

Group A Rotavirus Veterinary Vaccines

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Group A rotaviruses cause diarrhea in young livestock and poultry; consequently, vaccination strategies have focused on induction of active or passive immunity. Gnotobiotic pigs and calves serve as useful models to evaluate induction of active immunity by candidate animal or human rotavirus vaccines. However, live attenuated rotavirus vaccines lacked efficacy when administered orally to calves and pigs in the field, presumably because colostral antibodies inhibited vaccine virus replication. The widespread occurrence of rotavirus antibodies in colostrum led to strategies for maternal rotavirus vaccination to boost lactogenic immunity and transfer passive antibodies to the neonate via colostrum and milk. The variable success of maternal rotavirus vaccines in the field is influenced by vaccine dose, strain, inactivating agent, adjuvant, route of administration, and environmental rotavirus exposure levels. The use of genetically engineered rotavirus-like particle vaccines in cows to boost antibodies in mammary secretions shows promise. Such subunit vaccines possess potential advantages over existing vaccines.

In 1969, bovine rotavirus (BRV) was the first group A rotavirus to be isolated in cell culture, characterized, and confirmed as a cause of diarrhea in calves [1, 2]. Subsequent studies documented the widespread prevalence of group A rotavirus infections in young animals, including calves and pigs, and their association with the diarrhea disease complex in animals <1 month of age [3–7]. The enteropathogenicity of rotavirus field strains was confirmed in experimental studies using gnotobiotic pigs and calves [2, 7, 8]. It is now recognized that group A rotaviruses from all animals, including humans, share common group A antigens that define the rotavirus serogroup [4, 7]. Serotypes of group A rotavirus are determined by a dual typing system based on the outer capsid proteins VP7 (G type) and VP4 (P type), which induce neutralizing antibodies (NAs) [5–7]. Although it is unresolved whether animal rotaviruses are transmissible to humans, and vice versa, certain group A rotavirus serotypes are shared among humans, cattle, and swine [7], and these common serotypes may occur more frequently in regions where there is a close association between humans and animals [9, 10]. Furthermore, human group A rotaviruses are transmissible to and cause disease in gnotobiotic pigs and calves, which serve as important models in which to evaluate candidate human rotavirus (HRV) vaccines and protective immunity [11].

A current vaccination approach for prevention of rotavirus infections in calves and pigs relies on passive immunization [2,

7, 12–19]. However, species differences in transfer of passive immunity influence vaccination strategies and results (reviewed by Saif and Jackwood [14]). Unlike human infants, ungulates (pigs and calves) are born agammaglobulinemic and acquire immunoglobulins in the blood solely via ingestion of colostrum for up to ~48 h after birth. The predominance of serum-derived IgG in the colostrum of these species results in the postpartum transfer of mainly IgG antibodies into the serum, equivalent to the transplacental transfer of IgG to the fetus in rodents, primates, and rabbits. However, rodents differ from the latter 2 species in that they continue to absorb IgG from the intestine via colostrum and milk for up to ~3 weeks after birth. It is conceivable that the tubulovascular system for the prolonged uptake of immunoglobulins in mice might also permit the non-specific uptake of rotaviruses in mice [8] and thus explain their susceptibility to infection with heterologous rotaviruses administered only in very high doses ($>10^6$ pfu). In this regard, the maturation of this system by glucocorticoids decreased the susceptibility of suckling mice to EDIM rotavirus infection [20].

Whereas in ruminants (cattle), serum-derived IgG1 remains the predominant immunoglobulin in milk, in monogastrics (e.g., swine and humans), secretory (s) IgA is the primary immunoglobulin in milk [14]. Only a small portion of this sIgA is absorbed into the blood, its main function being provision of passive immunity to the mucosa. Moreover, in monogastrics, milk sIgA is produced locally in the mammary gland by plasma cells originating in the intestine (reviewed in [14]). This mechanism ensures that neonates receive sIgA antibodies in milk against enteric pathogens endemic in the population. The significance of these species differences in regard to the design of vaccines for passive immunization will be addressed in subsequent sections.

In livestock, rotavirus infections are endemic, and in the young, the peak prevalence of rotavirus diarrhea occurs during the nursing period at 1–3 weeks of age [4–6, 8]. Similar to

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the asymptomatic rotavirus infections seen in human infants <3 months of age [21], many rotavirus infections of neonatal pigs and calves are mild or subclinical, presumably due to the rotavirus antibodies present in mammary secretions. Thus, under ideal circumstances, an animal becomes subclinically infected with rotavirus while under the protective influence of passive antibodies and develops active immunity (or is primed), which prevents subsequent severe disease. However, the balance between passive immunity and disease has been disrupted by many aspects of intensive animal production systems, including exposing animals to high doses of virus in confined, contaminated environments [22]; providing animals with feed supplements at an early age, which dilutes milk antibodies; and weaning animals at an early age, which curtails milk intake. Concurrent with these factors is an overall decrease in antibody titers to rotavirus in milk during the lactation period [7, 12–14, 18, 19, 23]. Many of these same scenarios also influence passive immunity to rotavirus transferred via breast milk and enteric disease in human infants [22]. Thus, strategies for the prevention and control of rotavirus infections in animals have focused on passive immunization procedures to boost colostrum and milk antibody titers to rotavirus in mothers and on management practices that promote immediate consumption of colostrum at birth and that improve sanitation to reduce environmental exposure levels to rotavirus. This review evaluates current animal rotavirus vaccines and potential new vaccine approaches.

BRV Vaccines

Background. Since their discovery in 1969, group A BRVs have remained a major cause of diarrhea in young calves worldwide, with most cases occurring in 1- to 3-week-old calves in the field [1, 3, 4]. At least 3 distinct G types (G6, G8, and G10) and P types (P[1], P[5], and P[11]) have been characterized in cattle [7]. The age and immune status of the animals, the virulence and dose of the BRV strain, the presence of other enteropathogens, and various management and environmental factors influence the severity of the disease. Although management practices may reduce the exposure of susceptible animals to BRV, the high concentrations of BRV shed in feces, their long-term stability in the environment, and the low doses required for infectivity suggest that eradication of BRV from infected herds is unlikely, if not impossible [5, 6, 8].

Oral vaccines to induce active immunity in calves. Calves are born agammaglobulinemic, and antibodies transferred in mammary secretions are absorbed into the blood for a limited time after birth (up to ~48 h) and, thereafter, continue to function locally by providing passive immunity to the mucosa. Most adult cows are seropositive to BRV and transfer various degrees of passive immunity to their nursing offspring via mammary secretions [2, 13, 17–19, 22–28]. Because young calves are more severely affected by enteric viral infections, transfer of optimal passive immunity plays a vital role in their protection.

