies made in diverse cattle-rearing countries are generally in broad agreement with the findings summarized in Table 1.[12] These show that rotavirus is usually the predominant cause of calf diarrhoea, with coronavirus also significantly involved. Several other potentially enteropathogenic viruses have been described from calves, in particular calici-like viruses,[1] parvoviruses,[14] astroviruses,[19] and toroviruses.[6,20] However, evidence that any of these latter agents are significantly involved in the aetiology of calf diarrhoea is lacking. It should be noted also that concurrent infections of the intestinal tract with more than one pathogen are common,[11] but there is no information on possible synergistic effects of viral coinfection. This paper will therefore concentrate on rotavirus and coronavirus as the two viral infections of the calf intestine that are known to be important.

Table 1. Surveys of the aetiology of calf diarrhoea in the UK.

Micro-Organism Micro-Organism	Diarrhoeic Calves No of Positives/No of samples (%)		Healthy Calves No of Positives/No of samples (%)		Significance (p)
Rotavirus	338/741	46%	60/434	14%	<0.01
Coronavirus	85/722	12%	2/434	<1%	<0.01
Cryptosporidium	159/710	22%	37/428	9%	<0.01
Salmonella	53/273	7%	12/434	3%	<0.01
ETEC	20/592	3%	1/215	<1%	<0.05

Epidemiology of Viral Enteritis in Calves

Surveys of the occurrence of these infections and antibodies to them suggest that rotavirus and probably coronavirus occur in all herds. Infection with these agents, probably repeated infections, are a normal occurrence in young calves. Thus we have the intriguing situation in which most infections are subclinical with no disease produced, while occasionally the same organism produces major outbreaks of diarrhoea. The explanation for this probably rests in the tolerance of the calf's intestine to minor insults. Thus a healthy calf under benign environmental conditions can tolerate local and patchy damage to its small intestine, particularly if the large intestine is functioning normally. The factors leading to overwhelming infection and damage and thus disease are not clearly understood, but include the variety of adverse circumstances associated with diarrhoea such as poor colostral intake, climatic stress, mineral deficiency, and intercurrent disease. The single most important factor is poor hygiene, which allows replication of the organisms through frequent calf-to-calf passage to levels which produce overwhelming infection of the intestine. This is exemplified by the common observation of much increased problems towards the end of the calving period, which is the end result of a buildup in contamination.

Pathogenesis of Viral Enteritis in Calves

Rotavirus and coronavirus are epitheliotropic, cytopathogenic agents with a predilection for mature villous absorptive cells toward the tips of the villi in the small intestine, with infection of the large intestine being noted occasionally.[7] Infections are usually patchy, with only a proportion of the epithelial cells infected. Infected cells degenerate and are sloughed. This results in contraction of the *lamina propria*, and crypt cells migrate to fill the defect. The villus surface area is reduced, the number of cells per unit surface area is decreased, and the replacement cells are functionally immature. The end result is a generalized reduction in small intestinal digestive and absorptive functions.

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These pathogenetic mechanisms are relevant to both treatment and immunity. Some residual villous absorptive cells remain which can be effectively utilized in oral rehydration therapy; and as the crypt epithelium is intact, recovery can be rapid provided fluid and electrolyte balance and nutrition are maintained. As the infections do not progress beyond the epithelium, local immunity in the intestine is all-important.

Immunity of Viral Enteritis in Calves

Placentation in the ungulate does not permit transfer of immunity *in utero*. Passive transfer of immunoglobulins through colostrum is therefore of central importance to the well-being of the neonatal calf, and has been the subject of extensive study and review.[16]

In colostrum from cows, IgG1 is present in high concentrations of 75-100 mg/ml, with much lower amounts of IgA and IgM. After the transition from colostrum to milk, IgG1 is still the major Ig, but at comparatively negligible concentrations 100 to 200-fold lower than in colostrum. This IgG1 is derived from serum and the high concentrations of colostral Ig ensure effective passive transfer to the neonate as these Igs are absorbed non-selectively through the gut for approximately the first day of life. Ruminant IgG1 has many functional similarities to sIgA in other species: its resistance to proteolytic enzymes; its predominance in milk and specificity against enteric viruses; and the increase of IgG1 but not IgA antibodies in milk after intestinal antigen administration.

The short period of significant immunoglobulin presence in colostrum (over approximately 4 days *post partum*) can leave the neonatal intestine crucially unprotected after these first few days of life. This is of particular importance for the epitheliotropic agents that cause neonatal diarrhoea. The full pathogenic potential of rotavirus and coronavirus is expressed by invading no further than the mature columnar epithelial cells on the intestinal villi. As these agents are endemic, normal ruminant colostrum contains antibody, and as long as this antibody is present in the intestinal lumen it exerts a protective effect. Once the neonate is no longer ingesting colostrum, antibody in the circulation is secreted in only limited amounts onto the intestinal epithelium and the young animal reverts to susceptibility. This is supported by the observation that calf diarrhoea is relatively uncommon in the first 3-4 days while the calf is ingesting colostrum, and that the peak disease incidence occurs from 5 days to 3 weeks of age.