Passive immunity to BRV, which infects the villous enterocytes, is associated with the frequent ingestion of colostrum and milk, which contain high levels of specific antibodies [2, 16, 17]. Parenterally absorbed antibodies, unless present in very high titers, appear to be of lesser value than local colostrum and milk antibodies in protecting the enterocytes [2, 16, 19]. However, studies in calves have suggested that colostrally absorbed IgG1 antibodies are transudated from the serum back into the intestine [29] and thus may complement the role of milk antibodies in passive immunity to BRV [13, 19]. Whether a similar transfer of antibodies from the serum to the intestine occurs in other species is unknown. Hence, maximal passive immunity against enteric viral infections in calves under field conditions may involve the presence in the gut of both local colostrum and milk and colostrally derived serum antibodies.

Because infection by BRV is localized to the small intestine of young calves, vaccine strategies have focused on methods to induce active or passive immunity in the intestine of susceptible animals [2, 12, 14, 16–19]. The first rotavirus vaccines were developed and tested in cattle. Two vaccination strategies evolved to prevent BRV diarrhea in young calves. The first involved stimulation of active immunity by the oral administration of live attenuated BRV to newborn calves [2, 27, 30]. This approach appeared to be successful in experimental studies using gnotobiotic calves and in some field trials [2, 14, 30]; however, its efficacy in double-blind field trials or as reported from surveys of rotavirus diarrhea in vaccinated calves was poor [25, 27, 31]. Although a lack of cross-protection due to infection by different serotypes of BRV may occur, most investigators suggest that under field conditions, failure of the oral vaccine was mediated by interference by maternal antibodies [25, 27]. The widespread occurrence of these antibodies in cows and the degree of virus attenuation needed to ensure innocuousness of the live vaccine in neonates limit the feasibility of this approach. An additional problem is that neonates may be exposed to a virulent field strain of BRV before a protective immune response can be induced by the vaccine. The necessity to handle and vaccinate each calf shortly after birth also poses management problems in large beef herds.

Parenteral maternal rotavirus vaccines to induce passive immunity in calves. Failure of the oral vaccine prompted a passive immunization approach to prevent BRV disease in calves. Pregnant dams are vaccinated with BRV to increase titers of antibodies in colostrum and milk, thereby increasing transfer of those antibodies, via suckling, to neonates. The success of this approach is contingent upon the fact that BRV infections are endemic; hence, in normal colostrum, most cattle have naturally acquired antibodies to BRV (primarily IgG1) that are selectively transported from serum [18, 32, 33]. Although these colostrum antibodies may be protective for a short time after birth, or longer if fed as undiluted supplements on a daily basis [17], titers to such antibodies normally decline to low or undetectable levels in milk, rendering young calves susceptible to BRV diarrhea [2, 18, 27, 32]. Therefore, because

the predominant BRV antibodies in colostrum and milk are serum-derived IgG1, parenteral vaccination of cows represents a logical approach to boosting these antibody titers in mammary secretions and, thus, stimulating the prolonged secretion of high-titer antibodies in milk.

Experimental studies of maternal rotavirus vaccines in cattle. Attempts to significantly enhance antibody titers in mammary secretions by parenteral vaccination of cows with BRV vaccines have met with various degrees of success [13, 19, 23, 28]. In 1973, Mebus et al. [2] were the first to report increased serum antibody titers to BRV after vaccinating pregnant cows subcutaneously (sc) or intramuscularly (im) with a formalin-inactivated neonatal calf diarrhea virus (NCDV) BRV P[1], G6 vaccine. They further noted that in a field trial of the inactivated vaccine in 1 herd over a 3-year period, the prevalence of mortality and morbidity due to calf diarrhea was reduced compared with the prevalence before vaccination. Subsequently, Snodgrass et al. [33] detected enhanced antibody titers in mammary secretions of cows parenterally vaccinated with the inactivated UK strain of BRV P[5], G6. However, in their studies, calves born to vaccinated dams were only partially protected against homotypic BRV challenge. Possible explanations for this lack of protection included high challenge dose of virus, milk antibody titers below the protective threshold, and destruction of critical antigens by formalin inactivation.

Saif and colleagues [13, 14, 18, 19, 23] confirmed and extended these earlier vaccination studies, describing optimal methods for vaccination of pregnant cows with NCDV BRV P[1], G6 vaccines to enhance BRV antibody titers in mammary secretions and to provide passive immunity to suckling calves. They found that IgG1 antibodies to BRV were predominant in mammary secretions of cows before and after vaccination, increasing at least 20- to 100-fold (compared with unvaccinated controls) in cows vaccinated im and intramammarily (imm) with modified live or binary ethylenimine (BEI)-inactivated BRV in incomplete Freund's adjuvant (IFA). Colostral antibody titers were also significantly increased (~10-fold) in cows im vaccinated with BEI-inactivated BRV in IFA. Passive protection studies were done in newborn unsuckled calves fed pooled colostrum supplements from the vaccinated cows [13, 17, 19, 23]. Complete protection against BRV diarrhea and infection during the experimental feeding period was achieved by feeding (20 mL, two times a day) 1% supplemental pooled colostrum from cows vaccinated im and imm with live BRV or from cows vaccinated im using BEI-inactivated BRV. Moreover, feeding 2 mL of the pooled 0.1% colostrum three times a day resulted in partial protection (characterized by delayed onset and reduced diarrhea and decreased BRV shedding). These results demonstrated that high virus neutralization and IgG1 BRV antibody titers in colostrum correlate with passive protection against challenge with homotypic (NCDV) BRV.

Feeding calves colostrum from cows immunized im with inactivated BRV vaccines containing Lincoln P[1], G6 and KK-3 (BRV serotype 2) strains protected the calves against

subsequent challenge by both strains [34]. Others have reported similar results in evaluating passive immunity to BRV under experimental conditions [35, 36] (reviewed in [15, 22]).

Field trials of commercial maternal rotavirus vaccines in cattle. Various results have been reported from studies of the field efficacy of commercial BRV maternal vaccines. These findings may be complicated by a number of factors, including the overall field trial design (comparison of historical data or analysis of vaccinated and unvaccinated cows in the same herd), the low prevalence of morbidity due to diarrhea seen in some herds after introduction of rotavirus vaccines, and the presence of concurrent infections with other enteropathogens, such as *Escherichia coli*, cryptosporidia, and coronavirus. Saif and colleagues [17, 18] reported that a commercial live attenuated US BRV vaccine (P[1], G6) failed to significantly enhance BRV antibody titers in mammary secretions of vaccinated cows or to provide passive protection to experimentally challenged calves. Myers and Snodgrass [28] noted a similar lack of elevated serum, colostrum, or milk antibody titers to BRV in cows vaccinated in the field with the same commercial BRV vaccine. Similarly, colostrum antibody titers to BRV did not differ significantly between vaccinated and unvaccinated dams in a field trial of the same vaccine in dairy herds in Canada, nor was the vaccine effective in preventing calf diarrhea or mortality under the field trial conditions [37]. Results of experimental studies suggested that the lack of effective adjuvants and low BRV doses in the commercial vaccine contributed to vaccine failure [12-19].

Field trials were conducted in two studies using the inactivated UK BRV maternal vaccine [38, 39]. In one trial [38], interpretation of results was confounded because of a low (<10%) prevalence of morbidity in many vaccinated herds and concurrent diarrheal infections with cryptosporidia and rotavirus in other herds. In 2 herds (68 cows) without concurrent infections, the prevalence of diarrhea was significantly reduced in calves born to vaccinated cows. In a subsequent study of the UK BRV vaccine under farm conditions in a single herd, vaccination resulted in reduced BRV shedding and diarrhea in calves [39]. Because the BRV present on the farm was the same G type as the vaccine virus [39], heterotypic protection could not be evaluated.

Efficacy (as demonstrated by a decreased morbidity and mortality due to diarrhea and a lessened severity of diarrhea) of inactivated G6 BRV vaccines was demonstrated in beef and dairy herds in two large field trials in Argentina. In the trials, disease was monitored in calves from vaccinated and unvaccinated cows and results were compared with historical data [40, 41]. In 1 trial herd, a progressive decrease in the prevalence of mortality and morbidity due to diarrhea was observed after 3 successive years of vaccination. Repetitive doses of BRV vaccine also reduced the amount and duration of viral shedding by calves in the field, effectively reducing the overall BRV exposure levels in the herd [41]. Of interest, however, calves born to BRV-vaccinated heifers (primiparous) continued to

have a higher prevalence of morbidity due to BRV diarrhea than calves born to vaccinated multiparous cows (unpublished data). Thus, calves born to heifers may be a reservoir for BRV infections in a closed herd, and, therefore, more effective vaccination procedures, consisting of more frequent boosters or higher doses of vaccines, may be needed for heifers.

In other field trials, pregnant dairy cows were vaccinated sc with inactivated BRV vaccines in IFA, and their immune colostrum and the normal colostrum from unvaccinated cows were fed as daily supplements to experimentally or naturally BRV-exposed calves [36, 42]. Morbidity and mortality due to diarrhea were decreased in the immune colostrum-fed calves.

The impact of the introduction of BRV vaccines on serotypic changes of BRV has not been investigated. In the United States, G6 remains the predominant G type of BRV in the field, despite the widespread use of a G6 BRV vaccine [7]. Recently, however, several genetic "subtypes" of G6 BRV, characterized by high amino acid sequence homology (89%–91%) but lower nucleotide sequence homology (82%–87%) have been identified from both vaccinated and unvaccinated herds (unpublished data). Whether use of the G6 BRV vaccine has influenced their emergence is unclear since historic BRV field samples collected before vaccine application are not available. In contrast, although the BRV vaccine P type is P[1], it is uncommon in the field, where instead, P[5] is the predominant P type [7]. Again, it is not known what influence the BRV vaccine might have on the emergence and prevalence of different P types in the field.

Heterotypic immunity induced by maternal rotavirus vaccines in cows and passive protection in heterologous species. Few investigators have studied heterotypic immunity following BRV maternal vaccination. Several investigators [14, 43, 44] demonstrated that cows vaccinated with 1 serotype of rotavirus developed increased serum NA titers to the immunizing strain and to other serotypes for which they had preexisting NA titers. Thus there was a broadening of the immune response after vaccination. However the role of such antibodies in passive protection of calves against heterotypic challenge is not clear. Recent investigations [45–47] describing the use of heterotypic virus-like particle (VLP) vaccines in cows to induce antibody responses to BRV in colostrum and to provide heterotypic passive protection are described in the following section.

The ability of maternal rotavirus vaccines to stimulate homotypic and heterotypic antibodies to rotavirus in colostrum of cows has been exploited for the passive treatment of children at high risk of severe or fatal rotavirus infections or of children who are in day care centers. In a preliminary trial, bovine colostrum antibodies were prepared from cows immunized with HRV P1A[8], G1 and simian rotavirus SA11 P[2], G3 and then fed daily to gnotobiotic pigs [48]. The immune colostrum feeding effectively reduced or eliminated both rotavirus shedding and diarrhea in a dose-dependent manner after challenge of the pigs with a virulent P1A[8], G1 HRV. Similarly, feeding bovine colostrum or milk preparations from hyperimmunized

cows to children ameliorated rotavirus diarrhea or reduced virus excretion in some studies [49, 50].

Genetically engineered rotavirus subunit vaccines. Recombinant viral multiprotein subunit vaccines (VLP vaccines) have been produced recently by use of the baculovirus expression system. Such particles, described initially by Roy et al. [51] for bluetongue virus and by Labbe et al. [52] for rotavirus, represent a new generation of noninfectious, stable, antigenically authentic, and highly immunogenic vaccines. Crawford et al. [53] described the production of triple-layered VLPs by the coexpression (using recombinant baculoviruses) of BRV and SA11 simian rotavirus inner (VP2 and VP6, respectively) and SA11 rotavirus outer (VP4 and VP7) capsid protein genes in insect cells (SA11 VLP). Previous studies of VLPs, including the ones produced from bluetongue virus, indicated that they are antigenically authentic and that the immune responses they induced in animals mimic those induced by intact virions [15, 45, 46, 51, 53].

Recently, Saif et al. [45] and Fernandez et al. [46] showed that homotypic (SA11, P[2], G3) and heterotypic (IND BRV, P[5], G6) NA and ELISA antibody titers to BRV in serum, colostrum, and milk of cows were significantly enhanced by the im plus imm administration of an SA11 P[2], G3 VLP vaccine (100–250 mg in IFA) to pregnant cows. Furthermore, similar administration of rotavirus core-like particles (CLPs; VP2 and VP6) to pregnant cows also significantly elevated ELISA IgG1 but not NA titers to BRV in serum and mammary secretions [46]. In colostrum of the VLP-vaccinated cows, the geometric mean NA titers to SA11 rotavirus and IND BRV were at least 70-fold greater than in colostrum of control cows [45]. IgG1 antibodies to BRV were the predominant isotype in mammary secretions of all cows, and in colostrum of VLP- and CLP-vaccinated cows, the levels were at least 100-fold higher than those in control cows [46, 47]. Our preliminary results further suggest that the core and inner capsid rotavirus proteins (VP2 and VP6) can be used as "universal" carrier CLPs for assembly of outer capsid proteins from BRV, permitting generation of new VLP vaccines reflective of the BRV strains most prevalent in the cattle population (unpublished data).