Rotavirus Vaccines

A brief description of rotavirus structure and its relationship to immunity is necessary. There are major subdivisions of rotavirus into serogroups A-F, which have no known serological relationship to each other. The non-group A or atypical rotaviruses are uncommon in calves (<1% of strains detected).[10] They are not at this stage relevant to rotavirus vaccine production, and all subsequent discussion relates to group A rotaviruses.

Protection against rotavirus is conferred by immune responses to the two outer coat proteins VP4 and VP7. These epitopes define the dominant G serotypes (on the major glycoprotein VP7) and the minor P serotypes (on the protease-sensitive protein VP4). Intestinal infection confers protection against subsequent reinfection with rotaviruses which share either VP4 or VP7 with the initial virus.[5] Passive immunity from ingested antibody has also been shown to depend on the VP4 and VP7 specificities of the antibodies. Thus knowledge of the dominant serotypes in a species population is necessary to design effective vaccines.

It has become apparent that bovine rotaviruses have restricted serotype diversity. The common G serotypes are G6 and G10, and the common P serotypes are P5 and P11.[9] These exclusively or predominantly occur in cattle.

The two major approaches that have been taken to calf rotavirus vaccine development are use of modified live vaccines given orally to the neonatal calf (active immunization), and vaccination of the cow to boost antibodies in her colostrum and milk (passive immunization). A summary of the rotaviral and coronaviral vaccines available in the European Union is presented in Table 2.

Table 2 Vaccines against bovine enteric viral infections available in the European Union.

Vaccine	Components	Availability	Adjuvant	Dose
TRIVACTON 6 (Rhône Mérieux)	rotavirus coronavirus *E. coli*	France Germany Belgium Ireland	aluminium hydroxide & saponin	2 doses, second on day of calving
CORONIFFA (Rhône Mérieux)	rotavirus coronavirus	France	oil	2 doses, second on day of calving
SCOURGUARD 3 (Pfizer)	rotavirus coronavirus *E. coli*	France Germany Spain Italy Belgium Holland	aluminium hydroxide	2 doses during pregnancy
ROTAVEC K99 (Mallinckrodt)	rotavirus *E.coli*	UK Ireland	oil	single dose during preg.
LACTOVAC (Hoechst)	rotavirus (2 strains) parvovirus *E. coli*	Germany Belgium Holland Portugal	aluminium hydroxide & saponin	2 doses during pregnancy
SCOURVAX II (Pfizer)	rotavirus coronavirus	France Germany Spain Belgium	none	orally to calves at birth

Active Immunization of the Young Calf

The first modified live rotavirus vaccines had been developed and tested by 1973. However, with hindsight this rapid development accompanied by associated patent protection in North America rendered a substantial and long-term disservice to the generation of effective rotavirus vaccines. Experimental studies with the attenuated rotavirus vaccine confirmed that it was indeed attenuated, and that it could protect against subsequent virulent infection. Problems arose, however, in field use where contemporary comparison trials consistently failed to demonstrate protection. A key to understanding this conundrum was the observation that antibody to rotavirus is universal in normal bovine colostrum, and that the obligatory ingestion of this colostrum within a few hours of birth led to prompt inactivation of the live oral vaccine virus.[21]

The more recent studies on definition of and cross-protection between calf rotavirus serotypes suggest that serotype diversity would also be a problem for modified live rotavirus vaccines. Serotype G6 predominates in cattle populations, and the Lincoln vaccine strain also belonged to G6. However, a significant minority of G10 and occasionally other serotypes also occurs. Experience in calves, piglets and children suggests that a G6 vaccine would not be protective against these G10 strains.[18]

Thus effective modified live rotavirus vaccines for oral use in calves probably require two modifications: to contain at least two serotypes, i.e. G6 and G10; and to be delivered in a manner which will allow evasion of virus neutralization by colostrum.

Passive Immunization Through Maternal Vaccination

Passive immunization of the young calf offers an alternative route to effective prophylaxis. The concept is based on the observations that:

- rotavirus antibody present in the lumen of the intestine is an effective mediator of protection and,
- rotavirus antibody in colostrum and milk can be boosted significantly by vaccinating the cow.[8]

This led to the development and marketing of several rotavirus vaccines based on very simple technology, i.e. virus grown in cell culture, and incorporated in adjuvant. Such vaccines were shown to boost IgG1 antibody not only in colostrum but also in milk for several weeks after calving. Effective adjuvantation is probably critical.[3]

Under husbandry conditions where the calf is reared by the cow, there is obviously no problem in ensuring continual bathing of the intestinal epithelium with protective antibody. However, in typical dairy calf rearing, the calf ingests its dam's colostrum but then has no further access to her milk. This does not ensure the continual exposure to antibody critical to protection against rotavirus, and so alteration of management systems to continue feeding colostrum as all or part of the diet for 10-14 days is essential. Used in this way, effective clinical protection is achieved.