To evaluate passive immunity, 8 newborn colostrum-deprived calves were fed 1% pooled colostrum supplements from the SA11 VLP- or CLP-vaccinated cows twice daily for 7 days and then challenged with IND BRV [47]. All calves fed VLP-immune colostrum were protected against diarrhea, and only 1 animal shed BRV transiently in feces. Calves fed CLP-immune colostrum were only partially protected against diarrhea, and all calves shed BRV in feces. These results demonstrate the efficacy of a P[2], G3 VLP subunit vaccine for elevating NA titers to BRV in colostrum and show an association of increased titers with protective heterotypic passive immunity to BRV in calves. Greatly elevated IgG1 colostrum antibody titers in the absence of increased titers of NA (induced by a CLP vaccine) were associated with only partial passive protection in calves.

Thus, the VLP vaccines possess a number of important potential advantages over existing BRV vaccines, including exclusion of adventitious agents potentially present in live vaccines, the consistent presence of VP4 and VP7 expressed on particles in an authentic and immunogenic form (possibly altered or destroyed in inactivated vaccines), and the ability to modify BRV vaccines (by incorporation of new VP4 or VP7 types into VLP) to reflect serotypic changes in the field.

Porcine Rotavirus (PRV) Vaccines

Background. Initial reports of rotaviruses associated with diarrhea in pigs [54, 55] followed earlier studies implicating rotaviruses in similar outbreaks in calves and humans [1–7]. Clinical signs of infection in pigs include diarrhea, dehydration, anorexia, and depressed growth, but subclinical infections also occur [7, 55–59]. Group A rotaviruses are now recognized as a common cause of diarrhea in nursing pigs at 1–5 weeks of age (with peak prevalence at 1–3 weeks) and weaning pigs at 3–5 weeks of age and within 3–5 days of weaning [7, 55–58]. Intermittent or recurrent shedding of rotavirus may occur in nursing and weaned pigs [56, 57]. In addition, seropositive sows may shed rotavirus subclinically shortly before and after parturition [59]. Mortality due to rotavirus varies from 7%–20% in nursing pigs and 3%–50% in weaned pigs [7, 58]. Serologic data indicate that nearly 100% of adult swine are seropositive for antibodies to PRV [5, 7, 58]. Multiple rotavirus G serotypes (G3, G4, G5, and G11) and P types (P[6] and P[7]) have been detected and characterized in swine [5, 7, 60]. There is little or no cross-protection between PRVs with distinct G and P types [60], whereas viruses that share common G or P types induce at least partial cross-protection in experimental studies in gnotobiotic pigs [61].

Oral vaccines and active immunity in pigs. Like cattle, most adult sows are seropositive to rotavirus and transfer various amounts of rotavirus antibodies to the neonate via colostrum and milk. Pigs are also born agammaglobulinemic and absorb mainly IgG antibodies from colostrum into the blood for approximately the first 48 h after birth, resulting in a correlation between colostrum rotavirus antibody titers and serum antibody titers in neonates [7, 13, 14, 62].

At least two federally licensed PRV vaccines are available in the United States and are intended to stimulate active immunity and prevent rotavirus-associated diarrhea in weaning pigs. Little protection was afforded by a killed PRV vaccine administered im, ip, or orally to colostrum-deprived, 5- to 7-day-old pigs, presumably due to its failure to induce local intestinal immunity [63]. P[7], G5, a live attenuated oral PRV vaccine analogous to The Ohio State University (OSU) strain, protected nursing and weaned pigs under experimental conditions [63, 64] and in gnotobiotic piglet studies [60], but its efficacy in the field was questionable when tested by independent investigators [57, 65]. Likewise, the live attenuated NCDV BRV P[1], G6 commercial vaccine administered orally to nursing pigs

failed to replicate or protect against experimental challenge with a PRV [66]. These findings differ from studies by Zissis et al. [67], who reported that two im or im plus intragastric doses of the live attenuated BRV vaccine strain RIT 4237 (P[1], G6) induced seroconversion and partial protection against challenge with HRVs in colostrum-deprived piglets. It is probable that the dose and titer of the BRV vaccine used may have influenced its ability to replicate in the piglets. Although serotypic variability among PRVs and heterologous rotaviruses may also play a role in vaccine failures [7, 66], in at least one field trial [57] it was not a factor because the field strain isolated from weaned diarrheic pigs belonged to the same serotype P[7], G5 as the vaccine strain (unpublished data). As with the failure of the live oral NCDV BRV vaccine in field calves [17, 25, 27, 37], the most plausible explanation for a lack of field efficacy of the oral PRV vaccine is its inability to replicate in the piglets' intestine because of low virus titers ($\sim 10^4$ – 10^5 pfu) and a high degree of attenuation [60] and neutralization of vaccine virus by rotavirus antibodies present in the milk of most sows [7, 13, 14].

In studies using gnotobiotic piglets as a model for HRV infections, prior infection of piglets with a live attenuated HRV P1A[8], G1 induced only partial protection against challenge with the homologous virulent HRV, whereas complete protection was induced by prior infection with virulent virus [11, 68–70]. The failure of the attenuated rotavirus to replicate extensively in intestinal epithelial cells, even though it induced seroconversion, correlated with the low intestinal immune responses induced and the limited protection. In contrast, the virulent virus replicated extensively throughout the small intestine, induced villous atrophy, and stimulated significantly greater numbers of intestinal IgA antibody-secreting cells (ASC), which correlated with protection. These findings agree with those of previous investigations of active immunity to virulent PRV [71] or enteric coronavirus [72, 73] infections in swine: Virus-specific IgA ASC prevailed over IgG ASC in the intestinal lamina propria and were associated with induction of protective active immunity. Pigs inoculated oronasally with a live attenuated enteric or respiratory coronavirus developed significantly lower numbers of IgA ASC in the intestine than did pigs inoculated with the corresponding virulent strain of enteric coronavirus [72, 73]. After virulent enteric coronavirus challenge, only pigs previously exposed to the virulent coronavirus were protected against diarrhea: Primary exposure to attenuated enteric or respiratory coronavirus induced only partial protection against virulent virus challenge.

Maternal rotavirus vaccines to induce passive immunity in swine. As in cattle, passive immunization represents an alternative strategy to prevent rotavirus diarrhea in nursing piglets [7, 13, 14]. In swine, as in ruminants, IgG antibodies to rotavirus are predominant in colostrum and decline 8- to 32-fold in the transition to milk; however, unlike the case in ruminants, sIgA is the primary isotype of rotavirus antibody in the milk of swine, humans, and other monogastrics [7, 13, 14]. Previous

studies of infections of transmissible gastroenteritis virus (TGEV), an enteric coronavirus, in swine led to the concept of a gut-mammary immunologic axis whereby induction of colostral and milk sIgA antibodies in monogastrics requires primary intestinal antigenic exposure [7, 13, 14]. However, because rotavirus infections are endemic, most sows possess sIgA antibodies to rotavirus in milk due to prior natural enteric infection; hence, optimization of lactogenic immunity requires boosting rather than priming for milk sIgA antibody responses.

There were increased sIgA and IgG antibodies to rotavirus in the milk of sows after natural rotavirus infection of nursing piglets or following parenteral inoculation of pregnant or lactating sows with live attenuated rotaviruses [7, 13, 14]. Both homotypic and heterotypic rotavirus antibody titers were increased, but titers declined by the end of lactation, suggesting that repeated natural rotavirus infection of sows or parenteral boosting may be necessary to maintain high sIgA antibody titers to rotavirus in milk. This observation may account for the higher prevalence of rotavirus infections during the first week of life in pigs born to primiparous gilts (38%) than in those born to multiparous sows (3%) [56, 74]. These studies of lactogenic immunity in swine concur with reports of increased breast milk sIgA antibodies in women endemically exposed to cholera and parenterally boosted with a cholera vaccine [75], suggesting that parenteral vaccination is effective in boosting lactogenic immunity in naturally infected females.

There are few studies of maternal rotavirus vaccines in swine. Parenteral vaccination of pregnant or lactating sows with P[7], G5, an experimental live attenuated PRV vaccine analogous to the OSU strain, induced transient increases in sIgA and IgG rotavirus antibodies in milk and partial protection against field challenge, as reflected by a delayed onset and shorter duration of diarrhea and rotavirus shedding [7, 13, 14]. Such vaccines, although only partially effective under field conditions, may be useful in decreasing rotavirus morbidity and mortality, especially in herds with an early onset of rotavirus disease in neonatal pigs. Both of the federally licensed vaccines mentioned previously are approved for parenteral (killed) or oral and parenteral (live attenuated) administration to pregnant swine; however, few efficacy data from independent field trials of the vaccines have been published, and rotavirus diarrhea continues to be a problem in nursing piglets even in herds using these vaccines (unpublished data).

Maternal rotavirus vaccines for women. In children, the prevalence of rotavirus diarrhea peaks at 6–24 months of age [5, 6], but symptomatic infections occur in infants <6 months old in developing countries and regions where sanitation is poor [76]. Therefore, immunization of pregnant or lactating women may be feasible to protect young breast-fed infants, especially in developing countries where breast-feeding is common and of long duration. On the basis of studies in swine [7, 13, 14] and in women orally primed to cholera [75], oral vaccination of pregnant or lactating women with live attenuated vaccines [77] or parenteral immunization with inactivated or

VLP rotavirus vaccines appears likely to boost maternal antibodies to rotavirus in serum and existing sIgA antibodies in mammary secretions [78]. Increasing maternal serum rotavirus antibodies has the additional benefit of providing infants with enhanced levels of transplacentally acquired serum antibodies, which may play a role in addition to milk antibodies in the amelioration of symptomatic rotavirus infections in the neonate [79].

Recent vaccination trials using one dose of monovalent or tetravalent rhesus rotavirus vaccine in lactating women have demonstrated the safety and efficacy of this approach for boosting milk sIgA antibody titers to rotavirus for at least 4 months postpartum [77]. Although data on the protective effect of breast milk against rotavirus infections in infants conflicts [77], boosting rotavirus antibody titers in breast milk may enhance passive protection against rotavirus diarrhea at least in the first few months of life.

Correlates of protection and strategies for improved rotavirus veterinary vaccines. Investigators studying active immunity to rotavirus and enteric coronavirus infections in pigs have shown that the presence of high numbers of virus-specific IgA ASC in the intestine at challenge correlates with protection [11, 68, 71–73]. Similarly, in studies of rotavirus infections in mice, protection against a virulent homologous murine rotavirus challenge correlated with the presence of fecal or intestinal IgA antibodies to rotavirus [80]. Unfortunately, in several studies of immunity to enteric viral infections in pigs, the highest numbers of intestinal IgA ASC were induced only after enteric infection with the pathogenic virulent viruses; attenuated viruses induced lower numbers of intestinal IgA ASC and only partial protection [11, 68, 71–73]. These results and those from studies of mice [81] suggest that the efficacy of immunity induced by live attenuated oral rotavirus vaccines may relate to their degree of intestinal viral replication. Thus, the use of higher doses of oral rotavirus vaccines in combination with microencapsulation procedures to preserve virus integrity and stability during passage through the stomach and intestine and the development of mucosal adjuvants may be needed to improve clinical protection. In addition, there is a dearth of information on the efficacy of oral vaccines for priming followed by parenteral boosting (using inactivated or VLP vaccines) to elicit protective immune responses to rotavirus in neonates. Whether any of the factors discussed can overcome the interference by maternal antibodies of the replication of live oral rotavirus vaccines in pigs and calves remains uncertain and largely untested.

Correlates of passive immunity to enteric viruses, including rotaviruses and enteric coronaviruses, differ because of major differences in the immune systems of ruminants and monogastrics [14] and because of the serologic status of the host (endemic vs. epidemic infections). As discussed, colostrum and milk of ruminants contain mainly serum-derived IgG1 [32]; thus, im, imm, or sc inoculation of BRV-seropositive cows with live inactivated or VLP rotavirus vaccines significantly

boosted BRV antibody titers in serum and, consequently, mammary secretions [12–18, 23, 33–36, 40–42, 45, 46]. Vaccine titer and dose, virus strain, adjuvant, route of vaccine administration, and parity of the dam are all important variables for the design of optimal maternal BRV vaccines [7, 12–19, 23].