With passive immunization, some calves continue to experience subclinical infection. This is probably beneficial, resulting in active immunization of the calf under cover of clinical protection.

One unforeseen advantage of passive immunization is the fact that cross-protection between serotypes becomes much less of a problem. This is due to the fact that vaccination of a mature mother with wide natural rotavirus experience leads to cross-serotype stimulation of heterotypic antibodies.[2] Single serotype vaccination therefore stimulates antibody production to a wide range of rotavirus serotypes, obviating the need for multivalent rotavirus vaccines.

Vaccines containing both G6 and G10 strains are available, in addition to other vaccines containing only a single (usually G6) serotype. While a bivalent vaccine may seem attractive to ensure wider protection, the practical benefit from this increased cost has not been demonstrated.

Coronavirus Vaccines

Bovine coronavirus vaccines have been developed by several manufacturers as components of vaccines containing other viral and bacterial enteropathogens. However, published evidence for their experimental or field efficacy is signally lacking. Assessment of the antibody responses in serum and milk of cows vaccinated with coronavirus vaccines available in North America and Europe revealed minimal or no increase in titre.[4,17]

Effective coronavirus vaccines to protect calves through passive immunization should be possible to develop. The virus is endemic in cattle and hence cows have pre-existing antibody; bovine coronavirus has a similar tropism and pathogenic mechanisms to bovine rotavirus; all strains probably belong to a single serotype; and by analogy with both bovine rotavirus and porcine coronavirus (TGEV), passively-acquired antibody in the gut is likely to protect.

Problems with current bovine coronavirus vaccines may relate to difficulties in growing the virus to sufficiently high titre, and commercial unwillingness to use water-in-oil emulsions as adjuvants.

Vaccines Currently Available in the European Union

The enteric viral vaccines for cattle currently available are listed in Table 2. A detailed analysis of these vaccines is depressing. Passive protection of calves can be mediated only through stimulation of high antibody titres, and yet published evidence of serological efficacy for the rotavirus component for some of these products is lacking. There is no published evidence to support seroconversion to coronavirus in any of the current vaccines. Contrary evidence that some of these vaccines do

not produce seroconversion has been published independently. The supporting data from field trials are also woefully scanty. Published records of trials with concurrent controls exist for only one of the vaccines, the others using historical controls or having no published trial results. Not one of the products is supported by publication of results from blind trials with placebo control groups. Several of the vaccines have been tested in independent trials and found to be ineffective.

It is not the purpose of this paper to make comment in detail on the performance of individual vaccines, but further references on this matter can be sought.[4,13,15,17]

It has to be concluded that the enteric viral vaccines currently available in the EU do not offer the cattle veterinary practitioner an acceptable choice between products of demonstrable efficacy. The opportunity afforded by product reviews must be taken to ensure that the next generation of products can be recommended by veterinarians throughout the EU. As only 4 million of the EU's 33 million cows are vaccinated with the products currently available, the prize in expanded markets for effective products is large.

Future Prospects

The most significant challenge facing vaccine manufacturers is the development of an efficacious coronavirus component. A few strains of virus exist that do not grow to high titre in cell culture, but if these do not prove suitable it may be that a molecular biological approach to producing recombinant protein(s) will be necessary. The prospects for a recombinant protein raising viral neutralizing antibody through maternal vaccination are improved by the presumed previous endemic experience of the cows.

Some of the current rotavirus vaccines are effective. The challenge for the manufacturers is to match the standard of the best, which is probably dependent above all on adjuvantation and viral titre. Although these vaccines offer protection to home-reared calves, they have nothing to offer the calf rearer who buys in calves at a few days of age. Whether the modified live vaccines which are relatively ineffective in neonatal calves are capable of adaptation for oral use in these older calves remains to be seen.

Subunit rotavirus vaccines are a possible future development as the important neutralizing epitopes on VP4 and VP7 have been defined. However the epitopes on VP7 in particular are highly conformational, and most rotavirus recombinant proteins have proved less than optimal immunogens. It remains a long-term goal to develop vector-based systems for delivering rotavirus antigens to the intestine. For maternal vaccination and passive protection of the neonate, the key question regarding subunit vaccines will concern their ability to raise antibody to heterotypic rotavirus serotypes as effectively as whole virions do.

Summary

Rotavirus and coronavirus are significant causes of diarrhoea in young calves. The means by which efficacious vaccines can be developed are discussed. Most of the viral calf diarrhoea vaccines currently available in the European Union are unsupported by published evidence of their efficacy in field trials or even of their ability to produce seroconversion.

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