Vaccine efficacy has been studied in field trials and in unsuckled calves fed colostrum from vaccinated cows and challenged with BRV [7, 12–19, 23, 33–42]. The results suggest that colostrum containing high titers of NAs, mainly associated with IgG1, provided effective passive protection in a dose-dependent manner [12–19, 23, 33, 48]. Of interest, cows vaccinated with an SA11/BRV CLP (VP2 and VP6) vaccine had significantly elevated IgG1 antibodies to BRV (comparable to IgG1 antibody titers in colostrum from VLP-vaccinated cows) but not significantly elevated virus NA titers (CLP lack VP4 and VP7) [45, 46]. Only colostrum from the VLP-vaccinated cows (with significantly elevated NA and IgG1 antibody titers) passively protected calves from BRV diarrhea, although partial passive immunity against BRV diarrhea occurred in calves fed the CLP vaccine colostrum [47]. These data suggest that maternal VLP vaccines are a promising new vaccine approach to induce passive immunity to rotavirus in calves.

In monogastrics, IgA ASC, whose precursors originated from the intestine, mainly secrete sIgA antibodies in milk [13, 14]. Studies of TGEV infections in pregnant seronegative swine documented that only live virulent TGEV administered orally induced high NA titers associated with sIgA in milk, and the high titers correlated with passive protection against diarrhea in challenged piglets [13, 14]. Thus, live oral vaccines were required to induce sIgA in milk and passive immunity to enteric viruses in seronegative swine.

Studies of naturally infected rotavirus-seropositive sows also documented that mainly sIgA antibodies were present in milk, but titers declined during lactation [13, 14]. However, either oral (natural exposure) or parenteral inoculation of sows with live rotavirus vaccines induced increased sIgA antibody titers in milk and provided at least partial protection against field exposure to rotavirus [13, 14]. Similar observations pertain to humans: Increased sIgA antibodies were observed in the milk of women orally primed to cholera and parenterally boosted with cholera vaccines [75] and in women given live attenuated oral rotavirus vaccines [77]. Thus, high titers of NAs associated with sIgA in milk appear to be an important correlate of passive immunity in monogastrics [14].

References

- Mebus CA, Underdahl NR, Rhodes MB, Twiehaus MJ. Calf diarrhea (scours): reproduced with a virus from field outbreak. *Nebraska Agricultural Experimental Station Research Bulletin*, 1969;233:1–16.
- Mebus CA, White RG, Baas EP, Twiehaus MJ. Immunity to neonatal calf diarrhea virus. *J Am Vet Med Assoc* 1973;163:880–3.
- Bridger JC, Woode GN. Neonatal calf diarrhoea: identification of reovirus-like (rotavirus) agent in feces by immunofluorescence and immune electron microscopy. *Br Vet J* 1975;131:528–35.
- McNulty MS. Rotaviruses. *J Gen Virol* 1978;40:1–18.
- Theil KW. Group A rotaviruses. In: Saif LJ, Theil KW, eds. *Viral diarrheas of man and animals*. Boca Raton, FL: CRC Press, 1990:35–72.
- Holmes IH. Rotaviruses. In: Joklik WT, ed. *The Reoviridae*. New York: Plenum Press, 1983:359–423.
- Saif LJ, Rosen B, Parwani A. Animal rotaviruses. In: Kapikian AZ, ed. *Virus infections of the gastrointestinal tract*. 2nd ed. New York: Marcel-Dekker, 1994:279–367.
- Saif LJ. Comparative aspects of enteric viral infections. In: Saif LJ, Theil KW, eds. *Viral diarrheas of man and animals*. Boca Raton, FL: CRC Press, 1990:9–31.
- Bern C, Unicomb L, Gentsch JR, et al. Rotavirus diarrhea in Bangladeshi children: correlation of disease severity with serotypes. *J Clin Microbiol* 1992;30:3234–8.
- Urasawa S, Hasegawa A, Urasawa T. Antigenic and genetic analysis of human rotaviruses in Chiang Mai, Thailand: evidence for a close relationship between human and animal rotaviruses. *J Infect Dis* 1992;166:227–34.
- Saif LJ, Ward LA, Yuan L, Rosen BI, To TL. The gnotobiotic pig as a model for studies of disease pathogenesis and immunity to human rotaviruses. *Arch Virol* 1996 (in press).
- Saif LJ. Passive immunity to enteric viral infections: bovine rotavirus. *Adelantosen Microbiologia Enfermedades Infecciosas* 1988;7:86–109.
- Saif LJ. Passive immunity to coronavirus and rotavirus infections in swine and cattle: enhancement by maternal vaccination. In: Tzipori S, ed. *Infectious diarrhea in the young*. Amsterdam: Elsevier, 1985:456–67.
- Saif LJ, Jackwood DJ. Enteric virus vaccines: theoretical considerations, current status, and future approaches. In: Saif LJ, Theil KW, eds. *Viral diarrheas of man and animals*. Boca Raton, FL: CRC Press, 1990:313–29.
- Conner ME, Matson DO, Estes MK. Rotavirus vaccines and vaccination potential. *Curr Top Microbiol Immunol* 1994;185:285–337.
- Snodgrass DR, Wells PW. Passive immunity in rotaviral infections. *J Am Vet Med Assoc* 1978;173:565–8.
- Saif LJ, Redman DR, Smith KL, Theil KW. Passive immunity to bovine rotavirus in newborn calves fed colostrum supplements from immunized or non-immunized cows. *Infect Immun* 1983;41:1118–31.
- Saif LJ, Smith KL, Landmeier BJ, Bohl EH, Theil KW. Immune response of pregnant cows to bovine rotavirus immunization. *Am J Vet Res* 1984;45:49–58.
- Saif LJ, Smith KL. Enteric viral infections of calves and passive immunity. *J Dairy Sci* 1985;68:206–28.
- Wolf JL, Cukor G, Blacklow NR, Dambrauskas R, Trier JS. Susceptibility of mice to rotavirus infection: effects of age and administration of corticosteroids. *Infect Immun* 1981;33:565–74.
- Bhan MK, Lew JF, Sazawai S, Das BK, Gentsch JR, Glass RI. Protection conferred by neonatal rotavirus infection against subsequent rotavirus diarrhea. *J Infect Dis* 1993;168:282–7.
- Lecce JG, King MW, Dorsey WE. Rearing regimen producing piglet diarrhea (rotavirus) and its relevance to acute infantile diarrhea. *Science* 1978;199:776–8.
- Saif LJ, Smith KL. Keynote address: a review of rotavirus immunization of cows and passive protection in calves. In: *Proceedings of the 4th International Symposium on Neonatal Diarrhea* (Saskatoon, Canada). Saskatoon: Veterinary Infectious Disease Organization, 1984:394–423.
- Zygraich N, Georges AM, Vascoboinic E. Etiologie des diarrhies neonatales du veau—resultats d'une enquete serologique relative aux virus reo-like et corona dans la population bovine Belga. *Ann Med Vet* 1975;119:105–13.
- Acres SD, Radostits OM. The efficacy of a modified live reo-like virus vaccine and an *E. coli* bacterin for prevention of acute undifferentiated neonatal diarrhea of beef calves. *Can Vet J* 1976;17:197–212.
- Schlafer DH, Scott FW. Prevalence of neutralizing antibody to the calf rotavirus in New York cattle. *Cornell Vet* 1979;69:262–71.

27. DeLeeuw PW, Ellens DJ, Talmon FP, Zimmer GN, Kommerij R. Rotavirus infections in calves: efficacy of oral vaccination in endemically infected herds. *Res Vet Sci* **1980**;29:142-7.
28. Myers LL, Snodgrass DR. Colostral and milk antibody titers in cows vaccinated with a modified live rotavirus-coronavirus vaccine. *Am J Vet Res* **1982**;45:49-58.
29. Besser TE, Gay CC, McGuire TC, Evermann JF. Passive immunity to bovine rotavirus infection associated with transfer of serum antibody into the intestinal lumen. *J Virol* **1988**;62:2238-42.
30. Thurber ET, Bass EP, Beckenhauer WT. Field trial evaluation of a reo-coronavirus calf diarrhea vaccine. *Can J Comp Med* **1977**;41:131-6.
31. Hancock DD, Saif LJ, Bohl EH, Redman DR. Evaluation of response to field use of commercial oral-rotavirus-coronavirus vaccine in 10 Ohio dairy herds [abstract 242]. In: Programs and abstracts of the 62nd Conference of Research Workers in Animal Diseases (Chicago). Wooster, OH: CRWAD, **1981**.
32. Pierce AE, Feinstein A. Biophysical and immunological studies on bovine immunoglobulins with evidence for selective transport within the mammary gland from maternal plasma to colostrum. *Immunology* **1965**;8:106-23.
33. Snodgrass DR, Fahey KL, Wells PW, Campbell I, Whitelaw A. Passive immunity in calf rotavirus infections. Maternal vaccination increases and prolongs immunoglobulin G1 antibody secretion in milk. *Infect Immun* **1980**;28:344-9.
34. Tsunemitsu H, Shimizu M, Hirai T, et al. Protection against bovine rotaviruses in newborn calves by continuous feeding of immune colostrum. *J Vet Sci* **1989**;51:300-8.
35. Castrucci G, Frigeri F, Ferrari M, Aldrovandi V, Tassini F, Gatti R. The protection of newborn calves against experimental rotavirus infection by feeding mammary secretions from vaccinated cow. *Microbiologica* **1988**;11:379-85.
36. Tromp AM, Friedman A, Gallily O, Chen R, Perk K, Saif LJ. Prevention of bovine rotavirus infection through vaccination [abstract]. In: Program and abstracts of the Bologna XVIII World Buiatrics Congress (Bologna, Italy). Bologna: World Congress of Buiatrics, **1994**.
37. Waltner-Towes D, Martin SW, Meek AH, McMillan I, Crouch CF. A field trial to evaluate the efficacy of a combined rotavirus-coronavirus/*Escherichia coli* vaccine in dairy cattle. *Can J Comp Med* **1985**;49:1-9.
38. Snodgrass DR. Evaluation of a combined rotavirus and enterotoxigenic *Escherichia coli* vaccine in cattle. *Vet Rec* **1986**;119:39-43.
39. McNulty MS, Logan EF. Effect of vaccination of the dam on rotavirus infection in young calves. *Vet Rec* **1987**;120:250-2.
40. Bellinzoni RC, Blackall J, Baro N. Efficacy of an inactivated oil-adjuvanted rotavirus vaccine in the control of calf diarrhoea in beef herds in Argentina. *Vaccine* **1989**;7:263-9.
41. Cornaglia EM, Fernandez FM, Gottschalk M. Reduction in morbidity due to diarrhea in nursing beef calves by use of an inactivated oil-adjuvanted rotavirus-*Escherichia coli* vaccine in the dam. *Vet Microbiol* **1992**;30:191-202.
42. Castrucci G, Frigeri F, Ferrari M, Aldrovandi V, Angelillo V, Tassini F. Immunization against bovine rotaviral infection. *Eur J Epidemiol* **1989**;5:279-84.
43. Brussow H, Walther J, Fryder V, Sidoti J, Bruttin A. Cross-neutralizing antibodies induced by single serotype vaccination of cows with rotavirus. *J Gen Virol* **1988**;69:1647-58.
44. Snodgrass DR, Ojeh OK, Campbell I, Herring AJ. Bovine rotavirus serotypes and their significance for immunization. *J Clin Microbiol* **1984**;20:342-6.
45. Saif LJ, Todhunter KL, Smith KL, et al. Antibody responses in cows inoculated with recombinant rotavirus-like particles (VLP) as a subunit vaccine [abstract W4-7]. In: Proceedings and abstracts of the 13th annual meeting of the American Society for Virology (Madison, Wisconsin). Milwaukee: ASV, **1994**.
46. Fernandez FM, Conner ME, Parwani AV, et al. Isotype specific antibody responses to rotavirus and virus proteins in cows inoculated with subunit vaccines comprised of recombinant SA11 rotavirus core-like particles (CLP) or virus-like particles (VLP). *Vaccine* **1996** (in press).
47. Fernandez FM, Parwani A, Estes MK, Crawford, Conner ME, Saif LJ. Passive immunity to bovine rotavirus in newborn calves fed colostrum supplements from cows immunized with recombinant SA11 rotavirus-like particles (VLP) [abstract]. In: Proceedings and abstracts of the 76th conference of Research Workers in Animal Diseases (Chicago). Fort Collins, CO: CRWAD, **1995**:61.
48. Schaller JP, Saif LJ, Cordle CT, Candler E, Winship TR, Smith KL. Prevention of human rotavirus-induced diarrhea in gnotobiotic piglets using bovine antibody. *J Infect Dis* **1992**;165:623-30.
49. Ebina T, Sato A, Umezaki K, et al. Prevention of rotavirus infection by oral administration of cow colostrum containing anti-human rotavirus antibody. *Med Microbiol Immunol* **1985**;174:177-85.
50. Brussow H, Hilpert H, Walther I, Sidoti J, Mietens C, Bachmann P. Bovine milk immunoglobulins for passive immunity to infantile rotavirus gastroenteritis. *J Clin Microbiol* **1987**;25:982-6.
51. Roy P, French TJ, Erasmus BJ. Protection efficacy of virus-like particles for bluetongue disease. *Vaccine* **1991**;10:28-32.
52. Labbe M, Charpilienne A, Crawford SE, Estes MK, Coen J. Expression of rotavirus VP2 produces empty core-like particles. *J Virol* **1991**;65:2946-52.
53. Crawford SE, Labbe M, Cohen J, Burroughs M, Zhou YJ, Estes M. Characterization of virus-like particles produced by the expression of rotavirus capsid proteins in insect cells. *J Virol* **1994**;68:5945-52.
54. Rodger SM, Craven JA, Williams I. Demonstration of reovirus-like particles in intestinal contents of piglets with diarrhoea [letter]. *Aust Vet J* **1975**;51:536.
55. Woode GN, Bridger J, Hall GA, Jones JM, Jackson G. The isolation of reovirus-like agents (rotaviruses) from acute gastroenteritis of piglets. *J Med Virol* **1976**;9:203-9.
56. Fu ZF, Hampson DJ. Group A rotavirus excretion patterns in naturally infected pigs. *Res Vet Sci* **1987**;43:297-300.
57. Hoblet KH, Saif LJ, Kohler EM, Theil KW, Bech-Nielsen S, Stitzlein GA. Efficacy of an orally administered modified-live porcine-origin rotavirus vaccine against postweaning diarrhea in pigs. *Am J Vet Res* **1986**;47:1697-703.
58. Bohl EH, Kohler EM, Saif LJ, Cross RF, Agnes AG, Theil KW. Rotavirus as a cause of diarrhea in pigs. *J Am Vet Med Assoc* **1978**;172:458-63.
59. Benfield DA, Stotz I, Moore R, McAdaragh JP. Shedding of rotavirus in feces of sows before and after farrowing. *J Clin Microbiol* **1982**;16:186-90.
60. Bohl EH, Theil KW, Saif LJ. Isolation and serotyping of porcine rotaviruses and antigenic comparison with other rotaviruses. *J Clin Microbiol* **1984**;19:105-11.
61. Hoshino Y, Saif LJ, Sereno MM, Chanock RM, Kapikian AZ. Infection immunity of piglets to either VP3 or VP7 outer capsid protein confers resistance to challenge with a virulent rotavirus bearing the corresponding antigen. *J Virol* **1988**;62:744-8.
62. Fu ZF, Hampson DJ, Wilks CR. Transfer of maternal antibody against group A rotavirus from sows to piglets and serological responses following natural infection. *Res Vet Sci* **1990**;48:365-73.
63. Welter MW, Welter CJ. Evaluation of killed and modified live porcine rotavirus vaccines in cesarean-derived colostrum-deprived pigs. *Vet Microbiol* **1990**;22:179-86.
64. Fitzgerald GR, Welter MW, Welter CJ. Evaluating the performance of a porcine rotavirus vaccine. *Vet Med* **1986**;81:188-92.
65. Westercamp DH. Field trial of porcine rotavirus vaccine to combat postweaning scours in baby pigs. *Mod Vet Practice* **1986**;67:17-8.
66. Lecce JG, King MW. The calf reo-like virus (rotavirus) vaccine: an ineffective immunization agent for rotaviral diarrhea of piglets. *Can J Comp Med* **1979**;43:90-3.

67. Zissis G, Lamberg JP, Marbehant P, et al. Protection studies in colostrum-deprived piglets of a bovine rotavirus vaccine candidate using human rotavirus strains for challenge. *J Infect Dis* **1983**;148:1061–8.
68. Yuan L, Ward LA, Rosen BI, To TL, Saif LJ. Systemic and intestinal antibody-secreting cell responses and correlates of protective immunity to human rotavirus in a gnotobiotic pig model of disease. *J Virol* **1996**;70:3075–83.
69. Ward LA, Yuan L, Rosen BI, Saif LJ. Pathogenesis of an attenuated and a virulent strain of group A human rotavirus in neonatal gnotobiotic pigs. *J Gen Virol* **1996**;77:1431–41.
70. Ward LA, Yuan L, Rosen BI, Saif LJ. Development of mucosal and systemic T cell responses and protective immunity to human group A rotavirus in neonatal gnotobiotic pigs. *Clin Diagn Lab Immunol* **1996**;3:342–50.
71. Chen WK, Campbell T, VanCott J, Saif LJ. Enumeration of isotype-specific antibody-secreting cells derived from gnotobiotic piglets inoculated with porcine rotaviruses. *Vet Immunol Immunopathol* **1995**;45:265–84.
72. VanCott J, Brim TA, Simkins RS, Saif LJ. Isotype specific antibody-secreting cells to transmissible gastroenteritis virus and porcine respiratory coronavirus in gut- and bronchus-associated lymphoid tissues of suckling pigs. *J Immunol* **1993**;150:3990–4000.
73. VanCott J, Brim TA, Lunney JK, Saif LJ. Contribution of immune responses induced in mucosal lymphoid tissues of pigs inoculated with respiratory or enteric strains of coronavirus to immunity against enteric coronavirus challenge. *J Immunol* **1994**;152:3980–90.
74. Svensmark B. Prevalence rate of porcine rotavirus in Danish swine herds. *Annales de Recherches Veterinaires* **1983**;14:433–6.
75. Svennerholm AM, Holmgren J, Hanson LA, Lindblad BS, Quereshi F, Rahimtoola RJ. Boosting of secretory IgA antibody responses in man by parenteral cholera vaccination. *Scand J Immunol* **1977**;6:1345–9.
76. Velazquez FR, Calva JJ, Guerrero ML, et al. Cohort study of rotavirus serotype patterns in symptomatic and asymptomatic infections in Mexican children. *Pediatr Infect Dis J* **1993**;12:56–61.
77. Pickering LK, Morrow AL, Herrera I, et al. Effect of maternal rotavirus immunization on milk and serum antibody titers. *J Infect Dis* **1995**;172:723–8.
78. Yolken R, Wyatt R, Malta L, et al. Secretory antibody directed against rotavirus in human milk—measurement by means of ELISA. *J Pediatr* **1978**;93:916–21.
79. Chrystie I, Totterdell B, Banatvala J. Asymptomatic endemic rotavirus infections in the newborn. *Lancet* **1978**;1:1176–8.
80. Feng N, Burns JW, Bracy L, Greenberg HB. Comparison of mucosal and systemic humoral immune responses and subsequent protection in mice orally inoculated with a homologous or a heterologous rotavirus. *J Virol* **1994**;68:7766–73.
81. McNeal MM, Broome RL, Ward RL. Active immunity against rotavirus infection in mice is correlated with viral replication and titers of serum rotavirus IgA following vaccination. *Virology* **1994**;204:642–50.